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CHRONOLOGICAL LIST OF SESSIONS

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WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

Thematic List of Sessions

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389.	Chemical sensory systems IV	Poster	Fri AM
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2 WORKSHOP: VISCERAL AFFERENTS: SIGNALING AND INTEGRATION. **M. Kalia**, Thomas Jefferson Univ. (Chairperson), and **L. Kruger**, UCLA (Chairperson), **K. Fuxe**, Karolinska Inst. (Sweden), **A.R. Light**, Univ. North Carolina, **W.C. deGroat**, Univ. Pittsburgh, **G.F. Gebhart**, Univ. Iowa, **C. Sternini**, UCLA, **M. Kalia**, Thomas Jefferson Univ., **D.A. Ruggiero**, Cornell Univ. Med. Ctr.

Recent developments in the neurobiology of visceral and somatic afferents have provided new insights into their central organization and patterns of discharge. These studies have utilized multidisciplinary approaches to examine functional and morphological aspects of visceral and somatic afferents. Such an approach is of great value in understanding sensorimotor coordination. Investigators working on central projections of visceral and somatic afferents have seldom directed their attention to addressing issues that are common to both sensory systems. The main aim of this workshop is to bring together neurobiologists working on central projections of visceral and somatic afferent projections so that current knowledge of these two sensory systems can be compared.

Dr. Kruger will introduce the symposium by providing a general overview of somatic and visceral afferents. Similarities and differences between these systems will be discussed in broad terms. **Dr. Fuxe** will lay the groundwork for subsequent talks by presenting the neurochemistry of peptidergic and monoaminergic neurons in the medulla and spinal cord where primary visceral and somatic afferents are known to terminate. The question of coexistence between monoamines and neuropeptides (NPY) will be addressed for both medulla and spinal cord. The distribution of these putative neurotransmitters in relation to specific cytoarchitectonically distinct regions of the central nervous system will be described. **Dr. Light** will discuss similarities and differences between primary afferent innervation by somatic vs. visceral afferents. He will describe the distribution and synaptology of somatic nociceptive primary afferents in the trigeminal nuclei and spinal cord based on data derived from single unit recording coupled with intraaxonal and intracellular staining with horseradish peroxidase and other neuronal tracers. Recent results on the activation of substantia gelatinosa neurons by muscle and visceral afferent inputs will also be presented. **Dr. deGroat** will review the anatomical and physiological properties of afferent pathways from pelvic viscera. He will focus on the examination of receptive fields of these visceral afferents, their termination and processing of their inputs in the lumbosacral spinal cord, as well as the colocalization of peptidergic neurotransmitters in visceral afferent neurons. **Dr. Gebhart** will present the results of electrophysiological studies examining responses of spinal neurons in the thoracolumbar and lumbosacral spinal cord following colorectal distension. Characterization of these spinal neurons was also done with respect to converging receptive fields, rostral projections and chemosensitivity to intraarterial injections of bradykinin. The responses of these neurons in relation to sensations such as nociception will be discussed. **Dr. Sternini** will focus her presentation on an analysis of afferent innervation of the enteric nervous system with particular emphasis on the distribution of spinal afferent fibers containing calcitonin gene-related peptide and substance P immunoreactivity in the stomach and pancreas and their perikarya in dorsal root ganglia. **Dr. Kalia** will present ultrastructural analyses of the central terminals of primary pulmonary afferents, originating from slowly and rapidly adapting receptors and compare this data on visceral afferents with results derived from Dr. Light's work on somatic afferents. **Dr. Ruggiero** will focus his presentation on cardiovascular afferents. He will present data relating to the following anatomical substrates underlying tonic and reflex control of arterial blood pressure: the cardiopulmonary subdivisions of the nucleus of the tractus solitarius, dorsal horn of spinal cord, brain stem cholinergic neurons and bulbospinal projections from the rostral ventrolateral medulla to the intermediolateral cell column of the spinal cord.

3 SYMPOSIUM. A NEURAL SYSTEMS APPROACH TO THE ANALYSIS OF FEAR AND ANXIETY. M. Davis, Yale Univ. (Chairperson); R.F. Thompson (Stanford Univ.), P.G. Henke (St. Francis Xavier Univ.), B.S. Kapp (Univ. of Vermont), J.E. LeDoux (Cornell Univ. Med. Ctr.), T.S. Gray. (Loyola Med. Ctr.).

Over the last several years a great deal of new, compelling, and highly consistent data have shown that the central nucleus of the amygdala along with its direct connections to hypothalamic and brainstem target areas appears to form a crucial component of a central fear system in a variety of animal species. Electrical stimulation of the central nucleus of the amygdala elicits a pattern of changes in blood pressure, gastric ulceration, respiration, heart rate, and ongoing motor and reflex behavior that normally occur during fear and anxiety. In fact, this pattern of behavioral changes is typically used to define the presence of generalized anxiety clinically, and many of the individual components listed above form the basis of various animal models that are used to study anxiolytic drugs (e.g., cessation of ongoing bar-pressing in the operant conflict test). Electrolytic or chemical lesions of cell bodies in the amygdala prevent stressful or conditioned fear stimuli from producing this pattern of behavioral changes. Local infusion of different psychoactive compounds into specific nuclei within the amygdala produce anxiolytic effects in several different behavioral tests of anxiety. Electrophysiological studies show that aversive or conditioned fear stimuli preferentially alter single unit activity in the amygdala. With the advent of sensitive and selective anterograde and retrograde anatomical tracing techniques it has become possible to delineate efferent pathways and specific target areas of the amygdala that might mediate each of the various symptoms seen in fear and anxiety. Data from several laboratories have now shown that direct connections from the central nucleus of the amygdala to specific target areas mediate specific symptoms of fear. Thus projections to the hypothalamus and dorsal motor nucleus of the vagus mediate changes in blood pressure and heart rate. Projections to the parabrachial nucleus may mediate changes in respiration. Projections to the central grey and pontine reticular formation are critical for freezing and increased startle, respectively. New assay techniques have shown that these efferent projections utilize one or several different neurotransmitters, including several peptides which are known to be abundant in the amygdala. Taken together, the data indicate that the central nucleus of the amygdala and its direct projections to the hypothalamus and brain stem comprise a critical neural substrate of fear. This conclusion has been reached using anatomical, behavioral, physiological and pharmacological approaches and will ultimately have considerable impact on the design of more selective anxiolytic drugs.

4 WORKSHOP: CHEMICAL ARCHITECTURE OF THE CEREBELLAR CORTEX: STRUCTURE AND FUNCTION. J.S. King The Ohio State Univ. (Chairperson) E. Mugnaini, Univ. of Connecticut, A.J. Beitz, Univ. of Minnesota, N.H. Barmack, Good Samaritan Hospital, J.C. Strahlendorf, Texas Tech. Univ.

The neuronal circuitry of the cerebellum, as defined by anatomical and physiological techniques, has been well characterized. The cerebellar cortex is generally described as a uniformly layered structure in which the neural components are organized in a repetitive geometric arrangement. Recent studies of the intrinsic circuits and afferent systems have utilized immunohistochemistry and the iontophoretic application of putative neurotransmitters to provide new insights into cerebellar structure and function. Dr. Mugnaini will show that most, if not all, Purkinje cells, stellate/basket cells, and Golgi cells are GABAergic. In addition, glycine and several neuropeptides can be localized in certain cerebellar inhibitory neurons. The notion that the cerebellar nuclear outflow is exclusively excitatory is no longer tenable. Apparently most, if not all, small and a few medium-size nerve cells are GABAergic projection neurons. Dr. King will describe a differential distribution of enkephalin, cholecystokinin and corticotropin releasing factor within distinct populations of climbing fibers, mossy fibers, and/or a beaded plexus of axons located at the Purkinje cell-granule cell border. These data indicate that classic cerebellar afferent systems are chemically heterogeneous. Dr. Beitz will discuss the localization of two putative excitatory amino acid transmitters, glutamate within pontocerebellar and trigeminocerebellar projections and aspartate within the olivocerebellar projection. In addition, the localization of glutamate-like and aspartate-like immunoreactivities to certain populations of granule cells in the cerebellar cortex will be described. Finally, the localization of the amino acid taurine and its synthesizing enzyme, cysteine sulfinate decarboxylase, in certain Purkinje cells and Golgi cells will be considered. Dr. Barmack will describe cholinergic mossy fiber projections; monoclonal and polyclonal antibodies to the cholinergic synthesizing enzyme, choline acetyltransferase have been used in this study. The importance of this cholinergic projection in visual-vestibular interactions will be discussed. Dr. Strahlendorf will discuss the direct and modulatory actions of serotonin on Purkinje cells. Direct effects are pentobarbital-sensitive and dependent on the firing rates of these cells. Modulatory actions include attenuation of glutamate-elicited excitation, and augmentation of inhibitory effects of GABA mimetics at $GABA_A$ and $GABA_B$ receptors. The predominant action of serotonin appears to be modulatory, serving to suppress Purkinje cell activity by interacting with endogenous inhibitory and excitatory processes within the cortex.

*** Indicates nonmember of the Society for Neuroscience**

PO Indicates abstracts that are published only

5.1 IN VIVO DEMONSTRATION OF PRESUMPTIVE BLOB AND INTERBLOB REGIONS BY MANIPULATION OF STIMULUS SPATIAL FREQUENCY IN MACAQUE STRIATE CORTEX. *Roger B. H. Tootell and Gary G. Blasdel.* Dept. Med. Physiol., Calgary Sch. Med., Calgary, Alberta, Canada, T2N4N1.

Previous studies in macaque striate cortex have demonstrated a horizontal segregation in the responsiveness of cortical neurons to achromatic, sinusoidal gratings of high vs. low spatial frequency (Tootell et al, submitted). The anatomical segregation appears so far to be binary, with some regions preferring high spatial frequencies and others preferring low spatial frequencies. In layers 2 + 3, regions tuned to low spatial frequency correlate with the blobs revealed by cytochrome oxidase staining, and regions tuned to high spatial frequency correlate with the interblob regions. This anatomical segregation also extends beyond layers 2 + 3, vertically through cortex. In layers 4B, 5 and 6, where the blobs are faint or absent, patchy deoxyglucose patterns are produced in response to high vs. low spatial frequency gratings which are in vertical registration with those seen in the upper layers.

Given these previous deoxyglucose results, and the availability of optical techniques for visualizing cortical activity *in vivo* (Blasdel and Salama, 1986), we decided to try to visualize the cytochrome oxidase blobs *in vivo* by manipulating stimulus spatial frequency. Such a demonstration would be useful for a number of reasons. It would allow deliberate introduction of microelectrodes, tracers, etc. into blob and interblob areas, it would permit correlations of blob position to other anatomical organizations in optical studies, and it would function as a further test of the deoxyglucose findings related to spatial frequency.

In these experiments, paralyzed, anesthetized macaques were prepared as for single unit recording, with both eyes converged and focused on a stimulus display screen. The dye NK 2367 was superfused over parafoveal striate cortex via a recording chamber implanted earlier, and illuminated with light of 720 nm. A television camera recorded changes in absorbance of the dye in response to achromatic, sinusoidal gratings of either low (0.5-1.5 c/deg) or high (6.5 c/deg) spatial frequency. For each orientation (in 45^{o} steps), the absorbance topography produced by the low spatial frequency was subtracted from that produced by the high spatial frequency grating.

At each orientation, this difference topography showed patches which looked like thinner versions of orientation columns, spaced regularly over striate cortex. Control comparisons of high-minus-high (or low-minus-low) spatial frequency gratings produced no periodicities in the absorbance topographies within the same area. The sum of topographies from all high-minus-low comparisons, at all orientations, produced a composite topography which strongly resembled that produced by deoxyglucose uptake in response to high spatial frequency gratings at all orientations: a repetive annular pattern, as if all the interblob area was labelled. The "holes" in the annulae (the areas absorbing most light in response to low spatial frequencies; the presumptive blobs) lay in the center of the ocular dominance strips, when the strips were produced by dye manipulations in the same piece of tissue. The similarity of these patterns to those seen earlier in deoxyglucose studies strongly suggest that the presumptive blobs seen in this study correspond to the blobs revealed by cytochrome oxidase staining *post mortem*. These dye results also support the idea of a segregation of cells according to peak spatial frequency tuning in the blob vs. interblob regions of primate striate cortex. Supported by NRSA 5F32 EY05786.

5.2 STUDIES OF ORIENTATION AND MOTION SELECTIVITY IN MONKEY STRIATE CORTEX USING OPTICAL TECHNIQUES. Gary G. Blasdel and Roger B.H. Tootell. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada.

We used optical techniques based on video imaging technology and the voltage sensitive dye NK2367 to analyze the functional anatomy of orientation and motion selectivity in monkey striate cortex (macaca Nemestrina). Previous work using this approach (Blasdel and Salama, 1986) revealed a close relationship between the continuity of orientation mapping and the centres of ocular dominance columns: orientation shifts most continuously in patches of cortex that lie between O.D. column centres. Further analysis of this organization reveals that regions of greatest orientation continuity coincide with regions displaying the greatest selectivity for orientation. Since the boundaries of these regions, where orientation selectivity is much weaker, lie at the centres of ocular dominance columns, they include cytochrome oxidase blobs, the cells of which have been shown previously (Livingston and Hubel, 1984) to lack orientation selectivity. There is also the suggestion, from our optical data as well as from results achieved with 2-deoxyGlucose, that some of the observed differences in orientational selectivity may derive from differences in the absolute responsiveness of different regions of cortex to elongated black/white edges.

Since it is conceivable that end-stopped inhibition plays some role in the observed differences in responsiveness we assessed the effect of edge length on orientation preference and tuning by comparing patterns of activity generated by moving black and white edges with those generated by a field of random white dots (2% density with each dot subtending 0.1 degree of visual angle) moving uniformly in the same direction. Although the results of this comparison proved inconclusive with respect to our original question - effects on responsiveness and orientation tuning - they nevertheless revealed a surprising and robust difference in the direction of preferred movement for each of the two patterns. Maps of orientation/movement selectivity generated by moving fields of elongated edges (oriented perpendicular to the direction of motion) and random dots appear strikingly similar if the motional direction for one is rotated by 90 degrees. A cortical region responding best to the horizontal movement of black and white bars, for example, responds best to random dots moving vertically. And similar differences are apparent for all other possible orientations and directions of movement.

One possible explanation for this finding is that it derives from orthogonal direction selectivities for separate but co-mingling populations of neurons - one responsive to elongated edges, the other to dots. Alternatively, it could derive from some intrinsic property of all or most cortical cells - some property that causes them to prefer different directions of movement for different classes of stimuli. Preliminary studies, using micro-electrodes, of well isolated, single cells support the second possibility. All cells isolated to date, that responded well to elongated edges, also responded well to fields of moving random dots and preferred radically different (usually orthogonal) directions of movement for each.

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5.3 ORGANIZATION AND CONNECTIVITY OF COLOR-SPECIFIC CELLS IN CYTOCHROME OXIDASE-RICH PATCHES OF MONKEY STRIATE CORTEX. D.Y. Ts'o, C.D. Gilbert, and T.N. Wiesel. Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021

We have been studying the functional organization, receptive field properties and connectivity of color-selective cells found in the cytochrome oxidase-rich blob regions of the monkey striate cortex. Using multiple closely spaced penetrations into individual blob regions, we have previously shown that an individual blob seems to be dedicated to one color opponency system, either red/green or blue/yellow. In the present study, we examined the cells of layer 4C directly underneath blob regions to reveal relationships between the functional specificity of the cells in the two layers. Electrode penetrations first entered into blob regions and the functional properties of the blob cells were characterized. The electrode was advanced through a layer of oriented, non-color selective cells in layer 4B and then to layer 4C, where cells had monocular, unoriented, concentric receptive fields that were often color selective. In most cases (94%), the color opponency of the cells in layer 4C was identical to the blob cells found more superficially in the same penetration. These findings are consistent with idea that color-selective layer 4c cells contribute input to layer 2+3 blob cells with matching color selectivity. In addition, a few penetrations beginning in the superficial layer blobs and extending into layer 4C did not encounter oriented non-color selective cells in layer 4B, but instead encountered a continuous column of blob-like (unoriented, often color-selective, monocular) cells. Closely spaced neighboring penetrations did encounter the expected oriented cells of layer 4B. These penetrations suggest that there may exist a narrow columns of blob-like cells, perhaps centered in the middle of blobs, that span from layer 2+3 to layer 4C.

We have also done a preliminary study on the connectivity between color-selective blob cells and color-selective oriented cells. Using cross-correlation analysis between recordings from two electrodes, we have observed correlated firing between color-selective unoriented blob cells and oriented cells with matched color selectivity. The correlations reflect both monosynaptic and common input connectivity. The blob cells involved in these correlations had either Type I (color opponent center surround) or modified Type II (center color opponent with surround antagonism). No correlations between blob cells and oriented cells of different color selectivity have been found. These results suggest that color-selective unoriented blob cells may contribute to the construction of color-selective oriented fields in the monkey striate cortex.

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5.4 COMPARATIVE STUDIES OF THE COLOR CELLS IN LAYER IVC_b AND IN THE BLOBS OF THE MONKEY'S STRIATE CORTEX. Charles R. Michael, Dept. of Cellular and Molecular Physiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT. 06510.

Long, oblique tungsten electrode tracks were made through the monkey's striate cortex to examine the relationship of the double opponent color cells in layer IVC_b to the neurons in the cytochrome oxidase blobs in layers III and II. Small lesions marked the clusters of color cells in IVC_b; cytochrome oxidase (CO) stain revealed the blobs. The clusters were in register with the overlying blob pattern. As with the blobs, the cells within each color cluster were confined to a single ocular dominance column. When two oblique electrode tracks were made successively in the same plane through the same region of cortex, one in layer II/III and the other in IVC_b, the color properties of the blob cells were related to those of the cells in the clusters directly underneath them. The vertical blob/cluster pairs contained cells which shared the same chromatic organization, e.g. red on, green off center double opponents. Some blobs appeared to be entirely color specific while others apparently contained a mixture of color types. Oriented color cells were generally confined to the immediate area around the blobs but sometimes they formed bridges between adjacent blobs.

Intracellular recordings were made from double opponent color spiny stellate cells in IVC_b with micropipette electrodes filled with horseradish peroxidase (HRP). A double stain procedure for CO and HRP revealed that the axons of these cells terminated in the blobs. An axon arborized in the blob directly above its cell body. The terminals were confined within the blob. Sometimes an axon branched to end in two adjacent blobs which were probably in the same ocular dominance column. The contrast-sensitive cells in IVC_b were also spiny stellate cells. Their axons projected to layer III where they terminated in single interblob regions, spanning the boundary between two adjacent ocular dominance columns. Their arborizations sometimes extended into the blobs. These specific projection patterns indicate that the properties of the color cells in the blobs are influenced by the inputs from the spiny stellate double opponent cells in IVC_b.

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5.5 ORGANIZATION OF HUMAN VISUAL CORTEX DEMONSTRATED WITH MONOCLONAL ANTIBODY CAT-301. S. Hockfield and R.B.H. Tootell.[#] [$]Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT and [#]Dept. of Neurobiology, Harvard Sch. Med., Boston, MA.

Monoclonal antibody Cat-301 recognizes a surface-associated antigen on a subset of neurons in the mammalian CNS. Previously we have shown that in monkey cortical area V1 (area 17) patches of Cat-301 positive neurons line up to form rows that are in vertical register with the center of the ocular dominance stripes (Hockfield, et al, CSH Symp. Quant. Biol. 48:877). Cat-301 also demonstrates a substructure in monkey area V2 (area 18), as antibody positive neurons lie predominantly within the thick strips demonstrated with cytochrome oxidase histochemistry (DeYoe, et al, this volume). Conservation of antigens through evolution potentially allows reagents like Cat-301 to be used to analyze the organization of human cortical areas where electrophysiological and tracing techniques can not be employed.

In an effort to study the organization of human striate and extrastriate visual cortex, the occipital third of human post-mortem brains was flattened, sectioned tangential to the pial surface and stained immunohistochemically for Cat-301. The subcellular localization of the Cat-301 antigen in human was identical to that previously described in cat and monkey, on the surface of cell bodies and proximal dendrites. Throughout the human cortex, Cat-301 recognizes only a subset of neurons. In area V1, antibody positive neurons are found in layers IV and VI. In flattened, tangentially cut sections, antibody positive cells lie in patches that form rows across the area. The rows of Cat-301 neurons show a periodicity of 1mm within an area approximately 4cm in width. At the border of V1, a continuous line of Cat-301 positive cells is found, marking the boundary between V1 and V2. In V2, Cat-301 recognizes cells that form broad strips, approximately 1-2mm in width, separated by gaps of 4-6mm. These antibody-demonstrated strips in the human are likely to represent the thick cytochrome oxidase positive strips in monkey that have been demonstrated to contain Cat-301 neurons and neurons that project to area MT.

These studies demonstrate an organization of human cortical areas V1 and V2 that is analogous to the organization previously demonstrated in monkey. The functional properties of the human areas are likely to be related to, if not identical to, the properties of the monkey areas.

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5.6 CONNECTIONS OF MODULAR SUBDIVISIONS OF CORTICAL VISUAL AREAS 17 AND 18 WITH THE MIDDLE TEMPORAL AREA, MT, IN SQUIRREL MONKEYS, L. A. Krubitzer and J. H. Kaas. Vanderbilt University, Department of Psychology Nashville, TN 37240.

Modern methods for revealing patterns of connections have the potential for defining aspects of modular organization within areas of cortex, and suggesting functional roles for such modules. In the present study, small, single injections of wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) were injected in the middle temporal visual area (MT) of four squirrel monkeys, Saimiri sciureus. The cortex was removed, flattened, and cut parallel to the surface in order to reveal surface-view patterns of connections. Sets of alternate sections were processed for HRP, cytochrome oxidase (CO), or myelin. The CO and myelin sections identified the borders of areas 17, 18, and MT, and revealed CO-dense bands alternating with CO-light (and myelin dense) interbands in area 18. The injections were restricted to portions of myeloarchitectonically defined MT. In each case only portions of areas 17 and 18 contained labeled neurons and terminals. The label in area 17 was concentrated in several short, closely spaced, meandering rows (columns). Since area 17 is subdivided into systematic arrays of orientation selective neurons, and area MT is similarly subdivided into arrays of direction selective neurons, a logical interpretation of the connection pattern is that given direction selective columns in MT receive inputs from a limited number (4-6) of matched orientation columns in area 17. In area 18, label was in rows of punctate patches which were superimposed on the CO-dense bands. Label was largely confined to a limited number (3-5) of adjacent bands. This pattern appears to differ somewhat from that reported for macaque monkeys, where thick and thin CO-dense bands alternate, and the projection to MT seems to be largely restricted to the thick CO-dense bands. Nevertheless, in both squirrel and macaque monkeys connection patterns implicate the CO-dense bands and not the interband regions of area 18 in the visual subsystem related to MT. The injections also revealed patchlike patterns of interconnections with other visual areas and cortical regions including the superior temporal area, the rostral division of the dorsolateral area, the dorsomedial area, cortex in the superior temporal sulcus, the frontal eye field, and the frontal ventral visual region. In the contralateral cerebral hemisphere, foci of label were predominately in MT, but sparser connections were in the superior temporal area and the rostral division of the dorsolateral area. Supported by NIH Grant EY-02686.

5.7 SUBPOPULATIONS OF AXONS FROM AREA V1 TO V2 IN MACAQUE MONKEY, VISUALIZED BY PHASEOLUS VULGARIS (PHA). K.S. Rockland. Eye Research Inst. of the Retina Foundation, Boston, MA. 02114.

In macaque monkey, the connections from area V1 to V2 (or area 17 to 18) terminate in and around layer 4. This information is based on bulk injections of 3H-amino acids or HRP into area V1, with subsequent orthograde transport to area V2. No data are currently available, however, concerning the exact geometric configuration of individual cortical axons from V1 to V2: what is their lateral spread? is there any sublaminar stratification, such as described for geniculocortical terminations in V1? To address these questions, we have injected the orthograde tracer PHA in area V1 of 3 monkeys. This technique can produce a Golgi-like delineation of axons issuing from the injected area (V1 in this case). To date, we have evidence for 4 morphologically distinct types of cortical axons terminating in V2. The most common configuration (5 out of 12 reconstructed profiles) is a horizontally oriented profile, traveling 600u obliquely through the deeper cortical layers, bifurcating in layer 5, and then sending 2 daughter branches mainly to layer 4. In layer 4, these 2 branches overlap to some extent. They have a lateral spread of 350u, and are sparsely studded with both spines and beads. Two other distinct axonal profiles are associated with layer 4: one is characterized by strikingly parallel main branches, which run along the 3C/4 and 4/5A boundaries for 350u. Delicate sprays sparsely studded with spines dip about 30u into layer 4 from each branch. Another profile terminates in both layers 3 and 4, in a compact, bushlike shape, forming a cylinder of 400u diameter. Unlike the previous two types, this is densely studded with grape-like appendages. A fourth category has a parent trunk rising 600u obliquely from the white matter, which bifurcates in layer 5, and sends 2 daughter branches selectively to layer 3. In layer 3, these have separate terminal arborizations (100u across), with a center-to-center spacing of 300u. This spacing is reminiscent of autoradiographically demonstrated periodic peaks of label in layer 3. Interestingly, these PHA-delineated axons have lateral spreads of 350-400u in layer 4. This is one-half or one-third the width reported for different cytochrome oxidase (CO) compartments in area V2. This dimensional discrepancy may support other evidence suggesting the existence of tangential subdivisions within CO compartments. Whether these 4 axonal types can be associated with specific CO compartments in V2 is still under investigation. Preliminary results suggest that at least 2 occur in projection zones associated with all 3 CO compartments. These results emphasize the likelihood of precise stratification within the middle, input layers of V2, and suggest that each of the physiologically identified functional "streams" may be built-up from anatomically diverse cortical connections. (EY07058)

5.8 POSTNATAL DEVELOPMENT, STRUCTURE AND POSSIBLE FUNCTION OF LONG-RANGE HORIZONTAL INTRINSIC CONNECTIONS IN CAT AREA 17. H.J. Luhmann[$], J.M. Greuel* and W. Singer. Max Planck Institute for Brain Research, P.O. Box 710662, 6000 Frankfurt 71, F.R.G.

It has been shown in kitten striate cortex that an exuberant intrinsic system of far-reaching horizontal connections is already present in the 2nd postnatal week (Luhmann et al., Exp. Brain Res., 63:443, 1986). Single extracellular intracortical injections of different fluorescent dyes or horseradish peroxidase conjugated with wheat-germ agglutinin revealed in frontal, tangential and parasagittal sections a characteristic patchy pattern of retrogradely filled pyramidal cells and anterogradely labeled terminals. The spatial expression and the maximal lateral extent of these connections were strongly age- and experience-dependent. In 2- to 5-week-old normally reared (NR) and visually deprived (VD) kittens patches often fused to beaded, medio-laterally running bands and tangential connections of up to 11 mm length could be demonstrated in the superficial layers. During the critical period this exuberant pattern is reduced by an activity-dependent process. Selective cell death and/or elimination of long axonal collaterals are likely mechanisms. In adult NR cats horizontal intrinsic connections are limited to 2-3 mm in their lateral extent, whereas in adult VD animals they are no longer demonstrable. The spatial structure, columnar arrangement and postnatal development of these connections suggest a functional relation to the cortical system of orientation columns.

In order to test the hypothesis that tangential excitatory interactions are involved in the formation of large or unconventional receptive fields (RF), the RF properties of 608 neurons were studied in area 17 of 4- to $8^1/_2$-week-old kittens and adult cats at eccentricities between 0-20°. The width of the dominant discharge region and the overall size of the RFs of simple and complex cells were significantly (U-Wilcoxon-test, $p<0.01$) smaller in adult cats than in kittens. Furthermore, 18% (n=28) of the quantitatively investigated cells in kittens and 2.6% (n=1) in adult cats showed one or two facilitatory, often direction-selective ectopic fields, which were more than 4° separated from the classical RF. These neurons were mainly located in the superficial layers suggesting a relation to the system of long-range horizontal connections.

Current source density analysis of field potential responses to intracortical electrical stimulation in NR and VD kittens and in adult cats confirmed most of the anatomical data and revealed a layer-specific organization of tangential excitatory connections.

5.9 **RELATIONSHIPS BETWEEN CORTICO-CORTICAL PROJECTIONS, INTRINSIC CORTICAL CONNECTIONS AND ORIENTATION COLUMNS IN CAT PRIMARY VISUAL CORTEX.** C. D. Gilbert and T. N. Wiesel, Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

A common pattern of cortico-cortical connections is their clustered or patchy distribution, with clusters of cells in one area projecting to patches of terminals in the target area. When projected onto a plane parallel to the cortical surface, the cell clusters are seen as systems of branching bands, reminiscent of the orientation and ocular dominance columns. To determine the relationship between the clustering pattern and the orientation columns, we combined retrograde labeling of the cells participating in cortico-cortical connections with labeling of orientation columns by 2-deoxyglucose autoradiography.

We labeled the cells projecting from area 17 to area 18 in the cat by injecting rhodamine filled latex microspheres (beads) in area 18. Prior to injecting the label we mapped the distribution of orientation columns in area 18 over a small area (approximately 1mm in diameter), and injected 0.5µl of the beads in a column of identified orientation preference, maximizing the distance from columns of orthogonal orientation preference. After allowing sufficient time for retrograde transport of the beads to area 17, the animal was reanesthetized and infused with 14C 2-deoxyglucose (100 µC). During the infusion the animal was presented with a visual stimulus consisting of vertical lines of varying thickness, thus labeling the vertical orientation columns. The cortex was flattened and sectioned in a plane tangential to its surface and exposed to X-ray film. The sections were then used to map the distribution of retrogradely labeled cells, allowing us to compare directly the distribution of cortical projection bands and vertical orientation columns in the same sections. The two patterns corresponded very closely: when the injection was placed in a horizontal orientation column in area 18, the labeled cells in area 17 lay between the labeled vertical orientation columns. With injections placed in a vertical orientation column in area 18, the labeled cells in area 17 were in register with the labeled vertical orientation columns. These findings indicate that cortico-cortical connections relate columns of like functional specificity.

We used a similar approach to relate intrinsic cortical connections to orientation columns. In these experiments, the orientation preferences of cells were determined by intracellular recording and their axons and dendrites were labeled by fluorescent dye injection. Preliminary results have revealed a correspondence between the distribution of the axons of the injected cells and the orientation columns.

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IS THERE A TOPOGRAPHICAL RELATION BETWEEN OCULAR DOMINANCE AND ORIENTATION COLUMNS IN THE CAT STRIATE CORTEX? S. Löwel* and W. Singer (SPON: European Neuroscience Association). Max-Planck-Institut für Hirnforschung, P.O. Box 710662, D-6000 Frankfurt a.M. 71, F.R.G.

In order to visualize the topographic relationship between ocular dominance (OD) and orientation (OR) columns, the two systems were mapped with (3H)proline and (14C)deoxyglucose (2-DG) autoradiography. Two weeks after intravitreous injection of (3H)proline, cats were stimulated 1. binocularly with moving square wave gratings of one orientation or 2. monocularly through the right (injected) eye with one orientation in the left visual hemifield (HF) and all orientations in the right HF. The resulting patterns of increased 2-DG uptake and of terminal labeling were analyzed in flat-mount sections of the visual cortices.

Both columnar systems have the tendency to form regularly spaced parallel bands whose main trajectory is perpendicular to the 17/18 border. However, they differ in their spatial frequencies: adjacent OR-columns are more widely spaced than OD-columns. The graphical superposition of the two systems failed to show correlations beyond the degree that is expected from the superposition of independent but similarly organized patterns: none of the organizational features of OR-columns (start and stop points, bifurcations or beads) was specifically related to the centers or borders of OD-columns. Interestingly, monocular stimulation with many orientations failed to induce the pattern of OD-columns observed by Kossut, M. et al. (Acta Neurobiol. Exp., 43:273, 1983), but resulted in a more or less homogeneous increase of radioactivity over the entire extent and throughout all layers of the visual cortex. In addition, monocular stimulation with one orientation induced a pattern of OR-columns similar to that obtained after binocular stimulation (Löwel, S. et al., J. Comp. Neurol., 255:401, 1987). In both cases, regions of increased 2-DG uptake were not restricted to columns of neural tissue above and below the (3H)proline labeled OD-bands in layer IV.

Thus, in contrast to the striate cortex of macaque monkeys (Kennedy, C. et al., Proc. Natl. Acad. Sci. USA, 73:4230, 1976), visual stimulation through one eye seems to be sufficient to induce activity in the entire visual cortex of the cat. These results indicate that area 17 of cats and area V1 of macaque monkeys have a fundamentally different organization of binocular information processing.

5.11 TOPOGRAPHY OF CALLOSAL PROJECTIONS OF RAT PRIMARY VISUAL CORTEX. A. Burkhalter[1], J.P. Doyle*[2], R. P. Haugland*[3], and J.M. Nerbonne[2]. Depts. of Neurosurgery[1] and Pharmacology[2], Washington University School of Medicine, St. Louis, MO 63110. Molecular Probes Inc.[3], Eugene, OR 97402.

In rodents the main callosal projection of primary visual cortex (area 17) originates from layers 2/3, 5 and 6 at the border of area 17 and 18a, where the vertical meridian (VM) is represented, and terminates at a homotopical site in the opposite hemisphere. In addition, this projection receives a contribution from layer 5 and 6 outside the 17/18a border region, representing peripheral parts of the visual field, which could lead to topographical mismatch in the terminal zone on the opposite side.

To study the topology and simultaneously compare the callosal fiber terminals originating from the 17/18a border and the interior of area 17, we used anterograde tracing with fluorescent dextrans (Molecular Probes Inc.). The left area 17 of the hooded rat (3-5 weeks old) was injected at the VM with FITC-labeled dextran (D-1821, 25% in H_2O, 0.05ul) and a second similar injection labeled with Texas Red (D-1828) was placed into the peripheral representation (20-40°). After 2-4 days survival animals were perfused, the brains removed and sectioned.

Injection sites were 0.1-0.2 mm in diameter and included all layers. From the injection sites, green and red labeled fibers coursed through grey matter, descended into the optic radiation or crossed via the corpus callosum. Terminal arbors were observed in many cortical regions of the left (in areas 18a and 18b) and right (in areas 17 and 18a) hemispheres, visualizing red and green endings and enabling direct comparison of their distributions. Similarly, anterograde labeling was found in all known subcortical projections of area 17 (LGNd, LGNv, LP, pretectal nuclei, SC). Several of these were also marked with retrogradely labeled cells, confirming known reciprocal connections.

Across the corpus callosum, VM injections on the left side with green tracer resulted in terminal labeling in layers 1, 2/3 and 5 in a 0.1-0.3mm wide strip within right area 17 overlying the 17/18a border. This border was never labeled with red fibers nor have we ever seen red terminals within area 17 that originated from peripheral area 17. Instead, red terminal arbors mapped exclusively onto area 18a lateral to the 17/18a border and never overlapped the green callosal projection to the VM.

This is the first demonstration of anterograde and retrograde neuronal tracing with fluorescent dextrans in mammalian CNS. Specifically the results show, unlike Miller and Vogt's (Brain Res., 297:75, 1984), that injections away from the VM are not connected to mirror-symmetrical points of area 17 in the opposite hemisphere. Such transcallosal connections, however, do exist between areas 17 and 18a. (Supported by NIH grant EY05935).

5.12 **IN VIVO VISUALIZATION OF CALLOSAL PATHWAYS: A NOVEL APPROACH TO THE STUDY OF VISUAL CORTEX ORGANIZATION.** R. Malach* (Spon: T. Flash). Center for Neurosciences, Weizmann Inst. of Science, Rehovot, Israel 76 100.

A major challenge in cortical research is to directly relate the structure and function of cortical modules. An important step towards this goal is to reveal aspects of cortical organization while the animal is alive. I describe here the successful visualization of callosal pathways in the live mammalian cortex. The technique, called vital tract tracing, was prompted by the finding that cortical neurons, when labeled with fluorescent tracers under optimal conditions, stain sufficiently intensely to be visible even through the intact cortical hemisphere.

In the experiments reported here, rats had their left cortical hemisphere extensively injected with the fluorescent tracer bisBenzimide. After appropriate survival, the animals were reanesthetized and the right cortical hemisphere was exposed. Upon illumination with UV light, the fluorescing callosal pattern could be discerned under the network of blood vessels even with the unaided eye. The visualized callosal pattern was then used to guide WGA-HRP injections into a minute cortical region - the central callosal ring (CCR)- a 0.4 mm zone, whose boundaries are completely invisible under normal viewing conditions, situated rostral to area 17. The exposed cortex appeared normal in nissl stained material, and the presence of doubly labeled neurons indicated that the fluorescence labeling and UV exposure did not interfere with WGA-HRP transport.

By making precise injections into the CCR it was found to be reciprocally connected mainly to two zones: one on the lateral edge of area 18a and the other medially in 18b. Other, more minor projections were to posterior area 8, area 3 and the posterior edge of area 18a. Since these zones had fixed spatial relationship with the callosal system, vital tracing was used to guide WGA-HRP injections into the CCR- connected zone in area 18a. Again, there were two main labeled regions, one overlapped the hollow in the CCR, while the other was situated within area 18b. These results demonstrate the feasibility of using vital tract tracing to achieve great precision in studies of cortical organization.

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6.1 NORMAL PATHFINDING BY PIONEER MOTOR GROWTH CONES IN MUTANT ZEBRAFISH LACKING FUNCTIONAL ACETYLCHOLINE RECEPTORS. M. Westerfield, C.B. Kimmel, and C. Walker*, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The formation of orderly nerve connections during development of the nervous system requires correct pathfinding, synapse formation, and, in some cases, the elimination of extra synapses. A variety of studies have suggested that one or more of these processes is dependent upon neuronal activity since altering the normal pattern of electrical activity affects the final organization of nerve connections. Recent studies using tetrodotoxin to block sodium channels have shown that action potential activity is not required for normal pathfinding or synapse formation by developing neurons. Spontaneous release of transmitter from growth cones or from growing neurites, however, is usually not blocked by these procedures. Hence it is unclear if non-impulse mediated signalling between pre- and post-synaptic cells is a required component for appropriate pathfinding and synapse formation.

We have used a heritable mutation that eliminates functional ACh receptors in the embryonic muscles of the zebrafish to investigate the role of one aspect of neuronal activity, cholinergic neuromuscular transmission, on the establishment of peripheral nerve pathways by pioneer motoneurons. The mutation is a recessive lethal that was induced by gamma irradiation. It segregates as a single gene among progeny obtained parthenogenetically from heterozygous females.

Homozygous mutant embryos, although normal in outward appearance, are paralyzed and die a few days after hatching. Moreover, they are nonresponsive to tactile stimulation or to electrical stimulation of the spinal cord and no spontaneous or evoked neuromuscular activity can be recorded in mutant muscle fibers. Direct stimulation of the muscles, however, produces a contraction. Muscle fibers in mutant embryos do not bind bungarotoxin and fail to respond to cholinergic agonists. These observations suggest that the mutation eliminates functional ACh receptors.

To examine the accuracy of pathfinding by motor growth cones in mutant embryos, we studied the initial outgrowth of motoneurons by staining them with a neuron specific monoclonal antibody. We found that identified motoneurons are present and have axons in the muscles. Moreover, the growth cones of these pioneer motoneurons follow the same cell-specific pathways into the muscles that they establish in normal embryos. Thus, although motoneuron growth cones normally release transmitter and communicate with muscle fibers via ACh receptors during their initial outgrowth, this signalling is not required for the establishment of proper nerve projections. Supported by NS21132, GM22731, DCB8317049 and BNS12370.

6.2 THE EARLY DEVELOPMENT OF ROHON-BEARD NEURONS IN EMBRYONIC ZEBRAFISH. Paul Z. Myers*, Judith S. Eisen, and Charles B. Kimmel. (SPON: W.K.Metcalfe). Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Young zebrafish possess a small set of distinctive large neurons that mediate the simple, reflexive behaviors of the embryo. These neurons, called primary neurons, initiate axogenesis at roughly the same time, prior to 24 hours after fertilization (h) and quickly establish the basic framework of the embryonic nervous system. The development of one class of primary neurons, the primary motoneurons, has already been described. Primary motoneurons are characterized by a segmental distribution of their somata within the spinal cord and a precise, stereotypic pattern of axonal outgrowth. We describe here the morphology and early development of another class of primary neurons, the Rohon-Beard cells, which, together with trigeminal sensory neurons, form the tactile sensory component of the primary neuronal network.

Like the other primary neurons, Rohon-Beard cells begin axogenesis very early in development, at approximately 16 h. Their cell bodies are large (10-15 μm in diameter), and their peripheral arbors become extensive. Unlike the primary motoneurons, however, Rohon-Beard neurons are not individually identifiable and are not distributed segmentally. In contrast to the stereotyped growth of the primary motor axons, Rohon-Beard axons grow into the periphery in an apparently random fashion, with no recognizable pattern, and in particular, no segmental specificity. Thus, despite their initial similarities in size and time of development, Rohon-Beard neurons and primary motoneurons follow profoundly different programs of development.

We conclude that even among the set of primary neurons that develop synchronously within the spinal cord, there are at least two distinctly different patterns of neuronal organization and development: primary motoneurons are segmental and have stereotyped and specific axon morphologies, while Rohon-Beard neurons are nonsegmental and have seemingly nonspecific peripheral arbors.

Supported by NIH-NS 17963, the American Heart Association, and NIH-NS 23915-01.

6.3 TRANSIENT COUPLING BETWEEN IDENTIFIED MOTONEURONS IN ZEBRAFISH EMBRYOS DURING AXONAL PATHFINDING. J. S. Eisen and B. Debu. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Dye-coupling between neurons and other cells has been described during axonal pathfinding in several invertebrate systems, but not in vertebrates. We studied dye-coupling between identified motoneurons in embryonic zebrafish to learn whether it occurs during axonal pathway selection. In zebrafish each side of every trunk muscle segment is innervated by three large identified motoneurons, referred to as primary motoneurons because they are the first motoneurons to develop. During embryogenesis the growth cones of the three primary motoneurons exit the spinal cord in an invariant sequence in which CaP (Caudal Primary) precedes MiP (Middle Primary) and MiP precedes RoP (Rostral Primary). Thus CaP acts as a pioneer in establishing the motor nerve of each segment.

We investigated coupling between identified primary motoneurons by injecting low molecular weight fluorescent dyes (Lucifer yellow or sulforhodamine) directly into motoneuronal somata. We found that, prior to initiation of axonal outgrowth, many cells within the spinal cord are dye-coupled. Primary motoneurons become uncoupled from their neighbors at the time they initiate axonal outgrowth. The CaP growth cone then exits the spinal cord and extends ventrally, into the periphery. At about the time the CaP growth cone leaves the spinal cord, the MiP growth cone extends caudally within the spinal cord, growing towards CaP. Because the CaP growth cone extends into the periphery before the MiP growth cone, the CaP axon is well established in the periphery before the MiP growth cone contacts it. When the MiP growth cone contacts the CaP axon, MiP and CaP become dye-coupled. Dye-coupling is bidirectional, resulting from dye injection either into CaP or MiP. After coupling with CaP, the MiP growth cone turns ventrally, leaves the spinal cord, and extends into the periphery, following the pathway previously pioneered by the CaP growth cone. MiP and CaP become uncoupled at about the time the MiP growth cone leaves the spinal cord, or shortly thereafter.

Our results indicate a correlation between the temporal appearance of transient dye-coupling between CaP and MiP and the change in direction of the MiP growth cone, from growing caudally within the spinal cord to growing ventrally into the periphery. This correlation suggests that coupling may play a role in pathway selection by the MiP growth cone. Supported by NIH NS23915, the AHA, and an NSF PYI award to JSE.

6.4 ANTIBODIES TO THY-1 PROMOTE NEURITE OUTGROWTH FROM RAT SYMPATHETIC NEURONS IN VITRO, AND PROMOTE NEURITE INITIATION BY RAT ADRENAL CHROMAFFIN AND PC12 CELLS IN THE ABSENCE OF NGF. N. K. Mahanthappa* and P. H. Patterson. Division of Biology, 216-76, California Institute of Technology, Pasadena, CA 91125.

Thy-1 is considered to be a member of the immunoglobulin gene superfamily (Williams and Gagnon, Science 216:696, 1982), and is expressed on the surface of mouse T lymphocytes and certain neurons (Reif and Allen, J. Exp. Med. 120:413, 1964). While the function of Thy-1 in the immune and nervous systems is unknown, monoclonal antibodies (MAbs) to Thy-1 induce mitosis and lymphokine secretion by T lymphocytes (Gunter et al., J. Exp. Med. 159:716, 1984; MacDonald et al., Eur. J. Immunol. 15:495, 1984), and substrates coated with anti-Thy-1 MAbs promote neurite regeneration from dissociated rat retinal ganglion cells (Leifer et al., Science 224:303, 1984) and mouse cerebellar Purkinje cells (Messer et al., Cell. Molec. Neurobiol. 4:285, 1984).

We have found that affinity purified anti-Thy-1 MAbs promote neurite outgrowth from neonatal rat superior cervical ganglion neurons, and that this effect is not simply a product of enhanced substrate adhesion or nonspecific membrane perturbation. Addition of any of three anti-Thy-1 MAbs (OX-7, 19XE5, and 2G12) to serum-free neuronal cultures increases the percentage of process-bearing cells by 40-50% over controls in 24 h, and crosslinking with secondary Ab enhances this effect. Simple preincubation of the collagen substrate with MAbs is insufficient, and the addition of antibodies to other cell surface antigens (NILE, heparan sulfate proteoglycan, and class I histocompatibility antigen) at similar concentrations (5 μg/ml), with or without secondary Ab, has no effect. Furthermore, anti-Thy-1 MAbs also promote neurite initiation, in the absence of NGF, from both neonatal rat adrenal chromaffin cells and the PC12 cell line over the course of 4-7 d: a three-fold increase over controls for chromaffin cells, and a fourfold increase for PC12 cells. In a different approach to the question of function, we are generating Thy-1 deficient mutant PC12 cells. Preliminary observations reveal that the deficient cells show a very high incidence of spontaneous neurite extension in the absence of NGF. This finding suggests that Thy-1 may play a role in the stabilization of processes and/or the inhibition of new outgrowth, and that MAbs to this molecule may be blocking normal function in our experiments.

This work is supported by grants from the NINCDS and the McKnight Foundation to P.H.P., and a NSF Graduate Fellowship to N.K.M.

6.5 **MOLECULAR GENETIC ANALYSIS OF DROSOPHILA LAMININ.** D.M. Johnson* and C.S. Goodman (SPON: S. Glatzbach). Dept. Biol. Sci., Stanford University, Stanford, CA 94305.

Laminin is a high molecular weight glycoprotein complex present in extracellular matrices. The complex is comprised of three disulfide-bonded subunits, A, B1, and B2, of 400, 220, and 220 kD respectively. A variety of functions, including neurite outgrowth, cell migration, epidermal growth, and cell attachment, have been proposed for laminin based primarily on in vitro assays. However, it has not been possible to directly assess what roles laminin plays during embryonic development. To test its role it promoting or directing neurite outgrowth during development, we have turned to a molecular genetic analysis of laminin function in Drosophila. We report here on the purification and cloning of all three subunits of Drosophila laminin.

Antibodies against mammalian laminin do not cross-react with Drosophila. Thus, laminin was purified from Drosophila embryos using lectin affinity chromatography. Each of the subunits (A, B1, B2; 400, 220, and 180 kD respectively) was purified, and antisera raised against them. We used these antisera to screen a Drosophila embryo cDNA expression library. cDNA clones were isolated for all three subunits. We confirmed the identity of the B1 and B2 cDNA clones by (i) cross-hybridization between the Drosophila and mouse B1 and B2 clones (the kind gift of Y. Yamada), (ii) cross-hybridization between the Drosophila B1 and B2 clones, and (iii) sequence analysis of a 1.9 kb partial cDNA clone encoding the Drosophila B1 subunit. This sequence has 25% amino acid identity with the mouse B1 sequence. We are presently determining the complete structure of the Drosophila B1 and B2 subunits by cDNA sequence analysis.

Northern analysis reveals that the A, B1, and B2 subunits have mRNAs of ~9, 4.6 and 4.5 kb, respectively. All three mRNAs are coordinately expressed during development, and yet polytene chromosome localization by in situ hybridization shows that the three laminin subunit genes are not clustered in the genome. We have begun a genetic analysis to test the function of laminin during development by searching for mutations in the B2 subunit.

6.6 LOCALIZED INO ANTIBODY BINDING IN THE WING OF DROSOPHILA DURING PERIOD OF AXONAL OUTGROWTH. S.S. Blair and J. Palka. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195

The wing of Drosophila melanogaster is formed from the metmorphosing wing imaginal disc. During the first few hours of this process, identifiable sensory neurons arise within the wing. These neurons initiate axon outgrowth, forming stereotyped nerves which project towards the CNS along very precise pathways through the longitudinal veins of the wing. A series of experiments has shown that the epithelium underlying the neuronal pathways is in some manner specialized for the promotion and guidance of growing axons. If neuron-containing tissues are tranplanted into mutant, aneural host wings, axons grow out of the implant into a host wing lacking both its normal neurons and any indication of physically defined channels or veins. Nonetheless, implant neurons project axons in the correct, proximal direction and along the approximate course of the epithelium of the third longitudinal vein, one of the two normal nerve paths in wild-type wings. The cues are fairly non-specific, as neurons derived from leg, eye, antenna, and wing, as well as wing tissue from different Drosophila species, can follow them.

We have therefore examined a wide variety of antibodies to see if any bind to wings in a pattern similar to that expected of a putative guidance molecule. One of the antibodies examined was the INO-3 antibody (kindly provided by Dr. P.H. Patterson). This antibody was originally isolated for its ability in mammalian cell culture to block neurite-promoting factors derived from non-neuronal cells; in that system it recognizes a complex of laminin and heparan sulfate proteoglycan. We find that it also binds to Drosophila tissue. In developing wings INO binding first appears during the initial stages of axon outgrowth. Its spatial distribution becomes increasingly restricted, and eventually is limited to the nerve pathway of the radius and the third vein. By 24 hr after pupariation, when axon outgrowth is complete, the antibody no longer binds.

Thus, the INO antibody marks the pathway followed by both normal and implant axons. However, the earliest pattern of binding does not exactly match the course taken by the first axons, and in preliminary experiments the antibody did not appear to disrupt outgrowth under our wing culture conditions. Therefore, the role of the INO-binding antigens in axonal guidance in the wing is not clear.

6.7 RAT RETINAL NEURITES GROW ON SCHWANN CELL SURFACES BUT NOT SCHWANN CELL-ORGANIZED EXTRACELLULAR MATRIX. N. Kleitman, P.M. Wood*, M.I. Johnson, and R.P. Bunge. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Despite evidence that both glial cell surfaces and components of the extracellular matrix (ECM) support neurite outgrowth in many culture systems, the relative contribution of these factors has rarely been compared directly. Specifically, it remains to be determined which components of peripheral nerve support growth of central nerve fibers. We have directly compared neurite outgrowth from embryonic day 15 rat retinal explants placed onto 1) Schwann cells without ECM, 2) Schwann cells expressing ECM (including a basal lamina), 3) cell-free ECM remaining after removal of the cellular components of neuron-Schwann cell cultures, 4) nonglial cells (fibroblasts), 5) purified laminin, 6) rat tail type I collagen prepared by air-drying after exposure to ammonia vapors (ammoniated) or 7) collagen which was air-dried without exposure to ammonia (ADC). Length estimates were made from a subset of cultures fixed 5-6d after plating of retinal explants and stained with Sudan black. Results are summaried as follows:

Substratum	Length in mm[1] (n)	% w/growth	(no. tested)
Schwann cell only	3.4 ± 0.4 (5)	82%	(33)
Schwann cell + ECM	2.9 ± 0.2 (4)	100%	(12)
ECM only	0.5 ± 0.4 (2)	10%	(58)
Fibroblasts	0	0	(12)
Laminin	0	5%[2]	(62)
Ammoniated collagen	0	0	(64)
Air-dried collagen	3.4 ± 0.1 (18)	92%	(111)

[1]mean ± SEM of maximum neurite length from each explant
[2]neurites extended on laminin retracted before fixation

Outgrowth on Schwann cells expressing ECM was extensive, but not obviously different from that on Schwann cells alone. Neurite extension on isolated ECM was poor to nonexistent. Ultrastructural observations revealed that in cultures containing Schwann cells with ECM, 95% of the shafts of retinal neurites contacted other neurites or Schwann cell surfaces exclusively; they were not seen in contact with the basal lamina. Finally, retinal explants were placed onto a small (3 mm) island of ADC in a laminin-coated coverslip to provide more secure explant attachment. Neurites grew on the ADC but none extended onto the laminin. Growth on ADC may be related to the 3-dimensionality of this substratum. We conclude from these experiments that neurite extension from embryonic rat retina is supported by a factor found on Schwann cell surfaces; neither organized ECM nor several isolated ECM components provided comparable growth support. (Supported by NIH grants NS09923, NS21771, NS07071.)

6.8 8D9 ANTIGEN IS A SUBSTRATE FOR NEURITE OUTGROWTH. V.P. Lemmon and C.F. Lagenaur. Dept. of Neurobiology, Anatomy and Cell Science and Ctr. for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Using an assay system in which integral membrane proteins are attached to the surface of petri plates, it has been possible to directly assess the ability of purified cell adhesion molecules to promote cell attachment and neurite outgrowth. 8D9 antigen, a chicken derived molecule that is cross-reactive with mouse L1 (Lemmon, V. and McLoon, S., J. Neurosci., 6:2987, 1986) was able to promote rapid attachment and neurite outgrowth of both chick retinal neurons and mouse cerebellar neurons. Non-neuronal cells were unable to attach to 8D9 coated surfaces. In contrast, 1A6 antigen, a chicken derived molecule related to N-CAM (ibid.) was unable to attach cells derived from either chick retina or mouse cerebellum. Since 8D9 is strongly expressed by neuronal axons, these findings support the hypothesis that 8D9 and related antigens are important in axon fasciculation and in outgrowth of axons along existing axons. The detection of the related molecule L1 on Schwann cells distal to sciatic nerve transsections (Nieke, J. and Schachner, M., Differentiation, 30:141, 1985) and the activity of 8D9 in promotion of neurite outgrowth detected in the studies presented here suggests that one function of Schwann cells in regeneration is to provide a substrate for axon outgrowth.

6.9 IDENTIFICATION OF TWO DISTINCT NEURONAL RECEPTOR SYSTEMS THAT MEDIATE PROCESS OUTGROWTH ON ASTROCYTE SURFACES. K.J. Tomaselli*, K.M. Neugebauer, J.L. Bixby, L.F. Reichardt and J. Lilien*. Dept. of Physiology and Howard Hughes Medical Institute, UCSF, San Francisco, CA and Dept. of Zoology, Univ. of Wisconsin, Madison, WI.

Neuronal process outgrowth by chick ciliary ganglion (CG) neurons of two different developmental ages was examined in vitro on extracellular matrix (ECM)-derived substrates and on the surfaces of primary cortical astrocytes. E8 CG neurite outgrowth on laminin (LN) or astrocyte-conditioned medium (CM) substrates was prevented by monoclonal antibodies that block the function of ECM receptors (CSAT and JG22) while process outgrowth on astrocytes was not. E14 CG neurons were unable to extend neurites on LN or astrocyte CM but grew neurites on astrocyte surfaces; this reveals the presence of a neurite-promoting activity on astrocytes that is distinct from LN. This activity is unlikely to be a soluble factor since E14 growth cones were unable to transit from astrocyte surfaces onto surrounding LN substrates as E8 growth cones often did.

To identify the interactions mediating process outgrowth on astrocyte surfaces, E8 and E14 CG neurons were grown 16-20 hours on astrocyte monolayers in the presence of antibodies to ECM receptors or to the Ca^{2+}-dependent and -independent cell adhesion molecules N-Cal-CAM and N-CAM. N-Cal-CAM is a member of the cadherin family and is involved in the Ca^{2+}-dependent aggregation of avian neural retinal cells. Antibodies to N-Cal-CAM had dramatic and specific effects on both E8 and E14 CG growth on astrocyte surfaces, reducing the percentage of neurons with neurites by about 70% and the average neurite lengths of those neurons that did respond by about 60%. On E8 neurons, ECM receptor antibodies reduced the average length of neurites and when combined with anti-N-Cal-CAM had roughly additive inhibitory effects. E14 neurite outgrowth was not inhibited by anti-ECM receptor antibodies. Antibodies to N-CAM had no detectable effects either alone or in combination with ECM receptor antibodies on process outgrowth by neurons of either age.

Thus, E8 CG neurons use primarily two receptor systems for growth on astrocytes: ECM receptors belonging to the integrin family and a Ca^{2+}-dependent cell adhesion molecule belonging to the cadherin family. E14 neurons that have lost ECM receptor function use primarily the latter system for growth on astrocytes. The results presented here suggest that expression of the cadherins and ligands to which they bind play a role in nervous system development and regeneration. Supported by NIH grant NS-19090, NSF grant DCB 8511052, March of Dimes Birth Defects Foundation grant I-774 and the Howard Hughes Medical Institute.

6.10 TISSUE ENVIRONMENT INFLUENCES THE TIMING OF PREGANGLIONIC AXONAL OUTGROWTH IN THE SYMPATHETIC SYSTEM OF THE CHICK. J.W. Yip. Dept. of Physiol., Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261.

The spatio-temporal patterns of preganglionic outgrowth in the chick sympathetic system are highly specific. Preganglionic axons arising from the T1 spinal segment project predominantly in the rostral direction, whereas T4 preganglionic axons project predominantly in the caudal direction. Moreover, the timing of this outgrowth proceeds in the rostral to the caudal direction. Axonal outgrowth begins at stage 27 (5-5½ days). By stage 28 (5½-6 days) preganglionic axons arising from the T1 spinal level already project 3 segments, whereas T4 preganglionic axons project less than 1 segment.

Previous spinal cord rotation studies (T1 to T4 spinal cord segments were rotated 180° along the anterior-posterior axis) show that translocated neurons project in directions appropriate to their new locations. This suggests that there are specific cues in the tissue environment providing the guidance for these axons. Similar spinal cord rotation studies were recently performed to examine the effect of the tissue environment on the temporal pattern of axonal outgrowth. My results show that translocated neurons project on a schedule consistent with their new location. That is, T4 preganglionic neurons which have been translocated to the T1 spinal level extend their axons into the sympathetic trunk at stage 27 and by stage 28 they project 3 segments rostrally. Thus, the tissue environment determines not only the direction but also the timing of preganglionic axonal outgrowth.

To examine the influence of tissue environmental factors on preganglionic axonal outgrowth at different times, heterochronic spinal cord transplantations were performed. A segment of the spinal cord from the T1 to the T4 spinal levels was removed from donor embryos (stages 18 and 19) and transplanted into younger hosts (stages 13 and 14) from which an equivalent length of the upper thoracic spinal cord was previously removed. Even though the donor cord is at least 24 hours older than the host, preganglionic axons from the T1 level of the donor were not observed in the sympathetic trunk until stage 27 of the host. Furthermore, they also project 3 segments rostrally by stage 28 of the host.

These results suggest that the tissue environment outside the spinal cord influences not only the spatial pattern but also the temporal pattern of sympathetic preganglionic outgrowth.

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6.11 THE ROLE OF GLIAL "ANTI-CHANNELS" IN COMMISSURAL AXON GUIDANCE. D. Snow*, J. Dodd, T. Jessell, J. Silver. (SPON: Michel Kliot) Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106. The Center for Neurobiology and Behavior, Columbia University, New York, New York 10032.

Axons have been shown to grow through preformed glial channels in several areas of the developing nervous system. A glial region, the roof plate, is located at the dorsal midline of mouse and rat embryonic spinal cord. Axons never cross this area, but the reasons for this are unknown. Is there a specialized substructure present within the roof plate that acts as a boundary during development? In order to address this question, we have analyzed the developing dorsal spinal cord midline in mice with 1 um plastic serial sections and with EM. In addition, we have examined a particular subpopulation of commissural axons using a monoclonal antibody, 1C12, which is specific for a cell surface glycoprotein. Commissural fibers show a stereotypical trajectory away from the dorsal midline. During early stages of their outgrowth, we have observed a system of large extracellular spaces, surrounded by glial processes, which are strategically located in the roof plate.

A spatio-temporal analysis was done to visualize the developmental sequence of formation of the roof plate channels on embryonic days 8-14 in cervical cord. The alar commissural fibers, generated before E9, near the roof plate, course laterally away from the dorsal midline in close juxtaposition with the edges of this channel system, but never travel through it. The roof plate channels are transient. They are first observed as a single row of extracellular spaces at E9. After E11, as the dorsal root afferents approach the midline, the morphology of the roof plate undergoes a series of dramatic changes, but continues to serve as a boundary.

Our data suggest that the mere physical presence of large extracellular channels is not always predictive of a future axon pathway. Indeed, in the present case, the roof plate spaces seem to function as "anti-channels" to orient the ventrally-bound commissural fibers away from the midline. What makes anti-channels inhibitory? Previous studies in chick forebrain have shown that a glycosoaminoglycan, chondroitin-6-sulfate, is present in large quantities in channels beneath the cortical plate region where axons are not found (Palmert et al., Neurosci. Abs., Vol. 12, 1986). Whether this component of the extracellular matrix, or others, are inhibitory to axons and actually present in the anti-channels is currently under investigation (Supported by NIH EYO 5952, the Spinal Cord Society, and the Case Alumni Brumagin Memorial Fund).

6.12 ABNORMAL PURKINJE CELL HISTOGENESIS IN HUMAN RENAL AGENESIS. A GOLGI STUDY. Miguel Marin-Padilla. Department Pathology, Dartmouth Medical School, Hanover, New Hampshire 03756.

The neuronal differentiation/maturation of the cerebellum of four premature infants with bilateral renal agenesis (Potter's syndrome) has been studied with the rapid Golgi method. The infants were born at 32,33,33, and 36 weeks of gestation, and survived for only a few (3-7) hours. Their cerebelli were considered to be anatomically normal. Golgi preparations were made of the lateral lobes. Histologically, all cases show a 2-3 weeks delay in their differentiation and neuronal maturation, and abnormal Purkinje cell histogenesis. These neurons have retained their basal and perisomatic dendrites which should have disappeared by the 31st week of gestation (Marin-Padilla, J. Comp. Neurol. 235: 82-96, 1985). Subsequently, the basal/perisomatic dendrites undergo a pronounced hypertrophy, growing into the internal granular layer where they reach a considerable length. Some of these long dendrites give off apical branches that enter the molecular layer. The progressive growth of basal/perisomatic dendrites coincides with a reduction in the number and length of the original Purkinje cell apical dendrites. These Purkinje cell dendritic changes show a distinct developmental gradient. These developmental modifications are quite apparent when younger and older cases are comparatively studied. The anomalies are more pronounced in proximal than in distal regions of the same folium, and in older than younger infants. The appearance of these developmental dendritic changes coincides with the arrival of climbing fibers at the Purkinje cell plate. These fibers seem to retain their contacts with the basal/perisomatic dendrites failing to ascend the apical branches. In addition, these Purkinje cell dendritic anomalies could be related to maldevelopment of basket cells, since these neurons fail to establish recognizable pericellular nests around the Purkinje cell soma. The Purkinje cell anomalies found will be discussed in relation to other neuronal and fibrillar components of the cerebellum. Somewhat similar Purkinje cell dendritic changes have been reported in renal dysplasia and infantile polycystic kidneys (Kornguth et al., Acta Neuropath. 40:1-9, 1977).

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6.13 **THE ROLE OF PROTEIN KINASE C IN GOVERNING NEURITE OUTGROWTH IN CULTURED ADRENERGIC CELLS.** F. L. Hall*, P. Fernyhough*, P. R. Vulliet, and D. N. Ishii (SPON: K. Beam). Department of Physiology, Colorado State University, Fort Collins, Colorado 80523.

The formation of vertebrate neural circuitry is regulated in part by neurotrophic factors, such as nerve growth factor (NGF) and insulin; however, the biochemical mechanisms controlling neurite outgrowth remain incompletely understood. Both NGF and insulin initiate neurite outgrowth in addition to enhancing the survival of cultured sympathetic neurons. Phorbol ester tumor promoters (such as 12-O-tetradecanoyl-phorbol-13-acetate: TPA) are also known to alter or influence the extension of neurites in a variety of cultured adrenergic cells; and protein kinase C, the major phorbol ester receptor, is implicated in this process. To further assess the role of protein kinase C in the elaboration of neurites, sphingosine, a specific pharmacological inhibitor of the enzyme, was employed in a variety of in vitro adrenergic neurodevelopmental systems. In PC12 rat pheochromocytoma cells, SH-SY5Y human neuroblastoma cells, and embryonic chick sympathetic neurons, the extension of neurites was found to be reversibly inhibited by sphingosine in a dose-dependent manner (micromolar concentrations), whether the neuritogenic stimulus was NGF, insulin, or TPA. The antagonism observed between the effects of TPA and sphingosine was indicative of competitive inhibition. Previous studies have shown that the induction of neurites by NGF (Fernyhough and Ishii, Neurochem. Res., in Press, 1987) and by insulin (Mill et al., Proc. Natl. Acad. Sci. U.S.A., 82:7126, 1985) is correlated with an increase in the levels of tubulin messenger RNA; therefore, the effects of sphingosine on tubulin transcript levels were examined in an effort to delimit the locus of action. Sphingosine did not inhibit the NGF-directed elevation of tubulin mRNA levels in PC12 cells. Nor did sphingosine inhibit the insulin-directed elevation in tubulin mRNA levels in SH-SY5Y cells. Taken together, these results obtained from both normal and neoplastic cells 1) reveal the presence of a sphingosine-sensitive pathway in neurite outgrowth; 2) indicate that protein kinase C plays a regulatory role in mediating the neuritogenic effects of both NGF and insulin in cultured adrenergic cells; and 3) suggest that protein kinase C acts at a distal segment of the neurite growth pathway.

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NEURONAL DEATH I

7.1 CELL DEATH AND SENSORY FIBERS IN THE TROCHLEAR SYSTEM OF LARVAL AND ADULT AMPHIBIANS. B. Fritzsch and R. Sonntag*. Fac. Biol., Univ. Bielefeld, P.O. Box 8640, 48 Bielefeld 1, FRG.

The development of the trochlear system and its adult diversity was studied in Xenopus laevis and Ambystoma mexicanum . The trochlear nerve was selectively exposed under anesthesia and cut in adults. Larvae were enucleated. HRP was briefly applied to the cut nerves. After survival times of 2 - 7 days, the animals were reanesthetized and perfused. The brains were subsequently removed and reacted with diaminobenzidine either as sections or as wholemounts. Labelled trochlear motoneurons were counted and the numbers corrected. Myelinated trochlear nerve fibers were counted in semithick epoxy resin sections of the proximal nerve and unmyelinated nerve fibers were counted in the electron microscope.

A significant reduction ($p<0.05$) of the initially higher numbers of labelled larval trochlear motoneurons in A. mexicanum (8 +- 4, N = 7, stage 40; 4 +- 2, N = 13, stage 44/45) and X.laevis (11 +- 3, N = 24, stage 42; 7 +- 4, N = 19, stage 46) was followed by a numerical increase to adult values. HRP-labelled degenerating trochlear motoneurons were present only at the time of numerical decrease. More than twice the numbers of fibers were found in the trochlear nerve of adult X. laevis (73 myelinated fibers) as compared to larvae (35 myelinated and unmyelinated fibers; stage 49). This suggests a late larval formation of trochlear nerve fibers and motoneurons. The development of the trochlear system of amphibians may show both cell death and long term proliferation.

Numbers of retrogradely labelled motoneurons (60 +- 8, N = 8) were slightly lower than those of myelinated trochlear nerve fibers (73 +- 11; N = 17) in adult X. laevis but much lower in adult A. mexicanum (19 +- 5; N = 13; 51 +- 6; N = 6). In the latter species a group of Mesencephalicus V cells (20 +- 7; N = 6) was consistently labelled via the trochlear nerve. The combined numbers of the two groups of labelled cells (39) related rather well to the numbers of trochlear nerve fibers (51) in A. mexicanum . These data indicate the presence of sensory Mesencephalicus V fibers in the trochlear nerve of some amphibians but not in others. Supported by the DFG, SFB 223.

7.2 DE NOVO TRANSCRIPTION AND TRANSLATION OF NEW GENES IS REQUIRED FOR THE PROGRAMMED DEATH OF THE INTERSEGMENTAL MUSCLES OF THE TOBACCO HAWKMOTH, Manduca sexta. L.M. Schwartz and B.K. Kay*. Department of Biology, University of North Carolina, Chapel Hill, N.C. 27514

The intersegmental muscles (ISM) of the moth Manduca undergo a developmentally programmed cell death following adult emergence. The trigger for cell death is a decline in the haemolymph ecdysteroid titer. We have sought to define some of the molecular events associated with the muscle's commitment to degenerate.

Animals at different developmental stages were injected with ^{35}S-methionine and after equilibration, muscle proteins were separated by 1 or 2 dimensional polyacrylamide gel electrophoresis and visualized by autoradiography. At each stage examined prior to the muscle's commitment to degenerate, the pattern of newly synthesized proteins was identical. However, coincident with committment, a set of approximately 5 new proteins could be resolved (25-100 k daltons). When animals were treated before commitment with actinomycin D or 20-hydroxyecdysone, the muscles failed to degenerate at the normal time. Both of these treatments also blocked the appearance of the new proteins. Interestingly, actinomycin D (0.5ug/animal) did not disrupt development or block adult eclosion.

Poly A$^+$ RNA was isolated from muscles at various stages and translated in vitro in the the presence of ^{35}S-methionine. The protein patterns obtained from all pre-commitment staged RNAs were identical. Translations with later mRNA samples however, revealed an additional set of 5 new proteins. Taken together, these data suggest that the de novo transcription and translation of a small set of "cell death" genes is required for muscle degeneration. We are curently attempting to clone these sequences for expression and analysis.

LMS was supported by grants from the American Cancer Society and the North Carolina Center for Biotechnology and Molecular Biology.

7.3 DIFFERENTIAL SENSITIVITY OF TRANSMITTER-IDENTIFIED NEURONES TO THE LETHAL EFFECTS OF INHIBITION OF ELECTRICAL ACTIVITY IN DISSOCIATED CULTURES OF MOUSE SPINAL CORD AND DRG. G.A. Foster and D.E. Brenneman. (SPON: P.J.Roberts). Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

The survival of cells in vivo and in vitro is dependent on a variety of trophic factors. Differential survival of the same neurons transplanted to ectopic regions of the central nervous system illustrates that not all cells are equally sensitive to this trophic support. The level of refinement of this discrimination for specific neuron types is at present unknown. We have been using primary neuronal cultures to investigate the sensitivity of mouse spinal cord and dorsal root ganglion (DRG) cells to the neuron-depleting effects of tetrodotoxin (TTX), and their rescue by vasoactive intestinal polypeptide. There is now considerable evidence that approximately 30% of the spinal cord/DRG neurons are lost after treatment with TTX over the sensitive period of 9-14 days in culture (Brenneman, D.E. et al., Brain Res., 9:13, 1983). It was questioned, therefore, whether this attenuation represented the complete loss of one or more specific transmitter-identified sub-populations of neurons, rather than a partial reduction of all neurons in general.

Dissociated cultures of mouse spinal cord and DRG were treated with 10^{-6} M TTX, as described previously (Brenneman, D.E. et al., Brain Res., 9:13, 1983), and then fixed with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 30 min at room temperature. After rinsing in phosphate buffered saline (PBS), 10% normal goat serum in PBS containing 0.1% triton was applied for 1h at room temperature. The cultures were then incubated with rabbit antibodies against either neuron-specific enolase (NSE), neurofilament (NF), met-enkephalin (ENK) or calcitonin gene-related peptide (cGRP), diluted in PBS containing 1% normal goat serum and 0.1% triton, at 4^{0}C overnight. The ABC-peroxidase method was used for visualization (Vector Labs.). Positive neurons were counted in at least 100 fields per culture dish.

As described previously, approximately one third of all NSE- and NF-immunoreactive cells, ie. neurons, were depleted by the TTX treatment. In control cultures, ENK-positive cells constituted almost 10% of the total number of neurons, and their proportionate decrement after TTX treatment was 95%. There was also a large proportionate reduction in cGRP-containing neurons after TTX application: despite constituting only 1.5% of the neurons in control cultures, their number was depleted by 70% in TTX-treated dishes. When the cGRP-immunoreactive neurons from spinal cord were distinguished from those derived from DRG (on the basis of their morphology), there was found to be an almost 100% decrease in the former, and no significant change in the latter.

The present data show that TTX blockade of electrical activity in primary neuronal cultures can lead to a differential loss of transmitter-identified neurons. Cells containing ENK, and spinal cord cells storing cGRP are essentially depleted entirely, whereas cGRP-positive neurons from DRG are not reduced. The reduction in neuronal number resulting from the TTX-induced loss of ENK and cGRP cells amounted to approximately 20% of the overall decrement caused by this drug: the transmitter identity of the remainder is still unknown.

7.4 NMDA ANTAGONISTS INCREASE NEURONAL DEATH WITHOUT BLOCKING ELECTRICAL ACTIVITY IN DEVELOPING CULTURES. P.G. Nelson, D.E. Brenneman, I. Forsythe, T. Nicol* and G. Westbrook. Lab. Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892

Activation of the NMDA receptor, a glutamate receptor subtype, has been implicated in several animal models of acute neuronal death. We have explored the possibility that excitatory amino acid receptors may also modify activity-related neuronal survival and loss during normal development. We have shown previously that blockade of electrical activity with tetrodotoxin (TTX) increases neuronal death in spinal neurons. This effect was apparent when conditioning substances were removed and when electrical activity was blocked during a two week period in development when 50% of the neurons normally die. Since excitatory amino acids mediate a large proportion of the synaptic interactions in spinal cord cultures, we have investigated the possibility that NMDA antagonists might increase neuronal death similar to that shown for TTX. A comparison of the effects of (±) 2-amino-5-phosphonovalerate (AP5) and (±) 5-methyl-10, 11-dihydro-5H-dibenzo(a,d)cyclo-hepen-5,10-imine (MK-801) was made.

Dissociated spinal cord cultures were prepared from 13 day old fetal mice. Cultures were maintained in defined medium supplemented with 5% horse serum. Prior to drug treatment, a complete change of nutrient medium was performed. Drugs were added to the culture medium on day 9 and treatment was continued for 5 days. To assay for neuronal survival, the neurons were immunocytochemically identified with antibodies to neuron specific enolase and then counted. On day 14, electrical activity was assayed using the whole cell patch clamp method.

The survival of spinal cord neurons was significantly influenced by both NMDA antagonists. With the addition of 10 μM AP5 or 0.1 μM MK-801, the number of surviving neurons was reduced to 65% of that for controls. In sister cultures, 1 μM TTX treatment reduced neuronal survival to 55% of control. There was no additivity with TTX treatment plus AP5. A significant increase (15-25 %) in the number of neurons was observed with 0.1 μM AP5 or 0.01 nM MK-801 as compared to control cultures. Spontaneous excitatory post synaptic potentials were observed in all control cultures and in all cells treated with 30 μM AP5. Spontaneous action potential bursting was observed in 12 of 15 cells from control cultures but in 0 of 15 cells treated with 30 μM AP5. These data indicate that chronic NMDA receptor blockade can, depending on concentration, either enhance or decrease neuronal survival during development. These data suggest that the pattern of electrical activity rather than activity per se may be an important determinant for neuronal survival during development.

7.5 DIRECT EVIDENCE FOR AN INCREASE IN INTRACELLULAR FREE CALCIUM IN AMINO ACID NEUROTOXICITY OF SELECTIVELY VULNERABLE HIPPOCAMPAL NEURONS. L.S. Stodieck and J.J. Miller. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C. V6T 1W5.

Amino acid neurotoxicity has been proposed as a mechanism underlying selective vulnerability in the mammalian brain following such disorders as ischemia and status epilepticus. Indirect evidence has suggested that excitatory amino acids exert their cytotoxic effects by causing an increase in intracellular free calcium concentration ($[Ca^{2+}]_i$). In the current study, direct measurements of $[Ca^{2+}]_i$, using Fura-2 fluorescence, were made in cultured hippocampal cells exposed to 0.5 mM l-glutamate for periods ranging from 5 to 30 min. Measurements of $[Ca^{2+}]_i$ were correlated with cell death quantified 24 hours after glutamate exposure using the fluorescent compounds ethidium bromide and acridine orange. Ethidium bromide stains DNA from cells with disrupted plasma membranes while acridine orange stains nuclear material in viable cells. Appropriate excitation and emission filters were selected such that photographs could be taken of either dead or viable cells in the same microscope field. The percentage of dead cells were readily determined from these photographs.

During exposure to 0.5 mM l-glutamate, $[Ca^{2+}]_i$ was found to increase rapidly from a mean of 83nM to 1020nM in the neuronal cell bodies. Non-neuronal cells, identified by morphological criteria, exhibited only very slight increases in $[Ca^{2+}]_i$ from 72nM to 131nM and, surprisingly, $[Ca^{2+}]_i$ measured in areas consisting of neurites alone also increased only slightly from 114nM to 135nM. Following glutamate exposure, $[Ca^{2+}]_i$ returned to resting levels (mean 92.6nM) in many neuronal cell bodies. However, some neuronal somata continued to exhibit elevated $[Ca^{2+}]_i$ (mean 1080nM). $[Ca^{2+}]_i$ appeared to either remain above 800nM or drop to less than 150nM with very few cell bodies exhibiting intermediate calcium concentrations. The number of neuronal cell bodies with $[Ca^{2+}]_i$ remaining elevated varied from less than 10% to greater than 90% depending on the duration of glutamate exposure and correlated strongly with the percentage of dead cells at 24 hours.

Results from this study demonstrate a form of selective vulnerability in cultures of hippocampal cells; neurons are more vulnerable to amino acid toxicity than non-neuronal cells and some neurons are more vulnerable to glutamate exposure than others. Selective vulnerability in these cultures correlated strongly with elevated $[Ca^{2+}]_i$. Further work is still required to determine whether or not elevated $[Ca^{2+}]_i$ is the actual cause of death in the vulnerable cells.

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7.6 LONG-TERM CHRONIC ELECTRICAL STIMULATION OF SPINAL CORD AND MOTONEURON SURVIVAL IN THE CHICK EMBRYO. (Spon: J.F.Toole) D.Prevette*, C.Fournier-LeRay*, G.Le Douarin*, R.Oppenheim and D. Renaud*. Dept. of Anatomy, Wake Forest Univ. Med. Sch., Winston-Salem, NC 27103 and Group de Physiologie cellulaire, Faculty Sci., Nantes-Cedex, France F-44072.

Previous studies have indicated that the survival of limb-innervating motoneurons (MNs) during periods of normally occurring cell death (E6-E15) is influenced by neuromuscular activity. Blockade of neuromuscular function by a variety of pre- and post-synaptic acting pharmacological agents or neurotoxins during the cell death period increases MN survival whereas short-term (< 24 hrs) direct electrical stimulation of the hind-limb musculature in ovo anytime between E6 and E8-9 significantly decreases MN survival. In the present study, we now extend this work and examine the effects of longer periods of chronic electrical stimulation of spinal cord on MN survival.

Chick embryos were prepared for in ovo spinal cord stimulation on E7 according to the methods of Renaud, D., et al., (Exp. Neurol., 60:189, 1978) in which two 60 μm silver wires are inserted around the still cartilagenous rudiment of the vertebral column rostral and caudal to either the brachial or lumbar enlargement. Continuous spinal cord stimulation was delivered by a constant current stimulator (5 ms, 0.5 H_z). Efficacy of the stimulation was verified daily by observing that limb movements were induced by each electrical pulse. Control embryos received electrode implants but no stimulation. Stimulation was begun either on E7 or E10 and embryos sacrificed on E10 or E14. Only embryos without grossly observable or histologically detectable abnormalities of spinal cord development were used for further morphometric examination. Spinal cords were processed histologically and cell counts carried out according to standard procedures.

Stimulation of either the brachial or lumbar spinal cord from E7-E10, E10-E14 or E7-E14 had little, if any, effect on the survival of brachial or lumbar MNs. For instance, following stimulation of the brachial region from E7-E14 MN numbers on E14 were 6,206 vs 6,737 for control embryos; and following stimulation of the lumbar region from E7-E10 MN numbers were 12,739 vs 13,181 for controls. Preliminary results also indicate that neither MN size nor the survival of three other populations of spinal cord neurons known to undergo normal cell death during these stages (dorsal root and sympathetic ganglia, and sympathetic preganglionic neurons) were affected by electrical stimulation of spinal cord. Experiments are planned to investigate the factors responsible for the apparent difference between spinal cord and direct muscle stimulation on MN survival.

7.7 DIRECT NEUROTOXIC EFFECTS OF COLCHICINE ON CHOLINERGIC NEURONS IN MEDIAL SEPTUM AND STRIATUM. G.M. Peterson, M.L. Stover*, and J.F. McGinty. Dept. Anatomy, East Carolina Univ. Sch. Med., Greenville, NC 27858.

Neurons in the medial septal nucleus die following transection of the fimbria-fornix and supracallosal stria through which they project to the hippocampal formation. It has been suggested that the septal cell death is due to interruption of retrograde transport of a neurotrophic factor (NTF) which is necessary to maintain viability of neurons in the medial septum. Indeed, several groups have reported that nerve growth factor preserves the viability of medial septal neurons after fimbria-fornix transections. Walsh and Schmechel (personal communication) have observed that colchicine-induced destruction of granule cells in the dentate gyrus (the target neurons of septohippocampal afferents) leads to neuronal death of up to 40% of the cholinergic neurons in the medial septum. Such an indirect, or retrograde degeneration would be predicted if the granule cells are a source of NTF for the septohippocampal neurons. We have investigated the direct neurotoxic effects of colchicine on the medial septal neurons. Ten adult Sprague-Dawley rats were implanted with chronic cannulae in the right lateral ventricle and injected 5-7 days later with colchicine (10 ug in 2.5 ul saline) or an equal volume of saline. Eight days later the rats were perfused with a 4% paraformaldehyde-picric acid solution and the brains were removed. Frozen sections of the brain were cut through the septal region and the hippocampus. Cholinergic neurons were identified immunocytochemically. Sections were incubated in antisera to choline acetyltransferase (Boehringer-Mannheim) at a dilution of 1:2000 for 3 days followed by the avidin-biotin immunoperoxidase method. Sections through the hippocampus were stained for acetylcholinesterase. Adjacent sections were Nissl stained. Of the six rats which received infusions of colchicine, 5 showed an almost total loss of cholinergic neurons ipsilateral to the injection. Furthermore, lateral to the injection site, there was a gradient of cholinergic cell death in the striatum. The cell loss in medial septum was barely detectable in the Nissl-stained sections, that is, the colchicine did not affect all septal neurons. We conclude from these observations that colchicine injections into the lateral ventricle in the immediate vicinity of the medial septal neurons produce a direct neurotoxic effect on the cholinergic neurons in both the medial septum and in the adjacent striatum. Supported by N.C. United Way grant # 5-80272 (GMP) and DA 03982 (JFM).

7.8 DELAYED NEURONAL NECROSIS IN NEONATAL HYPOXIA-ISCHEMIA. W.F. Massarweh*, H.V. Vinters*, P.H. Schwartz, B.E. Dwyer*, D.G. Fujikawa, and C.G. Wasterlain*. (SPON: A. MORIN). Epilepsy Res. Lab., VAMC, Sepulveda, CA, 91343, Depts. of Neurology, Pathology and Neuroscience, UCLA Sch. of Med., Los Angeles, CA 90024.

Following ischemic insults in adult animals, neuronal necrosis is often delayed by 24-72 hr, and it has been suggested that this delayed cell death is calcium-dependent. We recently reported that the inner layers of the dentate gyrus (DG) are selectively vulnerable to hypoxia-ischemia in 7-day old rats (Neurology, 37, Suppl. 1:88, 1987). Using the same model we now report that the histological appearance of neuronal necrosis is delayed by 24-72 hr in dentate granule cells but not in CA1 pyramidal cells of the hippocampus.

Twenty-three 7-day old rat pups underwent bilateral carotid ligation followed by exposure to an atmosphere of 8% O_2, 92% N_2 for 60 min. They were perfusion-fixed at the end of hypoxic exposure (time 0) or 4, 24 or 72 hr later. Serial sections (8 u) were stained with hematoxylin-eosin. Total number of necrotic neurons and total neuronal numbers in dorsal hippocampus were counted in one section per animal. In DG, where all necrotic neurons were located in the internal granular layer, the number of cells showing ischemic changes evolved from 1 per section (0 hr, 4 hr) to 30 (24 hr, over 95% from one rat) and finally 104 $\pm$ 31 (72 hr). CA3 neurons followed a similar progression (2-2-8-51) but CA1 pyramids and subiculum displayed ischemic cell change much earlier (CA1: 3-15-16-15; subiculum: 0-54-32-14 at 0-4-24-72 hr respectively). In cortex, at time 0 there were a few ischemic neurons scattered through layers 3 and 4, with vacuolation and ballooning. At 4 hr, numerous "halo" cells with condensed nuclei surrounded by pale, dilated cytoplasm were often seen in aggregate. At 24 hr, these were replaced by aggregates of ischemic neurons with fragmented nuclear material. At 72 hr, frank infarcts were present in most animals. Similar evolution of the ischemic insult was observed in basal ganglia and thalamus (VPL and reticular nucleus).

These data indicate that delayed cell death following hypoxic-ischemic insults is seen in neonates as well as in the adult. The predominance of this insult in the most immature cells of the DG suggests that some protective factor may appear during cell differentiation and confer resistance to hypoxia-ischemia. In other brain regions, the evolution from selective neuronal necrosis toward full infarction appeared as a continuum rather than independent processes.

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7.9 PRESERVATION OF GABAERGIC INNERVATION IN THE GERBIL HIPPOCAMPUS EXHIBITING ISCHEMIA-INDUCED DELAYED NEURONAL DEATH. C. Nitsch*, G. Goping* and I. Klatzo Anatomy Dept., Munich University, D-8000 München, FRG and Lab. Neuropathology, NIH, NINCDS, Bethesda, MD.

In gerbils, 5 min occlusion of carotid artery results after a delay of 4 days in selective loss of hippocampal pyramidal cells of the field CA1. This so-called delayed neuronal death was postulated to be caused either by excessive excitatory synaptic activity or by a loss of inhibitory input. The major source of inhibition in hippocampus is derived from the local basket cells and the polymorphic neurons of the field CA4 which use GABA as transmitter. In an attempt to examine whether or not the GABAergic innervation in the hippocampus remains intact, vibratome sections of hippocampus were collected from gerbils submitted to 5 min ischemia and perfusion fixed 6 h, and 1, 2, 4, 6, and 14 days after the insult. Presence of GABAergic neurons and nerve endings was visualized immunocytochemically using an antiserum directed against the GABA-sythetizing enzyme GAD (courtesy of W. H. Oertel). The sections were further processed for light and electron microscopical analysis.

No change in the structure or innervation pattern of GABAergic elements was found 6 h, and 1 and 2 days after the ischemic event. After 4 days, the non-immunoreactive CA1 pyramids were severely shrunken, but the perikarya were still covered by GAD-immunoreactive boutons. After 6 days and even more impressive after 14 days, the CA1 pyramidal cells had virtually disappeared; GABAergic neurons and terminals, however, were still present and clearly delining the former stratum pyramidale. At the ultrastructural level, these terminals were seen to synapse with remnants of the degenerated neurons or with preserved GABAergic neurons, but most frequently, apposing membrane specializations were absent.

The present findings reveal that neither the GABA-synthetizing capacity nor the GABAergic innervation pattern is impaired by the ischemic episode. Thus, it may be suggested that delayed neuronal death is probably not caused by a loss of GABA-mediated inhibition.

7.10 EARLY POLYAMINE TREATMENT CAN ENHANCE SURVIVAL OF SYMPATHETIC NEURONS DURING DEVELOPMENT AND AFTER INJURY. V.H. Gilad,* M. Dornay* and G.M. Gilad.
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It was previously demonstrated that following injury of their axon sympathetic neurons of the rat superior cervical ganglion become dependent on polyamine (PA) synthesis for their survival. This is probably the basis for their enhanced regeneration after PA treatment. In addition treatment of newborn rats with biogenic polyamines can prevent the naturally occurring death of these neurons. In the present study, we sought to determine the effect of early PA treatment on the survival of developing sympathetic neurons after injuries. Groups of newborn rats were subjected to postganglionic nerve crush (axotomy) or to treatment with antiserum to nerve growth factor (NGF) (immunosympathectomy), two treatments which result in a massive loss of neurons in the ganglion. Another group was castrated at birth, a treatment which results in nerve cell loss to below normal levels. Daily injections of a PA combination (putrescine, spermidine and spermine, 10 mg/kg each), for 7 days after axotomy and for 9 days starting with the first NGF antiserum injection, attenuated the nerve cell loss. In castrated rats PA treatment, for the first 9d after birth, not only prevented the castration-induced cell loss, but resulted in increased cell numbers as was previously observed in intact PA-treated animals. These results further indicate that polyamines are important for the survival of sympathetic neurons and, while their mechanism of action is unknown, an indirect effect on the neurons via the testes or interaction with testosterone can be ruled out, but an interaction with NGF cannot be excluded. The increase in neuron numbers within the ganglion after PA treatment was not accompanied by changes in their axon terminals in one of their target organs, the iris. Furthermore, the reduction observed in the iris in [^{3}H]-norepinephrine uptake, a functional marker of innervation, after axotomy or immunosympathectomy was unproportionally small when compared to the large drop in the number of parent neurons. These results suggest that compensatory mechanisms exist which act to adjust the number of functional axon terminals per neuron so that the number of terminals innervating the target remains relatively constant.

7.11 **NON-NEURONAL CELLS PARTICIPATE IN REGULATING LOCAL CEREBRAL PERFUSION AFTER NEURONAL DEATH BY EXCITOTOXINS.**
C. Iadecola, S.P. Arneric, M.D. Underwood, H.D. Baker, J. Callaway and D.J. Reis,*
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In brain, a major factor regulating local cerebral blood flow (lCBF) is local neuronal activity. However, we have recently discovered that, in rat, 5 days after selective elimination of local neurons in a restricted region of the primary sensory-motor cortex (SMI) by local microinjections of the excitotoxin ibotenic acid (IBO), local cerebral blood flow within the lesion, is unchanged. (Iadecola et al., Am. J. Physiol, 1987 in press). In this study we sought to determine whether the maintenance of lCBF after neuronal death correlates with the appearance of non-neuronal cells within the lesion and/or local changes in microvascular density. Rats were anesthetized (halothane 1-2%), IBO (10μg in 1 μl) was locally microinjected in SMI, and animals were allowed to recover. Three, 5, 7, 11, and 30 days later lCBF was measured autoradiographically under chloralose anesthesia (40 mg/kg, s.c.) by the ^{14}C-idoantipyrine technique. Cellular density was determined on Nissl-stained sections by using a microscope equipped with an ocular grid (140x140μm) and microvascular area (MVA) was measured by computer-assisted image analysis on sections processed for the endothelial marker alkaline phosphatase. Reactive astrocytes and macrophages were identified, respectively, by glial fibrillary acidic protein (GFAP) immunocytochemistry and acid phosphatase histochemistry. By 24 H after IBO, no neurons were detected within the area of the lesion. From 3 to 11d after IBO, lCBF within the lesion did not differ from that of the homotopic cortical area of sham lesioned controls (lesion at 3d: 120±23 mlx100g/min, n=4; control: 119±6, n=5; p>0.05, analysis of variance). However at 15d (n=5) lCBF was reduced by 22±6% and 30d (n=5) by 48±6% (p<0.05). After IBO, the number of cells increased dramatically reaching a plateau at 7-11d (17.5 fold increase at 11d; p<0.01) declining to a 11.7 fold increase at day 30 (p<0.01). The cells were mostly macrophages, endothelial cells and, at the periphery of the lesion, GFAP reactive astrocytes. MVA increased from 3d up to 4.2 fold at 11d (p<0.01), and then declined to 2.7 fold increase at 30d (p<0.01). To determine whether the microvessels were patent, the red blood cells (RBC) within the microvasculature of the area of the lesion were detected by endogenous peroxidase histochemistry and the RBC area (RBCA) determined by image analysis. In contrast to MVA, RBCA did not increase until after 7d, reaching a 3.2 fold increase at 11d. In summary, after neuronal death, lCBF is maintained in normal range during the period in which cellular and microvascular density increases. However, the increased vascular density does not participate to maintain lCBF since the, presumably newly-formed, vessels become patent at the time when lCBF begins to decline. The findings suggest that after neuronal death, non-neuronal cells, most likely activated macrophages, participate in the regulation of local cerebral perfusion, possibly, by releasing vasoactive substances and/or by-products of their metabolism.

7.12 NEUROPATHOLOGY IN ALZHEIMER'S AND DOWN'S DISEASES: THE LOCUS COERULEUS. V.M.Ingram, Z.Aslamy*, S.Jhaveri*, C.H.Miller*, H.M.Moore*, B.J.Blanchard* and D.F.Parry*, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

The noradrenergic neurons of the locus coeruleus [LC] suffer massive degeneration in the early-onset type of Alzheimer's disease [AD] and in Down's syndrome [DS], accounting perhaps for the losses in arousal and attention in these conditions. The degeneration of the LC and its subdivisions are best investigated quantitatively by computer-assisted 3-dimensional reconstructions.

Autopsy material [brain stem] was cross-sectioned sequentially, and neurons of the locus coeruleus were easily visualized by immunocytochemistry with a polyclonal antibody to tyrosine hydroxylase, the regulating enzyme in the biosynthesis of noradrenaline. The X-Y coordinates for every tenth or every thirtieth section were entered with a microcomputer into the Bioquant three-dimensional reconstruction program. Rotation of the assembly of neurons allowed us to perform quantitative analysis and quantitative comparisons between specimens.

We have to date analyzed the LC from 5 normal [27-85 yrs], 4 AD [66-82 yrs] and 4 DS brainstems [37-64 yrs]. We find degeneration of the posterior, central and anterior locus coeruleus ranging between specimens from mild to extremely extensive. Two early onset AD specimens show extreme degeneration, while a much older AD shows mild degeneration [?late onset AD]. Two DS brainstems [37,57 yrs] have LCs that are largely intact, but two other DS brainstems [48,64 yrs] are extensively degenerated in their LC. Contrary to expectation there is no correlation between age and degree of degeneration of the LC nuclei in these 4 DS specimens.

We find complete or relative sparing of the ventral LC which connects to the spinal cord and cerebellum [reported also by Marcyniuk et al. (1986) J Neurological Sci 76:335]. Interestingly, the trigeminal motor nucleus which is adjacent to the caudal part of the LC shows no loss of neurons at all in either the AD or the DS specimens. Thus there appears to be considerable selectivity in the degeneration of the LC in Alzheimer's and Down's diseases, at least in these specimens.

In our present normal LC reconstructions we see a considerable excess of LC neurons in the right caudal part of the LC[central] over the left.

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CONTROL OF POSTURE AND MOVEMENT I

8.1 AFFERENT FEEDBACK IN POSITION CONTROL OF THE HUMAN MANDIBLE IN VARIOUS LOAD CONDITIONS. F.Bosman* and C.J.Erkelens* (SPON: A.B.A. Kroese) Dept. of Oral Pathophysiology, Univ. of Utrecht, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

Apart from vital functions as chewing, swallowing, speaking, etc. the mandibular motor system is also dealing with maintaining an adequate mandibular position in various positions of the head or during movements of the head. Inherent mechanical properties of jaw muscle and other tissues involved as well as afferent feedback from several receptor groups play a role in mandibular motor control. Due to the complexity of the system special experiments and simulation studies are required to obtain more insight in the relative contribution of both factors. In the present study results from both approaches are compared for force-displacement relations of the human mandible.

In the experiments subjects were resisting a background load on the mandible while maintaining a requested jaw position. After the subjects were asked not to react a new stationary state was reached as result of a sudden change of load. Force, position and surface EMG of elevator and depressor muscles were recorded. The collection of states associated with several load changes makes up a force-displacement curve.

In the computer simulations the group of elevator muscles and the group of depressor muscles were each represented by a muscle model and the mandible by a mass. The muscle models contain a contractile element, a series elastic element, a parallel elastic element and a viscous element. The elastic elements have exponential characteristics. Two types of feedback have been incorporated in this model. The first type showing similar properties as muscle spindles has an excitatory influence on the elevator muscles. The second type has features of periodontal receptors and supplies positive feedback to jaw opening muscles and negative feedback to jaw closing muscles. The basic values of the parameters of the model have been derived from in vivo measured closing movements as a result of external force while feedback from periodontal receptors was excluded by local anaesthesia.

The experimental force displacement curves appeared to depend on the starting conditions (background load and jaw position). The slope of the curves near their starting positions depended on the preload but not on the initial jaw position. Considerable changes of EMG activity in closing and opening muscles were observed after a change in load with latencies between 10 and 40 ms. Moreover the curves for the jaw are not invariant in contrast to torque-angle relations of the arm.

The force displacement curves could be predicted well by the computer simulation. Comparison of the results from both studies revealed that feedback from muscle spindles and periodontal receptors is involved in mandibular motor control.

8.2 CONTROL OF ELEVATOR MUSCLE ACTIVITY DURING CHEWING IN MAN. H.W.van der Glas, L.W.Olthoff*, A.van der Bilt* and F.Bosman*. Lab. of Oral Pathophysiology, Univ. of Utrecht, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands.

The low level of rhythmic muscle activity for moving the jaw during chewing is probably generated by a central pattern generator (CPG). The additional elevator muscle activity required to overcome the resistance of the food may either be preprogrammed, i.e. intentionally or by a CPG, or may be induced and terminated by peripheral stimuli. In order to investigate the origin of this additional muscle activity, the relationship between events in recordings of the mandibular position and EMG activity was examined.

Silicone-rubber and chewing gum were used as standardized artificial testfoods in 7 subjects. Using bipolar surface electrodes, EMG was recorded from the masseter and anterior temporal muscles on both sides. The mandibular position was recorded with a Selspot system. In full-wave rectified and smoothed EMG, a computer program determined the onset, maximum and offset of the muscle activity. The moment of jaw-closing and opening, the first engagement of the food and the first tooth contact were detected in the jaw position signal and its derivatives (velocity and acceleration). Rank correlations (Kendall) were calculated between variables that made a link between events in the jaw position and the EMG recordings. In order to examine whether events in previous chewing cycles influenced the control of muscle activity in the actual cycles, the rank correlations were calculated between variables in actual as well as subsequent cycles.

The control of elevator muscle activity was based upon information about the height of the food bolus, gained in actual rather than previous chewing cycles. Part of the additional muscle activity in the various cycles preceded the first engagement of the food, suggesting a preprogrammed induction. However, since variables related to the first engagement of the food and the onset of those muscle activities following this engagement co-varied most significantly, these later muscle activities were predominantly peripherally induced by the food engagement as a mechanical stimulus. The first tooth contact following breakage of the food was a main factor in initiating the offset of all muscle activity.

8.3 HUMAN JAW MOVEMENT KINEMATICS IN MASTICATION AND SPEECH. D.J. Ostry and J.R. Flanagan (SPON: F. Wilkinson). McGill University, Montreal H3A 1B1 Canada

A central problem in motor control is the relationship between voluntary movements, such as reaching, grasping and talking, and more primitive motor functions, such as locomotion or mastication. We have addressed this problem in the present paper by examining the movements of the mandible, a single multi-function articulator, which, in humans, supports both mastication and speech. The study focuses on the geometric form of the velocity curve of movements as this has been shown to be invariant under transformation of rate, movement amplitude and inertial load in a variety of different tasks and species. As reported previously, movement amplitudes and durations were found to be greater in mastication than in speech. Nevertheless, detailed similarities were observed in the form of the normalized velocity curves. For jaw closing movements, the normalized curves were similar in form over differences in rate, movement amplitude (speech movements) and the compliance of the bolus (mastication). For opening movements, the curves for mastication and speech were also similar in form. In both, the shape of the curve differed for fast and slow movements. Normalized acceleration and deceleration durations were approximately equal in rapid movements, whereas, for slower movements, deceleration took substantially more time than acceleration. The similarity of the normalized velocity curves points to underlying similarities in aspects of the control of mastication and speech. The biomechanics of the mandible, the role of central function generation, and the possible similarity of neural control regimes are considered in this context.

8.4 STUTTERING: IS IT AN INSTABILITY IN THE COMPLEX SPEECH MOTOR CONTROL SYSTEM? H.B. Nudelman, K.E. Herbrich‡ B.D. Hoyt*and D.B. Rosenfield* Stuttering Cntr. Dept. Neurol., Baylor Col. Med. Houston, Tx 77030.

Speech production is a complex motor control problem. We model the complexity of this motor system by representing it as two nested control loops. The inner loop is the sound production loop and the outer linguistic loop uses this inner loop for the production of phones, words and sentences. We propose that a stuttered event is a momentary instability in this system.

We effectively open the outer loop and quantify the dynamics of the inner loop by using a vocal frequency tracking paradigm that has no linguistic content. Computer generated frequency modulated tones are played for the subject and he is asked to hum and track the frequency modulations. As in other motor tracking systems two types of behavior are observed: i) predictable tracking to periodic inputs, and ii) normal reaction time behaviors to non periodic inputs. Here we will deal only with the predictable data since we feel it is more analogous to speech where the subject knows what he is going to say. Model predictions, using the gain and phase parameters measured from sine wave frequency modulations, are made for square wave frequency modulations. The predictability of the model indicates that the parameters are valid descriptors of the predictable tracking system. Analysis of these parameters shows that stutterers have a larger phase lag at two Hz (frequency modulations near normal running speech) than do fluent speakers.

If the total phase lag equals 180 degrees at a frequency where the gain is greater than or equal to 1, the system will go unstable. This suggests that if the outer loop adds enough phase lag during linguistic processing the total system will go unstable. For instance, a time delay in the outer loop would add phase lag and could drive the system unstable. At two Hz a time delay of 250 ms equals 180 degrees. The data indicate that fluent speakers have on the average 247 ms processing time in the outer loop while stutterers have only 160 ms. This data indicates why the therapies that use forms of slowed speech work. At a frequency of 1 Hz a stutterer now has 500 ms for processing before an instability occurs.

Repeated measures indicate that while a person operates around an average gain and phase these parameters can show large variations. Stutterers tend to have larger variations than do fluent speakers.

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8.5 CEREBRAL ACTIVITY ASSOCIATED WITH VOCALIZATION D.H. York and S.C. Dulebohn*, Department of Physiology, School of Medicine, University of Missouri, Columbia, MO 65212

Previous studies, in man have defined specific patterns of cerebral activity which occurs preceding movement of a limb. The present study was undertaken to evaluate whether specific patterns of scalp-recorded cerebral activity could be obtained preceding vocalization.

Subjects were normal volunteers ranging from 22 - 40 years of age. The subject was seated in a semi-reclined position with recordings obtained from the vertex (Cz) referenced to left ear lobe (A1), and a ground placed on the opposite ear lobe (A2). Recordings were obtained with a high gain amplifier, with filter band pass set at 3 - 1000 Hz. The analog signal was sampled at 5000 Hz with data acquisition and analysis performed on an IBM-XT microcomputer. Single trials of EEG recorded 500 msec preceding vocalization and 100 msec following vocalization were averaged after 50 repetitions of a particular word. Multiple trial blocks were combined to form grand averages of several humdred trials. A voice activated trigger established a reference from which cerebral activity was time locked to voice onset.

Consistent patterns of cerebral activity were obtained preceding vocalization of particular words across different subjects. Different words had unique patterns of activity which were specific for a particular utterance. A peak identification algorithm defining number of slope changes in a 10 msec window was used to create histograms of activity. In specific time domains which preceded vocalization, consistent patterns were observed in subjects voicing the same word, but different patterns were seen with different words. A moving window cross correlation analysis demonstrated that these patterns were highly significant ($r = 0.9$) at specific time domains for comparisons of the same word, but not significant when compared across different words or a control of randomly averaged EEG.

This study has demonstrated that consistent scalp recorded cerebral activity patterns can be obtained preceding vocalization of particular words. The patterns are unique for each word and contain specific areas of high temporal correlation.

8.6 FUSIMOTOR SET: MUSCLE SPINDLE SENSITIVITY INCREASES IN NOVEL AND DIFFICULT MOVEMENTS. A.Prochazka, P.Trend*, M.Hulliger* & N.Durmuller*. Depts. of Physiology, Univ. of Alberta, Edmonton, Alta., Canada & St. Thomas's Hospital, London, U.K.; Brain Res. Inst., Zurich, Switzerland.

Chronic recordings from spindle afferents have indicated that CNS control of spindle responsiveness is highly flexible and task-related. In routine tasks such as gait, fusimotor (γ) action was hypothesised to be set to low levels, largely γ_s in type. In novel, difficult or strenuous movements γ_d "wind-up" was implicated (Prochazka et al., Brain Res., 339:136, 1986).

We have now recorded spindle firing in cats performing a range of tasks: stepping at different speeds, walking on beams and ladders of different widths and stabilities, accidental slips and paw shaking. The underlying fusimotor action was inferred in separate experiments: a length servo cyclically replicated the chronically recorded muscle length changes in soleus muscles of anesthetized cats. The responses of spindle endings in these muscles were made to match the chronic spindle firing by concomitant, computer-controlled γ_s and γ_d stimulation. Matches were optimized by iteration.

The results confirmed that fusimotor action is set to negligible or very low levels at rest, and winds up to low, static-dominated levels in slow gait. In fast locomotion, further γ_s wind-up occurs along with some modulated γ_d action. In imposed movements, paw shakes, difficult beam walking and accidental slips, γ_d set can increase greatly, not necessarily in parallel with γ_s set.

The neural substrate of "motor set" (Evarts et al., Neurophysiological Approaches to Higher Brain Functions, N.Y.: Wiley, 1984) may thus be extended to include the fusimotor system and, by implication, other sets of spinal and brainstem neurons. Strong fusimotor wind-up of spindle Ia afferent sensitivity is evidently held in reserve for novel, difficult, or strenuous movements.

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8.7 INHIBITION OF SALICYLATE-INDUCED VOMITING IN SQUIRREL MONKEYS BY HEAD FIXATION. C. R. Wilpizeski and L. D. Lowry *. Dept. of Otolaryngology, Jefferson Med. Col. of Thomas Jefferson Univ., Philadelphia, PA 19107.

We found previously that motion-induced vomiting can be inhibited in Bolivian-phenotype squirrel monkeys by firmly fixing the head to a rigid frame during 30-rpm horizontal rotation. It is the opinion of aerospace physiologists that restricting head movement is a means for preventing air and space sickness by reducing sensory input. This investigation was conducted in order to determine whether or not head fixation is effective for inhibiting vomiting of nonmotion origin.

During surgical anesthesia, 10 adult squirrel monkeys were fitted with machine screws attached, head down, to the skull with acrylic cement. After recovery from surgery, each was injected IP with 250 mg/kg body weight of sodium salicylate mixed with sterile water. Dose volumes ranged from 0.75 to 1 ml. Half of the sample was observed while nonrestrained in a transparent plastic test chamber for 2 hr after injection. The other half was restrained by bolting the head to the frame for 1 hr beginning at the time of injection and released for the second hr. After 1 week, the experiment was repeated with reversed restraint conditions. The original mobile subsample received a second injection with head fixed, whereas the subsample with head fixed during the first hr received the repeat injection while nonrestrained. One subject did not vomit during either phase of the experiment and was eliminated from statistical analysis.

Results showed that head fixation significantly increased vomiting latency and significantly decreased emesis frequency. The midpoint of the distributions of vomiting latencies was increased sixfold by head fixation. Some subjects who did not vomit during the 1-hr fixation did so soon after release from the restraining frame. We conclude that head fixation is not specific to preventing or retarding motion-induced vomiting but seems to be a more general phenomenon. Therefore, its effectiveness for inhibiting motion sickness may be more than simply a consequence of reduced sensory input caused by cupular mechanics.

8.8 INDEPENDENT SPECIFICATION OF RESPONSE FEATURES IN A REACTION TIME PARADIGM. M. Favilla*, W.Hening & C.Ghez, Ctr. for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY, 10032.

We previously demonstrated that, when aiming rapid isometric responses to a target, subjects can specify amplitude independently of direction (Favilla et al, Neurosci Abstr 1985). This was shown using a novel paradigm in which subjects synchronized their responses to a predictable auditory signal (the last of a series of 4 tones); the required amplitude and direction were signalled by a visual target presented at random intervals (0-400ms) before the last tone. Subjects were to produce single uncorrected force impulses which matched one of six randomized target shifts (3 flexions, 3 extensions). Responses initiated at the shortest S-R intervals were clustered around the middle targets in flexion and extension with about half in the wrong direction. Starting from these two default values, subjects progressively specified the amplitude of responses made both in the correct and the incorrect direction, while the number of wrong direction responses also decreased gradually. The time course of the specification of amplitude was not different from that observed when targets in only one direction were presented. Thus, specification of amplitude did not take longer when direction also had to be specified. These findings conflict with earlier data suggesting that in a reaction time task the processes of specification of different features of motor responses occur serially.

The present study was done to determine if independent specification of responses features could also occur in a reaction time paradigm. In the same subjects (N=5) as our earlier study, we examined the latencies and trajectories of isometric force impulses aimed to unpredictable visual targets under two conditions. In one (Unidirectional condition), subjects were presented with 3 flexion targets, while in the second (Bidirectional condition), subjects were presented with 3 flexion and 3 extension targets. Subjects were urged to respond "as soon as possible" and not to correct responses if either amplitude or direction seemed wrong. In both conditions response amplitudes varied with target size but their range was constricted. In the bidirectional condition, 4 subjects made between 9 and 36% of their responses in the wrong direction. Responses made in the wrong direction by 2 of these subjects showed highly significant specification of amplitude ($p<.01$). When comparing uni- and bidirectional conditions, we found that reaction times were similar and the degree of amplitude specification was the same in 4 subjects . The other subject was the one who did not produce wrong directional responses. This subject did show longer reaction times in the bidirectional condition, but response amplitude was better specified. These results further support the idea that response amplitude and direction are specified by independent neural processing channels.
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8.9 **ADAPTIVE VISUAL-MOTOR MODEL FOR RECOGNIZING OBJECT ORIENTATION IN 3-D.** M. Kuperstein, Biology Dept., Wellesley College, Wellesley, Ma., 02181

Studies by Held, Hein and others in the last two decades have shown that, in the kitten, visually guided behavior develops only when changes in visual stimulation are systematically related to self-produced movement. This work extends these studies by hypothesizing that object recognition emerges out of the correlation between object sensation and object manipulation. Within this hypothesis, recognition of object orientation in 3-D occurs when the intended grasping posture of a tilted object in 3-D space is represented and dissociated from all other 3-D orientation.

The general strategy for accomplishing this representation is the **sensory-motor neural circular reaction**. In this reaction, motor activity for the entire range of grasping postures of an object are generated one at a time by some activating source. During each posture the two eyes see the 2-D projection of the 3-D orientation of light contrast made by the grasped object. Visual map activity which is sensitive to contrast orientation, is then correlated with whatever motor map activity was used to grasp the object. The correlation occurs from the changes of synaptic weights between visual inputs and motor outputs. After the correlation is learned, any object that is seen can trigger the visual maps to activate the motor map for the intended grasping posture of that object, apart from other postures. It is the representation of the particular intended grasping posture for an object that becomes the perceptual recognition for that object.

There are three key aspects to the model: 1) A distributive, topographic architecture is used to combine the orientation sensitive visual activity from an object, from both eyes. This motor-target architecture is organized into columns with a global retinal topography. Each column contains representations from all arm muscles. 2) Local computations, that vary across the model's topography, are piece-wise linear. But convergence of all the local computations yields the global, nonlinear representation of 3-D orientation for any object. 3) Learning is achieved by incrementally modifying the distributions of all input weights to the target map over many performance trials. On any trial, only the weights of the active inputs in the trial are changed.

Computer simulations show that the model converges to good representations of object grasping postures. The model is shown to maintain accuracy and stability across many different parameter choices.

8.10 THE EFFECT OF SKIN DESENSITIZATION ON MOTOR UNIT RECRUITMENT AND FIRING RATES. C.J. De Luca, Y. Noda* and I. Matsuzawa*. NeuroMuscular Research Center, Boston University, Boston, MA 02215 USA.

Previous studies from our laboratory showed that skin desensitization increased the amplitude of the H-reflex and modulated the recruitment threshold of motor units. Hence, a study was conducted to investigate the modulating effect of skin afferents on the firing rate of motor units.

The skin over the hand and wrist was desensitized by application of 10% Xylocain. Myoelectric signals were detected from the First Dorsal Interosseus muscle using the selective quadrifilar needle electrode while the subjects generated a isometric force reaching 50% of their maximal voluntary contraction (MVC). The signals were decomposed into their constituent motor unit action potentials using a proven accurate signal Decomposition technique described elsewhere. The experiment was repeated with fixed needle position before, and 15, 30, 45 and 60 minutes after the application of topical anesthesia. The force threshold of progressively recruited motor units and their average firing rates during a plateau of 50% MVC were determined and compared among subsequent contractions.

The results showed that the force threshold of motor units recruited below 20% MVC increased, while their average firing rates (which are among the largest in value) decreased. On the contrary, the force threshold level of motor units recruited above 25% MVC decreased while their average firing rates (which are among the lowest in value) increased. In other words, the dynamic ranges of recruitment thresholds and firing rates became narrower after skin desensitization. Control studies (without application of anesthesia) revealed no significant change of the recruitment threshold or the average firing rates.

The complimentary behavior of the recruitment threshold and average firing rate indicates that the earlier recruited (< 20% MVC) motor units have a diminished net excitation, whereas the latter recruited (> 25% MVC) have an increased net excitation when the skin afferents are desensitized. This interconnected compensatory relationship between recruitment threshold and average firing rate provides one possible explanation for the relatively low firing rate found in higher threshold motor units, which according to the common drive theory receive a lower net excitation.

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8.11 COMMON DRIVE BEHAVIOR OF FIRST DORSAL INTEROSSEOUS MOTOR UNITS. Sandra Solar,* D. Stashuk,* C. J. De Luca (SPON: S. Roy). NeuroMuscular Research Center, Boston University, Boston, MA 02215

The common 1-2 Hz fluctuations of mean firing rates of active motor units during isometric contractions has been termed "Common Drive". In previous studies, significant cross correlation values were found to exist between firing rates of motor units within muscles such as the Deltoid, Tibialis Anterior, Extensor Pollicis Longus (EPL), Flexor Pollicis Longus (EPL), Extensor Carpi Ulnaris (ECU), Extensor Carpi Radialis Longus (ECRL) and First Dorsal Interosseous (FDI). Significant cross correlation values were obtained for motor unit pairs selected across the EPL and FPL muscles during either antagonistic or synergistic activation. Furthermore, in studies during synergistic contractions of ECU and ECRL muscles, common fluctuations in mean firing rates between motor units of the two muscles were also observed, but with a significant time shift of the peak cross correlation values. This time shift suggested the existence of a mechanical (proprioceptive) linkage between the muscles. Currently, we are conducting an investigation to determine whether the existence of common drive between muscles is related to a common functionality or to a mechanical linkage.

Myoelectric signals were detected from both FDI muscles of healthy subjects during two sets of experiments. One, functionally linked the subjects FDI muscles by attempting to simultaneously follow identical trapezoidal force trajectories. In the second, the subjects pressed their indices against each other producing a net zero force and mechanically and functionally linking the muscles. The myoelectric signals were recorded by quadrifilar needle electrodes and stored on FM tape. The signals were then digitized and the firing times of their constituent motor unit action potentials were obtained via a proven accurate decomposition algorithm. Mean firing rates of active motor units and their cross-correlations were then estimated.

Preliminary results of cross correlation values (.4 to .9) for motor units within each FDI have shown significant common drive. In addition, no common drive (.2 to .4) has been found between the motor units of right and left FDI's when functionally linked but some common drive (.3 to .6) has been found when they are functionally and mechanically linked. For the latter case the times of occurrence of the peak cross correlation values were found to lie between 0-100 ms. While these results are still preliminary, they seem to suggest that common drive has both a supraspinal as well as a peripheral (proprioceptive) component.

This work was supported by Liberty Mutual Insurance Co.

FEEDING AND DRINKING I

9.1 ATTEMPTS TO DEPRESS BRAIN SEROTONIN (5-HT) RELEASE DO NOT ENHANCE FEEDING INDUCED BY INJECTING NOREPINEPHRINE (NE) INTO THE PARAVENTRICULAR NUCLEUS (PVN). E. de Rooy* and D.V. Coscina. (SPON. P. Li). Sect. of Biopsychol., Clarke Institute of Psychiatry, and Depts. of Psychology and Psychiatry, University of Toronto.

Recent work suggests that NE and 5-HT interact on common systems in their control over feeding in the rat. The studies which led to this hypothesis showed that drug treatments which enhance brain 5-HT function block the feeding produced by NE in the PVN to a greater extent than that seen when 5-HT was enhanced alone. If this apparent interaction operates bidirectionally, then injecting NE into the PVN after 5-HT function has been suppressed might produce greater feeding than that seen after either treatment alone. To test that possibility, we measured the 40 minute feeding response of satiated adult male rats with unilateral cannulae aimed at the PVN after each of 4 treatments run in a counterbalanced design: (1) 40 nmoles NE in 0.5 ul injected into the PVN, (2) 250 ug/kg (+)-8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT) s.c., which is thought to inhibit endogenous 5-HT release by stimulating 5-HT-1A autoreceptors, (3) both treatments, or (4) no active treatment (i.e., isotonic saline; n = 19/condition). NE in the PVN alone or 8-OH-DPAT s.c. alone elicited significant feeding compared to only saline. However, the combination of treatments produced no more feeding than that seen after NE alone. In a second study, the same rats were tested for this PVN-NE feeding response over a 4 week period after half had received an intracisternal injection of the 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT; 200 ug in 20 ul 1% ascorbate following i.p. pretreatment with 25 mg/kg desipramine). Compared to vehicle-injected controls, NE produced a tendency toward enhanced feeding 2-3 wks after 5,7-DHT, but this trend was not sustained nor statistically significant. In a third study, satiated normal male rats were tested for their hourly feeding response for 6 consecutive hours after one of 4 treatments: (1) clonidine (30 ug/kg s.c.), (2) 8-OH-DPAT (250 ug/kg s.c.), (3) both treatments, or (4) neither treatment (i.e., isotonic saline; n = 8-9/condition). As previously reported, clonidine elicited reliable feeding compared to saline. However, neither 8-OH-DPAT alone nor in combination with clonidine enhanced feeding over saline. Since clonidine is believed to produce feeding by acting on alpha-2 adrenoceptors in the PVN, these data all imply that it may not be possible to enhance NE-induced feeding by impeding brain endogenous 5-HT release. Alternatively, the broad changes in 5-HT metabolism induced by systemic 8-OH-DPAT or 5,7-DHT may have masked effects of subnormal 5-HT release in the PVN to interact with NE on feeding. Further studies of NE/5-HT manipulations confined to the PVN are needed to address this possibility.

9.2 HYPOTHALAMIC SEROTONIN IN THE CONTROL OF EATING BEHAVIOR. S.F. Leibowitz, G.F. Weiss*, G. Shor-Posner*, U.A. Walsh* and D. Tempel*. The Rockefeller University, New York, N.Y. 10021.

Serotonin (5-HT) has long been known to have an inhibitory effect on food intake. Recent evidence points to central, as well as peripheral, mechanisms in the mediation of this phenomenon. However, essential information is lacking concerning specific brain site(s) involved, as well as specificity and physiological significance of 5-HT's central action. With the use of the brain cannula technique, electrolytic lesions and systemic drug injections, we have obtained evidence in the rat which argues strongly for a potent, behaviorally specific, and physiologically meaningful effect of 5-HT in the hypothalamus.

1) Injection of 5-HT directly into the paraventricular nucleus (PVN) reduces spontaneous, as well as deprivation-induced, feeding. 2) This effect of 5-HT, which is mimicked by PVN injection of compounds which release endogenous 5-HT, is behaviorally specific, as indicated by the finding that 5-HT reduces the size, but not the frequency, of meals taken. 3) Moreover, when animals are permitted to select their food from three simultaneously available macronutrient diets, PVN-injected 5-HT selectively reduces intake of carbohydrate, while sometimes actually enhancing protein intake. 4) This effect of 5-HT on macronutrient intake occurs at doses at least as low as 0.3 nmoles, in contrast to higher doses (> 5.0 nmoles) where the specificity of this effect is lost. 5) While PVN 5-HT reduces spontaneous food intake in both the early and late phases of the dark (active) cycle, it produces a stronger and more selective suppression of carbohydrate ingestion at the onset of the dark. At this time, rats exhibit a natural preference for carbohydrate, and as revealed with the microdialysis technique (B.G. Stanley, this meeting), a peak in PVN content of the 5-HT metabolite 5-HIAA also occurs. 6) Low doses of peripherally injected d-fenfluramine, through release of endogenous 5-HT, produces similar effects to PVN-injected 5-HT. This further supports a role for endogenous 5-HT in control of appetite for specific macronutrients. 7) This serotonergic control appears to be anatomically specific, as indicated by our evidence that electrolytic lesions in the PVN, as opposed to the perifornical lateral hypothalamus, strongly attenuate (by 60-80%) the suppressive effect of d-fenfluramine on feeding.

These results, along with additional findings showing potent effects of PVN electrolytic lesions on carbohydrate and protein ingestion, support the hypothesis that 5-HT innervation to the PVN is critical in coordinating appetite for specific macronutrients, and in mediating the influence of peripherally injected serotonergic drugs on patterns of eating behavior.

9.3 INHIBITION BY 5-HT-2 ANTAGONISTS OF THE ANOREXIC ACTIONS OF PERIPHERALLY ADMINISTERED 5-HYDROXYTRYPTAMINE (5-HT) AND 4-HT IN RATS. K. J. Simansky, B. I. Cohen* and K. Eberle. Dept. of Pharmacol., Med. Coll. of Pennsylvania, Philadelphia, PA 19129

This study examined the structure-activity relationship and the antagonist profile for the anorexic action of peripherally administered serotonin (5-HT) in rats. Male albino rats (350-450 g) were maintained individually with free access to water but with a sweetened milk diet available from 1100-1700 each day. The volume of milk consumed during the first 30 min access to the liquid diet served as the testing period for determining the effects of drugs on feeding. When injected i.p. 6 min before milk was provided, 5-HT (2.5-10 umol/kg) produced a dose-related inhibition of feeding with an ID-50 of 3.8 umol/kg. 4-HT (5, 10, 20, 28 and 40 umol/kg) reduced food intake (by 15-83%) less potently (ID-50=14.8 umol/kg) than 5-HT although the slopes for the anorexic actions of these two hydroxylated tryptamines were not different. By contrast, 7-HT (10-40 umol/kg) had no effect on food intake (e.g., VEH, 19.1 ± 2.4 ml, X ± SE; 40 umol/kg, 18.1 ± 1.6).

The anorexic action of 10 umol/kg 5-HT was antagonized by 60 min pretreatment (i.p.) with 10 umol/kg of xylamidine, methysergide, LY53857 and ketanserin, by 3.4 umol/kg of ritanserin and by 10 umol per kg of pipamperone. For example, meal sizes for the ketanserin experiment were: VEH/VEH, 15.4 ± 2.1 ml; VEH/5-HT, 2.6 ± 0.7 KET/VEH, 14.3 ± 2.3; KET/5-HT, 10.7 ± 1.7. Ketanserin (10 umol/kg) similarly antagonized the anorexic effect of 4-HT (40 umol/kg): VEH/VEH, 18.0 ± 2.7; VEH/4-HT, 1.1 ± 0.6; KET/VEH, 15.9 ± 3.2; KET/4-HT, 9.1 ± 2.6. In a separate experiment, xylamidine (10 umol/kg) reversed the anorexia produced by 4 umol/kg 5-HT and by 20 umol/kg 4-HT: V/V, 18.6 ± 1.4; X/V, 20.3 ± 1.3; V/5-HT, 10.4 ± 1.0; X/5-HT 17.7 ± 1.7; V/4-HT, 9.0 ± 1.3; X/4-HT, 15.3 ± 1.0 ml). The same dose of xylamidine did not, however, reduce the anorexic effect of epinephrine (1 umol/kg): V/E, 5.4 ± 1.4 (-81%); X/E, 4.8 ± 1.3.

Each of the antagonists used inhibit actions of serotonergic drugs at 5-HT-2 receptors. Further analysis revealed that these effects were dose-related and that doses as small as 0.3 umol/kg of xylamidine significantly reversed the action of 5-HT (e.g., VEH/5-HT, 1.3 ± 0.5; XYL/5-HT, 8.9 ± 1.9 ml). By contrast, however, doses as large as 10 umol/kg of the 5-HT-3 antagonist, ICS-205-930, or 20 umol/kg of the 5-HT-1a/1b:Beta receptor blocker, (±) pindolol failed to alter the anorexic action of 5-HT. In concert, these data demonstrate that peripherally acting hydroxylated tryptamines with 5-HT-2 agonist properties (i.e., 4-HT and 5-HT) exert anorexic effects via 5-HT-2-type mechanisms. These data complement results reported elsewhere at this meeting demonstrating that the selective 5-HT-2 agonist dimethoxy-iodophenylaminopropane produces anorexia (see Schechter and Simansky).

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9.4 EVIDENCE FOR SEROTONERGIC MODULATION OF SHAM FEEDING IN THE GASTRIC-FISTULATED RAT. J.C. Neill* and S.J. Cooper. Department of Psychology, University of Birmingham, Birmingham B15 2TT, U.K.

Considerable evidence indicates that serotonergic mechanisms are involved in the control of eating responses. In general, drugs which increase serotonergic activity decrease food consumption (Blundell, J.E., Int. J. Obesity, 1: 15, 1977). Sham feeding in the gastric-fistulated rat provides an important model for investigations of factors involved in the maintenance of ingestion. Sham feeding animals exhibit a pronounced satiety deficit. Our present aim is to investigate serotonergic mechanisms in the control of sham feeding, with particular emphasis placed upon the identification of specific serotonin (5-HT) receptor subtype involvement.

Adult, male hooded rats were used (General strain, bred in our laboratory). Each was prepared with a chronic gastric fistula, and trained to consume a 5% sucrose over a 2h test period, according to previously-described procedures (Kirkham, T.C. and Cooper, S.J., Pharmac. Biochem. Behav., 26: 497, 1987). D-fenfluramine (which increases 5-HT activity), over the dose-range 1.0 - 10 mg/kg, i.p., produced a pronounced dose-dependent reduction in sham feeding. The ED50 (dose which produced 50% reduction in intake) was about 1.75 mg/kg. Interestingly, intact rats which had been trained to drink the sucrose solution, were less sensitive to d-fenfluramine's anorectic effect (ED50 > 3.0 mg/kg). The 5-HT receptor agonist, quipazine, also produced a dose-related reduction in sham feeding, and, again, nonfistulated animals were less sensitive. Thus, 3.0 mg/kg quipazine reduced sham feeding by over 40%, while sucrose consumption in nonfistulated animals was reduced by about 10%. Hence, an anorectic effect of 5-HT agonists appears to be enhanced in animals with a satiety deficit.

The selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) may act at 5-HT cell body autoreceptors when given in small doses, and so decreases serotonergic activity. In support of this hypothesis, we found that 0.1 mg/kg 8-OH-DPAT produced a small increase in sham feeding when injected alone (s.c.). Furthermore, the same dose markedly attenuated the anorectic effect of 3 mg/kg d-fenfluramine in sham feeding rats. These data support the view that 5-HT mechanisms are involved in the control of feeding, and we propose that they may play a part in the maintenance, as distinct from the satiation, of feeding.

(D-fenfluramine was kindly supplied by Institut de Recherches Internationales Servier).

9.5 THE SEROTONIN ANTAGONIST METERGOLINE BLOCKS THE ANORECTIC EFFECT OF CHOLECYSTOKININ. D. Stallone*, S. Nicolaïdis*, J. Gibbs . Laboratoire de Neurobiologie des Régulations, Collège de France, 75231 Paris cedex 05, France.

Serotonin (5HT) and cholecystokinin (CCK) have been separately implicated as factors influencing food intake. Administration of CCK and of drugs such as fenfluramine, which increase the release of 5HT, produce an anorexia characterized by a reduction in meal size and the behavioral responses associated with satiety. Evidence that 5HT and CCK are localized together in APUD cells and have some common physiological effects suggested that the mechanisms of the anorectic action of these two substances may be related. The present study examined the possibility that CCK-induced anorexia depends on serotonergic function.

Testing began after it was demonstrated that metergoline, the 5HT antagonist, significantly attenuated fenfluramine-induced anorexia under our test conditions. The food intake of 4-hour-fasted male rats injected intraperitoneally either with metergoline (1 mg/kg 3 hours before food presentation), with the C-terminal octapeptide of CCK (2, 4 or 8 µg/kg immediately before food presentation), with both metergoline and each dose of CCK-8, or with saline, was measured 30 minutes after refeeding.

CCK-8 alone produced a dose-related suppression of food intake (2 µg/kg, 36.5% decrease ($p \leq 0.05$); 4µ/kg, 63.4% decrease ($p \leq 0.01$); 8µ/kg, 79.7% decrease ($p \leq 0.001$)). When the administration of each dose of CCK-8 was preceded by metergoline injection the anorectic effect of CCK-8 was totally blocked. In addition, injection of metergoline alone produced a 52% increase in food intake ($p \leq 0.01$).

These results indicate that the anorectic action of CCK-8 is dependent upon intact function of serotonergic systems. The facts that metergoline (a) completely blocked the satiety action of every dose of exogenous CCK-8 and (b) increased baseline food intake when administered alone suggest that the increase of baseline food intake by metergoline alone is the result of a metergoline-induced blockade of endogenous CCK. Further studies should clarify the nature of the relationship between 5HT and CCK-8 (whether the serotonergic system is necessary and/or sufficient for the anorectic action of CCK-8) and the site(s) of their interaction.

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9.6 5HT AND CA PRECURSOR LEVELS DURING PROTEIN SELECTION. E.L. Gibson*, D.J. Barber* and D.A. Booth. Department of Psychology, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

Genuinely protein-specific dietary self-selection requires recognition of both bodily cues to protein need and, at the same time, sensory cues to the protein content of a food (Booth, Appetite, 8: in press, 1987). Pharmacological manipulation of serotoninergic transmission that has been claimed to affect dietary selection has not been shown to play a role in protein selection when replicated on learned protein-specific appetite (Booth et al., Soc. Neurosci. 12: 593, 1986). Nevertheless, the protein need cue might be signalled over neurotransmitter specific pathways or even as changes in 5HT or CA activity induced by plasma amino acid patterns. To explore these possibilities, we compared rats in the protein need state which triggers the protein appetite with rats in which the development of the need had been prevented by protein infusion.

On the day of the experiment, adult male Sprague-Dawley rats were deprived of maintenance chow for the last 5 hours of the dark period and gavaged with a 2.5 ml solution at 2 hr, 30 min and 5 min before test: the solution was either non-nutritive (Sham), 10% maltodextrin (Starch) or 10% casein hydrolysate (Casein), 3 rats per condition. We have previously shown that, after learning, rats select protein-predictive dietary cues in this mildly deprived state when Sham and Starch preloaded on this schedule, but not when Casein preloaded (Baker et al., Nutr. Res., in press; Gibson & Booth, Experientia 42: 1003, 1986). Gut and plasma levels of the large neutral amino acids (LNAAs) were greater following Casein than following Starch (Table 1) or Sham (not shown). The ratio of plasma levels of each LNAA to those of the other five increased for the three branched-chain LNAAs (BCA) valine, leucine and isoleucine and decreased for the three monoamine transmitter precursors tyrosine (TYR), phenylalanine (PHE) and tryptophan (TRP) (Table 1). Similarly, brain levels of the precursor LNAAs decreased while those of the branched-chain LNAAs increased. There were no statistically significant differences between Starch and Sham. The results are consistent with other indications (Gietzen et al., Physiol. Behav. 36: 1071, 1986) of a role for noradrenaline more than 5HT in the learned control of protein consumption by protein need cues in the rat.

TABLE 1

Site		Ventral brain[a]		Blood plasma[b]		Ratio to LNAAs		Gut lumen[c]		Casein hydrolysate[b]
Preinfusion		Sta	Cas	Sta	Cas	Sta	Cas	Sta	Cas	
Mean levels*	TRP	6	3	59	97	.27	.18	18	157	290
	PHE	43	21	23	32	.09	.05	52	388	800
	TYR	63	32	30	48	.12	.08	--	--	290
	BCA	130	159	164	459	.26	.33	238	2511	4490

*: [a]pmoles/mg [b]nmoles/ml [c]nmoles/g wet weight

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9.7 DIURNAL RHYTHM OF MEDIAL HYPOTHALAMIC SEROTONIN METABOLISM IN RELATION TO EATING BEHAVIOR. B.G. Stanley, D.H. Schwartz, L. Hernandez, S.F. Leibowitz and B.G. Hoebel. Dept. of Psychology, Princeton Univ., Princeton, NJ and The Rockefeller Univ., New York, NY.

Evidence suggests that a reciprocal relationship may exist between brain serotonin (5-HT) activity and feeding behavior, with increased medial hypothalamic 5-HT activity suppressing food intake and food ingestion increasing brain 5-HT synthesis. The present experiment addressed these issues by examining diurnal rhythms of 5-HT activity, with repeated measures of changes in extracellular levels of the 5-HT metabolite 5-hydroxyindole acetic acid (5-HIAA), in the medial hypothalamus of freely-feeding and food deprived animals.

The subjects were 11 adult male Sprague-Dawley rats, housed in dialysis test chambers on a 12/12 hr light dark cycle. Microdialysis probes (Hernandez et al. Life Sci., 39, 1986, 2629) were inserted through an indwelling guide cannula into the region of the paraventricular hypothalamus, and samples of dialysate (20 µl/20 min) were collected every 2 hrs for 3 days. 5-HT and 5-HIAA in the samples were measured by HPLC-EC. For the first 2 days the subjects were allowed unrestricted access to food. Results indicate that the quantity of 5-HT in the samples was below the threshold for detection (less than 3 pg). In contrast, 5-HIAA levels averaged 156±41 pg across subjects, and there was a clear diurnal rhythm of this 5-HT metabolite. Specifically, there was a large and reliable increase in the levels of 5-HIAA at the beginning of the nocturnal feeding period (1 hr after lights out), compared to stable levels observed at all other times during the 2-day test period. The mean levels of 5-HIAA increased 45% on day 1 and 75% on day 2 relative to values obtained 1 hr before the beginning of the dark period. This pattern is virtually identical to that we recently reported for medial hypothalamic norepinephrine release (EPA, 58, 1987, 9).

On day 3, we food deprived the subjects for 24 hr and continued to measure 5-HIAA as before. Food deprivation completely abolished the peak of 5-HIAA observed at the beginning of the nocturnal period, without affecting the levels of this metabolite at other times. Subsequent to deprivation, during the light phase, the subjects were fed and 5-HIAA release was measured for 4 hr. Eating behavior following 24 hr of food deprivation had no effect on 5-HIAA release in the medial hypothalamus.

These findings suggest that medial hypothalamic 5-HT activity is specifically altered during the early portion of the nocturnal feeding period and demonstrate that this change is dependent upon the presence of food.

9.8 INGESTION OF SUCROSE INCREASES HYPOTHALAMIC DOPAMINERGIC ACTIVITY IN THE 7 DAY OLD RAT. G.P. Smith, M. Albert*, R.D. Shindledecker*, C. Jerome* and S.H. Ackerman. Dept.of Psychiatry, Eating Disorders Institute and Bourne Lab., NY Hospital-Cornell Medical Center, White Plains, NY 10605.

In the adult rat, sham feeding of 10% and 40% sucrose for 9 minutes increased hypothalamic dopaminergic metabolism (DOPAC/DA), but did not change DOPAC/DA in n. accumbens, olfactory tubercle, frontal cortex, amygdala-pyriform cortex or striatum (Smith et al, Pharmacol. Biochem. Behav. 26:585, 1987). It was not clear in these experiments (1) whether the increase in DOPAC/DA was correlated with the sensory or reinforcing effects of sucrose, and (2) whether the increase in hypothalamic DOPAC/DA depended on prior ingestive experience with sucrose. To attempt to answer these two questions we investigated the neurochemical effect of ingesting sucrose for the first time in 7-8 day old rat pups.

Anterior sublingual cannulas (PE10) were implanted under light ether anesthesia. After 4 hours of food and fluid deprivation, 34 rats were tested individually in a warm, humidified chamber. Rats were infused with water or 10% sucrose for 20 min at a rate of 0.072 ml/min. Volume ingested was inferred from body weight gain. At the end of the intake test, each rat was decapitated and the brain was regionally dissected under microscopic control to obtain hypothalamus, olfactory tubercle, amygdala-piriform cortex and striatum for measurement of monoamines and metabolites by HPLC and electrochemical detection.

Although rats drank equal volumes (ml) of water (0.70 ± .07) and 10% sucrose (0.73 ± .04), hypothalamic DOPAC/DA was larger after sucrose ingestion (0.45 ± .04) than after water ingestion (0.33 ± .03, $p < .05$). Sucrose ingestion did not increase DOPAC/DA in any other brain region and sucrose ingestion had no effect on 5-HT, 5-HIAA or NE in any brain region.

These results demonstrate that the effect of sucrose ingestion on hypothalamic DOPAC/DA is independent of prior experience. The results also demonstrate that the increase of hypothalamic DOPAC/DA is not correlated with ingestive movements because rats ingested equal volumes of sucrose and water. Finally, these data suggest, but do not prove, that the increased hypothalamic dopaminergic activity is associated with the sensory effect of sucrose rather than with its reinforcing effect.

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9.9 ALTERATIONS IN NEUROTRANSMITTER METABOLISM BY TOTAL PARENTERAL NUTRITION OF TUMOR-BEARING RATS. W.T. Chance, L. Cao,* J. Nelson, T. Foley-Nelson and J.E. Fischer.* Dept. of Surgery, University of Cincinnati, Cincinnati, OH 45267-0558.

We have observed increases in regional brain concentrations of dopamine (DA) and serotonin (5-HT) metabolites in anorectic tumor-bearing (TB) rats. If these alterations are causes of anorexia, correction of the nutritional imbalance with total parenteral nutrition (TPN) should not affect brain levels of these neurotransmitters. Therefore we assessed neurotransmitter metabolism by HPLC in the nucleus accumbens, hypothalamus, corpus striatum and amygdala of TB (n=6) and control (n=6) rats maintained on rat chow as well as in TB rats (n=4) maintained on TPN (isocaloric and isonitrogenous with amount of chow eaten by control rats). The jugular vein was cannulated 14 days after the induction of methylcholanthrene sarcomas in F-344 rats and TPN was initiated 3 days later. After 14 days of TPN (day 32 post tumor induction), all rats were sacrificed by decapitation, the brains were removed, dissected and frozen in liquid nitrogen prior to biochemical assay. Alterations in precursors (TYR & TRP) and metabolites of DA (DOPAC, 3-MT & HVA) and 5-HT (5-HIAA) for the nucleus accumbens, a representative region, are presented below.

Group	TYR (µg/g)	3-MT (ng/g)	DOPAC (ng/g)	HVA (ng/g)	TRP (µg/g)	5-HIAA (ng/g)
Control	15.9±0.7	151±6	1004±31	390±11	4.1±0.2	586±22
TB-Chow	34.1±3.3*	120±16	1176±58**	420±41	5.9±0.5*	688±35**
TB-TPN	13.4±3.5†	53±21*¶	802±64*†	277±30*¶	7.4±0.3*¶	804±42*¶

*p<0.01, **p<0.05 vs control; †p<0.01, ¶p<0.05 vs TB-Chow.

Thus, TB rats exhibited elevations in the levels of TYR and DOPAC as well as in TRP and 5-HIAA, suggesting increased metabolism of these neurotransmitters. Administration of TPN normalized the catecholamine alterations, while exacerbating the indoleamine changes. These results suggest that both DA and 5-HT metabolism follow precursor availability. The administration of TPN reduced brain TYR, while increasing TRP availability, probably through alterations in transport of these amino acids into the brain. Therefore, increases in DA metabolism may not be of primary importance in the development of anorexia.

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9.10 EFFECTS OF PVN CORTICOSTERONE AND NOREPINEPHRINE ADMINISTRATION ON NATURAL FEEDING PATTERNS. D. Tempel*, L. Tomkow* and S.F. Leibowitz (Spon: M. Grumet) Rockefeller Univ. N.Y., N.Y. 10021.

The glucocorticoid corticosterone (CORT) is known to be important in the control of food intake. Evidence indicates that CORT is also necessary for normal α_2-noradrenergic receptor function within the paraventricular nucleus (PVN) and for norepinephrine (NE)-induced feeding to occur. Further, both CORT and NE exhibit circadian rhythms, simultaneously peaking at the start of the nocturnal cycle when natural feeding begins, suggesting that they may interact at this time to influence food intake. In support of this hypothesis, we have observed that rats given pure macronutrient diets exhibit an increased preference for carbohydrate at dark onset. Further, PVN injection of NE and peripheral administration of CORT preferentially stimulate carbohydrate intake.

In light of recent evidence for a dense concentration of glucocorticoid receptors in the PVN, we examined the possibility that CORT regulates feeding, as well as PVN α_2-noradrenergic activity, by direct action upon PVN receptor sites. In these experiments, we studied the effects of direct PVN administration of CORT and NE, in crystalline form, on natural feeding patterns in animals given pure macronutrient diets. These manipulations were made just prior to dark onset, and food intake was recorded for the first hour of the nocturnal cycle.

The reduction of CORT levels after bilateral adrenalectomy (ADX) reduced daily food intake by 28% relative to SHAM-operated rats. During the first hour of the night, ADX reduced feeding by more than 50% ($p<0.05$), due mainly to a strong and selective decline (-75% $p<0.01$) in carbohydrate intake. Fat or protein ingestion was not significantly affected. PVN administration of crystalline CORT (approximately 200µg) in ADX rats produced a significant increase in food intake by specifically enhancing carbohydrate consumption (from 0.9 to 5.7 Kcal, $p<0.001$). Fat and protein intake was unaltered. Thus, PVN CORT completely restored the rats' normal feeding pattern, suggesting that CORT is required for the expression of this pattern at this time.

PVN administration of crystalline NE, in SHAM rats, increased food intake by selectively enhancing ($p<0.05$) carbohydrate ingestion. Consistent with other studies, ADX rats were unresponsive to PVN NE. However, the administration of NE and CORT in combination, in ADX rats, increased food intake by a preferential rise in carbohydrate consumption. This response was similar to that produced in SHAM rats by PVN NE alone, and was also comparable to that seen in ADX rats after the administration of CORT alone. This lack of response additivity suggests that CORT and NE may act through a common mechanism, within the PVN to regulate normal feeding patterns.

9.11 RESPONSES OF DIFFERENT TYPES OF PARAVENTRICULAR NEURONS IN VITRO TO NOREPINEPHRINE (NE) AND OTHER FEEDING-RELEVANT TRANSMITTERS. L.-M. Kow and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

In rats, NE infused into the hypothalamic paraventricular area (PVA) can act through a2-adrenergic receptors to induce feeding behavior (Goldman et al., '85, Eur. J. Pharmacol., 115:11). To investigate the NE action at the neuronal level and to see what type(s) of neurons are responsive, hypothalamic slices were used to record single-unit activity from the PVA.

Of 237 units studied, 28% were silent, 25% fired intermittently or phasically, and the remaining 47% fired continuously. When subjected to bath application of NE (12.5uM) 62% of the 212 units responded with excitation (n=61 units), inhibition (n=53), or biphasic inhibition-excitation (n=18). The excitatory actions of NE were mimicked in 22 out of 23 units by phenylephrine (PhE, an a1 agonist), but not (0/12 units) by clonidine (Clon, an a2 agonist), and were blocked or attenuated by prazosin (an a1 blocker) in 8/8 units. In contrast, the inhibitory actions of NE were mimicked by Clon (18/18 units), but rarely by PhE (1/27 units), and were blocked or attenuated by yohimbin (an a2 blocker) in 13/13 units. B-adrenergic agonist and blocker, isoproterenol and timolol, respectively, had very little effect or inconsistent effects on NE responses. The majority of inhibitory responses (40/53) were observed from continuously-firing units; whereas the majority of excitatory responses (43/61) were shown by other types of units; the correlation is statistically significant (Chi-square test, $p<0.001$, 2-tailed).

In addition to NE, some neurons were further tested with histamine (Hist, 12.5-25uM) and serotonin (5HT, 25uM), which inhibit feeding, and GABA (50uM), which induces feeding, to see whether they act preferentially on NE-inhibited neurons. Hist excited 70% of the 143; and 5HT excited 43% and inhibited 16% of the 74 units tested. None of these responses correlated with any type of NE responses. GABA inhibited 28% and caused excitation, perhaps indirectly, in 9% of the 54 units tested. The inhibitory responses to GABA occurred significantly (Chi-square test, $p<0.02$, 2-tailed) more often in NE-inhibited than in other types of neurons.

Thus, extending from behavioral studies, our results suggest that in the PVA: 1) NE induces feeding by inhibiting continuously-firing neurons; 2) NE-inhibited neurons could mediate feeding-inducing effect of GABA; and 3) the feeding-inhibiting effects of Hist and 5HT and the feeding-inducing effects of NE are mediated through different neuronal populations.

9.12 ESTROGEN-CATECHOLAMINE INTERACTIONS: INDEPENDENT AND DEPENDENT EFFECTS AT ESTROGEN-SENSITIVE BRAIN SITES IN RATS. C. WAYNE SIMPSON *(Spon: C. Nyquist-Battie) Div. of Structural and Systems Biology, Univ. of Missouri-Kansas City, K.C., Mo. 64108.

Alteration of peripheral estrogen levels in rats is well known to influence dopamine (DA) and norepinephrine (NE) levels at anterior hypothalamic locations. Stumpf and his colleagues and others have demonstrated high densities of estrogen sensitive neurons at lateral preoptic (LPO), stria medullaris (SM) and ventromedial hypothalamic (VMH) sites. Catecholamine injections at these same brain sites have been shown to increase short term food intakes. Simpson has shown that high peripheral doses of estradiol benzoate (EB), inhibit the food intake increases seen following DA injections, at a SM site, but not following NE injections at the same site. In the present report additional hypotheses concerning estrogen catecholamine interactions that influence feeding and body weight were investigated. To study the effects of initial body weight on these effects, 3 different groups of rats at significantly different levels of body weight, were injected centrally, with DA and NE at an SM site, with and without concurrent low peripheral doses of EB.

Without estrogen injections, NE and DA elicited significantly increased feeding in 1-2 hours following central injection but there was no increase in 24 hr. body weights in all three weight groups. Peripheral injections of EB did not reduce the short term increases in food intake following NE or DA central injections but did produce significant reductions in body weights in all groups. In a different set of experiments all injections of control material, cholesterol estrogen and NE or DA were delivered at central estrogen-catecholamine sensitive sites specifically in the LPO, SM and VMH. Compared to cholesterol control injections estrogen at doses >5ug elicited significant reductions in body weights and 24 hour food intakes at all three brain sites. In a final series of experiments, EB at estrogen sensitive sites was coupled with NE and DA injections at a SM feeding. NE injections in the SM site elicited 1-2 hour increases in food intakes when EB was placed at three EB sensitive brain sites. DA elicited short term feeding only when EB was placed in the LPO site and DA was injected in the SM site. EB placed in the VMH or SM sites inhibited DA increases in short-term feeding. One set of experiments demonstrated independent effects of EB on body weights and DA or NE on short-term food intakes. In additional experiments however, when EB and the catecholamines are placed at the same site, ie SM dependent interactions are observed in short-term feeding. The combination of results from all experiments suggested complex interactions between estrogen and catecholamines at different brain sites in the control of food intake and body weight.

NEUROENDOCRINE CONTROLS: PITUITARY I

10.1 EVIDENCE FOR TESTICULAR FEEDBACK AT THE LEVEL OF THE ANTERIOR PITUITARY IN HYPOPHYSECTOMIZED, PITUITARY-GRAFTED RATS RECEIVING PULSATILE LHRH. J.E. Levine, C. Gilmore,* R. Simov,* and F.J. Strobl.* Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

The purpose of this study was to examine the extent to which gonadal negative feedback actions are exerted directly at the level of the anterior pituitary (AP) gland. To address this question we developed an in vivo isolated pituitary paradigm to determine whether castration leads to increased gonadotropin secretion under "hypothalamic-clamp" conditions, i.e. during unvarying intermittent LHRH infusions. Since it was found previously that LHRH pulse frequency is increased in short-term castrate rats, we also tested the hypothesis that post-castration increases in LH result from increased LHRH pulse frequency. Hypophysectomized male rats received single AP transplants under the kidney capsule. Animals were fitted with a novel concentric atrial catheter system which allows for intermittent infusions of LHRH (250 ng/5 min/h) and chronic blood sampling. Rats received LHRH infusions for 4-5d before and throughout experimental sessions. On the day of experiments, blood samples were obtained 2h prior to treatment and at every 2h interval for 24h following treatment. Treatments at time 0 were as follows: group 1, sham-surgery; group 2, castration; group 3, LHRH pulse frequency increased to 1 pulse/30 min. Plasma LH levels in all groups were determined by RIA using the NIADDK RIA kit (LH RP-2 standard). In each animal the sella turcica was inspected post-mortem for completeness of hypophysectomy. Viability of pituitary transplants was verified histologically. In sham-operated animals LH levels were 0.3 - 0.7 ng/ml and remained unchanged throughout the 24h post-surgery period. By contrast, LH levels in the castrate animals increased steadily during the 24h following surgery to a level 3- to 5-fold higher than initial values (0.58 $\pm$ .26 ng/ml, pre-castration; 2.00 $\pm$ .48 ng/ml, post-castration). The post-castration increase in LH secretion in these experiments closely resembled that seen previously in pituitary-intact animals in time-course and magnitude. In group 3, a change in LHRH pulse frequency resulted only in transient increases in LH secretion within 4-8h following the frequency shift. Our data demonstrate that most, if not all, of the short-term effects of castration on LH secretion can be accounted for by the withdrawal of gonadal negative feedback at the level of the pituitary gland. Although regulation of the LHRH pulse generator may also operate as a component of an integrated gonadal negative feedback system in male rats, our results support the hypothesis that direct pituitary effects of testosterone are of much greater importance in the acute feedback regulation of LH secretion. Supported by NIH grant HD20677.

10.2 EFFECTS OF PULSATILE GNRH ADMINISTRATION ON α-SUBUNIT AND LHβ GENE EXPRESSION AND ON PULSATILE LH SECRETION DURING LACTATION. Lee, L.-R.*, D. Haisenleder*, J.C. Marshall and M.S. Smith, Dept. of Physiology, Univ. of Pittsburgh, PA 15261 and Dept. of Internal Medicine, Univ. of Michigan, Ann Arbor, MI 48109.

In the rat, suckling of 8 pups results in a suppression of pituitary GnRH receptors (GnRH-R), α-subunit and LHβ gene expression, and pulsatile LH secretion. Twenty-four hr after pup removal from intact rats, GnRH-R have increased 3-4 times, but there are no changes in α-subunit or LHβ mRNA concentrations nor evidence of pulsatile LH secretion. These studies were designed to determine whether administration of pulsatile GnRH could stimulate increases in these parameters. Intact rats suckling 8 pups were implanted with two right atrial catheters on day 8 postpartum and pups were removed on day 9. Blood samples were collected at 10 min intervals for 4 hr at the time of pup removal and 24 hr after pup removal. Pulsatile GnRH (2 ng/pulse every 50 min) was begun at 16 hr after pup removal (GnRH-8 hr) or at the time of pup removal (GnRH-24 hr). After collection of blood samples, pituitaries were removed and GnRH-R was determined or α-subunit and LHβ mRNAs were measured by an RNA dot blot hybridization assay.

There were few detectable LH pulses in saline-infused animals 24 hr after pup removal. The initial GnRH pulses administered at the time of pup removal were generally ineffective in stimulating pulsatile LH secretion. However, by 24 hr after pup removal, significant LH pulses were observed in response to each pulse of GnRH. LH peak heights (10-30 ng/ml) were similar to basal LH pulses observed during diestrus of the estrus cycle. GnRH-R (fmol/pit) and α-subunit and LHβ mRNAs (pg cDNA bound/100 µg pit DNA) were as follows:

GROUP	GnRH-R	α-SUBUNIT	LHB
INTACT + 8	44 ± 8	49 ± 2	5 ± 0.4
INTACT - 8	144 ±12	43 ± 2	5 ± 0.5
INTACT - 8, GNRH-8 hr	--------	44 ± 4	5 ± 0.8
INTACT - 8, GNRH-24 hr	139 ± 5	51 ± 3	5 ± 0.6

These studies demonstrate that restoration of GnRH-R alone is not sufficient to activate LH synthesis. Administration of small pulses of GnRH results in pulsatile LH secretion after upregulation of GnRH-R has occurred. However, induction of pulsatile LH is not accompanied by increased expression of α-subunit and LHβ mRNAs. In contrast, the pituitary of ovx rats has increased content of LH subunit mRNAs at 24 hr after pup removal and releases large amplitude LH pulses in response to the 2 ng dose of GnRH. Thus, in intact rats, the elevated levels of progesterone may inhibit α-subunit and LHβ gene expression (supported by grant HD14643).

10.3 THE EFFECT OF SURGICAL DISCONNECTION OF THE MEDIOBASAL HYPOTHALAMUS (MBH) ON LHRH RELEASE IN VITRO. C.P. Phelps and P.S. Kalra. Dept. of Anatomy, Univ. South Florida, Tampa, Fl and Dept. of Obstetrics and Gynecology, Univ. of Florida, Gainesville, FL.

The reproductive consequences of severing MBH pathways on pituitary luteinizing hormone (LH) release are anovulation and acyclcity in the female rat, whereas changes in basal gonadotropin release in the male are variable. We and others have observed growth of immunoreactive LHRH-containing neurons into scar tissue 1-2 months after anterior hypothalamic lesions. We have now compared basal and secretogogue-evoked LHRH release in vitro from the preoptic area (POA)-MBH of adult male rats at short (10 days) and long (60 days) intervals after frontal cuts (FC). A Halasz-type knife (radius 1.5mm) was used to sever medial LHRH pathways immediately behind the optic chiasm. FC was produced by fifteen 180° rotations of the knife. Additional rats received either sham operations or no surgery. At 10 or 60d after surgery rats were decapitated and the POA-MBH was rapidly removed. Individual POA-MBH fragments were halved longitudinally and perfused continuously for 6 hr at 37°C with a balanced salt solution (pH 7.4) containing 5 mM glucose, 0.1% BSA and 10^{-5} M Bacitracin. After a 60 min preincubation period, fractions (200 µl/10 min) were collected for LHRH determination by RIA. After 100 min of perfusion to estimate basal LHRH secretion rates, 60 mM KCL was added to the medium for 10 min. In a second experiment, POA-MBH of rats bearing FC for 60d and of control and sham operated rats were perfused similarly except that the opiate antagonist naloxone hydrochloride (1 mg/ml) was added to the medium for 30 min during the third hr and 60 mM KCL was added for 10 min during the fifth hour of perfusion. At the end of the incubation each POA-MBH was weighed and assayed for total LHRH content. Although there was considerable variation in individual rats at 10d after FC, the average LHRH release rate (2.9±1.1 pg/10 min) and concentration (40.5±14.4 pg/mg) were reduced when compared to shams (5.4±1.4 pg/10 and 78.8±5.8 pg/mg, respectively). At 60d after FC, the POA-MBH LHRH concentration (29.1±2.6 pg/mg) and basal LHRH release rate (2.6±0.2 pg/10 min) were also significantly reduced as compared to sham operated rats (59.3±7.2 pg/mg, 3.7±0.5 pg/10 min). However, there was no significant difference in LHRH concentration and release rates in short vs. long term FC rats. Although naloxone and KCL stimulated at least a two-fold increase in LHRH output, the response of POA-MBH from 60d and 10d FC rats was significantly lower than that from their respective sham operated rats. Higher release rates were associated with higher individual POA-MBH LHRH concentrations. In summary, severing the anterior neural connections of the MBH not only reduces LHRH content, but also basal and stimulated secretion rates and there was no evidence of functional recovery. Supported by The Whitehall Fnd. Inc. and NIH HD11362.

10.4 NOVEL DECONVOLUTION MECHANICS UNMASK THE NATURE OF SPONTANEOUS LH SECRETORY BURSTS IN VIVO. J.D. Veldhuis and M.L. Johnson. Endocrinology & Biophysics, Depts. of Medicine & Pharmacol., Univ. of Virginia Medical School, Charlottesville, VA 22908.

The exact nature of glandular secretory events is difficult to probe in vivo, since underlying patterns of hormone release are confounded by metabolic clearance (MC). Moreover, MC rates vary among different individuals. Accordingly, to discern the nature of endogenous LH secretory events, we propose a deconvolution model in which circulating hormone concentrations are determined simultaneously by 4 distinct parameters: (i) the location(s); (ii) amplitude(s); and (iii) half-duration(s) of all discrete secretory bursts, which are acted upon by (iv) exponential clearance kinetics. Secretory bursts are modeled as Gaussian distributions of instantaneous molecular secretion rates. The resultant convolution integral that describes the spontaneous fluctuations in plasma hormone concentrations over time can be solved by numerical integration. Moreover, non-linear, least-squares parameter estimation provides mean and bounded probability estimates of endogenous clearance kinetics and underlying secretory impulse amplitudes, durations, and locations. The physiological implications of this model were examined by analyzing spontaneous LH (RIA) pulsatility in 8 men sampled every 5 min for 8 hr. Deconvolution disclosed endogenous LH half-lives (monoexponential) of 87 ± 8.5 min (± SEM), which agreed well with independently determined half-lives of 44-106 min (67% confidence limits) for exogenous LH disappearance in 4 LH-deficient men given 35 mcg human LH by IV bolus injection. Further analysis revealed that underlying LH secretory impulses exhibited half-durations of only 7.8 ± 0.52 min; occurred at a frequency of 6.9 ± 0.55 pulses/8 hr (interpulse intervals of 75 ± 6.3 min); and achieved amplitudes of 0.41 ± 0.05 mIU/min/ml. By deconvolution, the calculated production rate of endogenous LH was 180 ± 14 mIU/min, which accords with that estimated by steady-state LH infusions (228 ± 28 mIU/min). In summary, the present analytical deconvolution model has disclosed discrete Gaussian bursts of LH release that are temporally delimited (half-duration 7.8 min) and occur approximately every 75 min. Moreover, this model correctly estimates endogenous LH production and metabolic clearance rates from measurements of plasma LH concentrations alone. We conclude that rates of endogenous LH production, half-times of native LH clearance, and the nature of spontaneous LH secretory bursts can be unmasked in vivo by this novel deconvolution model without injecting radiolabeled or "cold" hormone.

10.5 **LH SURGE AFTER ESTRADIOL AND PULSATILE GNRH IN HYPOPHYSIAL STALK-TRANSECTED (HST) FEMALE PIGS.** **J. S. Kesner*, M. J. Estienne*, R. R. Kraeling* and G. B. Rampacek*. (SPON: T. G. Reigle). R. B. Russell Agricultural Research Center, USDA-ARS, Athens, GA 30613 and University of Georgia, Athens, GA 30602.**

The objective was to determine sites of action whereby estradiol induces the preovulatory LH surge in pigs. Six domestic pigs (weight = 118 ± 12 kg; mean ± SE) were subjected to HST and ovariectomy (OVX). Pigs received either 1 µg GnRH pulses iv every 45 min from day -5 to 4 and an injection of estradiol benzoate (10 µg EB/kg BW) im on day 0 (TRT 1) or GnRH pulses only on days -5 to 0 and 2 to 4 and EB (TRT 2) or oil vehicle (TRT 3) on day 0. Pigs were reassigned to treatments according to an incomplete latin square design, so that each treatment was administered to four pigs, and so that no individual pig received the same treatment twice. Subsequently, during a third treatment period, all HST gilts received GnRH pulses only from days -5 to 0 and EB on day 0 (TRT 4). Four contemporary OVX pigs, one of which underwent sham-HST, served as positive controls and received saline pulses and EB during each of the three aforementioned treatment periods, which were conducted at 4 week intervals. In stalk-intact pigs, EB inhibited LH release for 48 h, then induced an LH surge that peaked 60 to 84 h after EB. Serum LH in HST pigs increased from undetectable concentrations (<.17 ng/ml) to a plateau at .42 ± .03 ng/ml after 5 days of GnRH stimulation. In TRT 1, LH response to GnRH was inhibited for 12 h after injecting EB, but then serum LH concentrations gradually rose for 36 h to .69 ± .09 ng/ml; surpassing those noted before EB (P<0.05). Interrupting the GnRH pulses immediately after EB (TRT 2), so as to mimic the secretory hiatus of GnRH/LH observed in intact pigs prior to the LH surge, rendered serum LH concentrations undetectable. Resuming GnRH pulses 48 h later stimulated LH concentrations to increase progressively over 4 h to .71 ± .09 ng/ml. When corrected for the compromised capacity of HST pigs to secrete LH, this response was comparable to the preovulatory surge in intact pigs. Without EB treatment, resumption of GnRH stimulation after a 48 h interruption (TRT 3) elicited a resurgence of LH release that was quantitatively similar to that of TRT 2, but peaked much more rapidly; within 1 h. Discontinuing GnRH pulses at the time of EB injection (TRT 4) caused LH concentrations to drop and remain below .17 ng/ml for 96 h. These data indicate that estradiol induces the preovulatory LH surge in pigs by first inhibiting, then restoring GnRH release, while acting at the pituitary to moderate the rate at which the resumed GnRH stimulation accelerates LH release.

10.6 ULTRASTRUCTURAL ANAYLIS OF LHRH NEURONS IN FEMALE RHESUS MACAQUE. J.W. Witkin, M. Ferin* and A.J. Silverman. Depts. Anat. & Cell Biol., Obstet. & Gynecol., Columbia Univ. Coll. P & S, New York, NY 10032.

Adult long-term ovariectomized rhesus macaques were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde through the left cardiac ventricle. A block of tissue encompassing the preoptic area and hypothalamus was postfixed for 2 h and cut at 60 um. Ultrastructural demonstration of LHRH was performed as previously described (Witkin and Silverman, Peptides 6:263,'85). In some cases, the DAB reaction product was silver-intensified to maximize visualization of intracellular sites. Comparisons were made of LHRH neurons in 4 areas: (1) medial preoptic (MPOA); (2) dorsal to the supraoptic nucleus (dSON); (3) the arcuate region (arc); and (4) medial basal hypothalamus (MBH). The distribution of reaction product within individual neurons was not uniform. In some neurons the reaction product was scattered throughout the cytoplasm, but always exclusive of the Golgi apparatus. In others, reaction product was only associated with secretory vesicles. These variations occurred in all regions, suggesting that instead of acting in concert, LHRH neurons are concurrently in various stages of synthesizing, processing and packaging the neuropeptide. Reaction product was never associated with the nucleolus, but in some neurons the nucleus was immunoreactive. This consistency leads us to believe that the nuclear staining, though inexplicable, is not an artifact. In addition to subcellular distribution of reaction product, the synaptic input and amount of glial ensheathment of LHRH soma and dendrites was measured and compared to non-identified neurons. Few synapses onto LHRH neurons were found (none in the MPOA or arc, an average of 0.4 per cell in 5 neurons in the dSON and 0.6 in 5 neurons in the MBH). Hypertrophied glial processes ensheathed an average of 54% of the membrane of LHRH cells in MPOA. Considerably less ensheathment was found in dSON, arc and MBH (an average of 17% of the membrane). There were no synapses onto LHRH dendrites in the MPOA, a few in dSON and arc and most in the MBH. About 1/3 of LHRH dendritic membrane in all regions abutted onto glial processes. Nonidentified neurons and their processes had more synapses than LHRH neurons in all regions and much less glial ensheathment. A most striking finding were a few axodendritic and axosomatic LHRH-LHRH synaptic interactions in the MBH. USPHS NIH AG05366 and HD10665.

10.7 CELLULAR LEVELS OF MESSENGER RNA ENCODING GONADOTROPIN-RELEASING HORMONE (GnRH) ARE ELEVATED AFTER THE LH SURGE ON THE DAY OF PROESTRUS. R. T. Zoeller and W. S. Young, III. Lab. of Cell Biol, NIMH, Bethesda, MD 20892.

Gonadotropin-releasing hormone (GnRH) is synthesized by specific neuronal cells distributed throughout the septum and preoptic area of the rat brain. We have shown previously that levels of mRNA encoding GnRH in cells within the OVLT are influenced by estrogen in a manner suggesting that GnRH synthesis is coupled to GnRH release. Specifically, GnRH mRNA levels are supressed by estrogen under conditions of negative feedback (Zoeller, Seeburg and Young, Endocrine Abstract #32, 1986). If GnRH mRNA levels are coupled to GnRH release, then GnRH mRNA levels should rise after a spontaneous surge release of GnRH to replenish GnRH stores. To test this prediction, we measured GnRH message levels in 4-day cycling rats throughout the day of proestrus. Adult female Sprague-Dawley rats were maintained under temperature and light-controlled conditions; only females exhibiting two consecutive 4-day cycles, as defined by daily vaginal smears, were included in this experiment. Five females each were ether anesthetized and perfused intracardially with 4% formaldehyde at 1000h, 1300h, 1600h, and 1900h on the day of proestrus. Frozen sections (12 µm) were prepared and hybridized according to Young et al. (Neurosci. Lett. 70:198, 1986). Sections were hybridized with an ^{35}S-labelled 48-base oligonucleotide probe complementary to the mRNA region encoding GnRH. Sections and ^{35}S-impregnated brain-paste standards were apposed to emulsion-coated coverslips for 2 weeks; mRNA levels were derived by measurement of light reflectance off silver grains using an image analysis system and normalize as percent 1000h values. GnRH mRNA levels on the day of proestrus were: 100±9.2%, 84±8.6%(ns), 76±7.3%(ns), and 123±13%($p<0.01$) for 1000h, 1300h, 1600h, and 1900h, respectively. Since these animals exhibit an LH surge at approximately 1400-1600h, these results demonstrate that GnRH mRNA levels become elevated after a spontanous surge release of GnRH and suggest that the same signal stimulating GnRH release may stimulate GnRH synthesis in order to replenish GnRH stores.

10.8 CONTROL OF PULSATILE LH RELEASE IN THE OVARIECTOMIZED GUINEA PIG: ROLE OF OPIATERGIC, SEROTONERGIC AND ADRENERGIC SYSTEMS. A.C. Gore*, J.A. Keller-Halbe* and E. Terasawa (SPON: J. Kemnitz). Neurosciences Training Prog. and Wisconsin Reg. Primate Res. Ctr., Univ. of Wis., Madison, WI 53706.

Despite numerous studies in the rat, monkey and human, little information is available on the control mechanism of LHRH release in the guinea pig. In the present experiment involvement of opiatergic, serotonergic and adrenergic neuronal systems in control of pulsatile LH release, presumably by their interaction with the LHRH neurosecretory system, was examined using various agonists and antagonists. Long-term ovariectomized (OVX) female guinea pigs received an indwelling catheter and blood samples were obtained through the catheter at 5 min intervals for a 6 h period. Drugs were injected i.v. after 1.5 h of control samplings. LH release in plasma determined by RIA in OVX guinea pigs was pulsatile, with mean LH levels, interpulse interval and LH pulse amplitude at 951±76 ng/ml, 29.2±1.5 min and 781±127 ng/ml, respectively. In the 1st experiment effects of an opiate agonist (morphine sulfate, 10 mg/kg or fentanyl, 50 µg/kg), opiate antagonist (naloxone, 2 mg/kg) serotonin agonist (quipazine, 15 mg/kg) or serotonin antagonist (methysergide, 15 mg/kg) were tested. Both fentanyl and morphine sulfate suppressed LH release completely, while naloxone significantly enhanced mean LH release 2-3 fold. Although methysergide suppressed LH release, quipazine did not result in any change in LH release. In the 2nd experiment a drug-induced LH suppression was reversed by injection of its corresponding agonist or antagonist 30 min later. The fentanyl-induced LH suppression was reversed by subsequent naloxone administration, and the methysergide-induced LH suppression by quipazine. In the 3rd experiment the interaction of the opiate and serotonin neuronal systems was examined by injecting naloxone 30 min after methysergide, and quipazine 30 min after fentanyl. While naloxone reversed the methysergide-induced LH suppression, quipazine did not overcome the effects of fentanyl. In the 4th experiment the effects of an α_1 antagonist (prazosin, 1 mg/kg), an α_2 agonist (clonidine, 0.1 mg/kg), an α_2 antagonist (rauwolscine, 1 mg/kg) or a β antagonist (propranolol, 2 mg/kg) on LH release in OVX guinea pigs were tested. While both prazosin and rauwolscine suppressed LH release, clonidine did not change LH release. Propranolol also did not affect LH release. Moreover, it was found that, while the rauwolscine-induced LH suppression was not reversed by clonidine, injection of clonidine prior to rauwolscine treatment prevented the rauwolscine-induced LH suppression. These results suggest that 1) the opiatergic system is inhibitory to LHRH release; 2) α-adrenergic, but not β-adrenergic systems, are facilitatory to LHRH release; 3) the serotonergic system is facilitatory, perhaps by inhibiting the opiatergic system. Thus, the opiatergic neuronal system appears to be localized more proximally than the serotonergic system to LHRH neurons. Further experiments investigating interactions between opiatergic and adrenergic systems are in progress. (Supported by NIH Grants RR00167 and HD15433.)

10.9 RAPID RELEASE OF SUBSTANCE P AND LHRH FROM SYNAPTOSOMES PREPARED FROM THE MEDIAL BASAL HYPOTHALAMUS AND SUBSTANTIA NIGRA. M. Selmanoff and C. Shu*. Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

In the present study we investigated the Ca^{2+}-dependent, depolarization-induced release of substance P (SP) and LHRH from medial basal hypothalamic (MBH) and substantia nigra (SN) synaptosomes in male rats. SP and LHRH release under basal (5mM K^+) and depolarizing (75mM K^+) conditions was determined over 1-20 sec time intervals using a rapid filtration technique. Synaptosomes were perifused with a physiological saline solution at 30°C containing 0.2mM bacitracin, 5mM KCl, 75mM NaCl, 70mM N-methyl-D-glucamine (a Na^+ substitute), 1mM $MgCl_2$, 10mM glucose, 0.5mM pyruvate, 1mM Na_2HPO_4 and 10mM Hepes-HCl buffer (pH 7.4). Basal efflux was measured in response to a pulse of this 5mM K^+ solution. Depolarization was elicited by adjusting the external KCl concentration to 75mM by iso-osmotic substitution of N-methyl-D-glucamine in the presence of 1mM $CaCl_2$. Release medium was exposed to a 95°C water bath and lyophylized in a vacuum centrifuge. SP and LHRH were quantitated by specific radioimmunoassays sensitive at 1-4pg/tube. Endogenous neuropeptide content was determined in 2N acetic acid extracts of sonicated synaptosomes.

Depolarization of MBH synaptosomes evoked release of SP from 13.0±0.7 (5mM K^+) to 23.0±1.9 (75mM K^+) pg released/10 sec. In contrast, LHRH was not released by depolarization (11.6±0.8 to 12.9 ±1.1) and also did not occur in the presence of 10^{-4} or 10^{-6}M norepinephrine, 10^{-7}M 12-O-tetradecanoylphorbol-13-acetate (TPA), 10^{-5}M forskolin or in female rats.

Significant SP release was seen from both MBH and SN synaptosomes at 20,15,10,5 and only 1 sec of depolarization. The initial rate (1 sec) of SP release was 4.5-fold greater from SN than from MBH synaptosomes [k_{rel}=2.7 (SN) and 0.6 (MBH)]. SP release from SN synaptosomes was 2-3-fold greater at any given time interval compared with release from MBH synaptosomes. We have previously reported that TPA (10^{-7}M) potentiates evoked ^{3}H-dopamine release from striatal and median eminence synaptosomes, however, TPA did not potentiate SP release at this concentration.

Release of LHRH and SP from MBH synaptosomes may occur by different mechanisms. Evoked SP release was more pronounced from SN than from MBH synaptosomes. The release kinetics and/or the readily releasable pools may differ in these two populations of SP nerve terminals. TPA potentiated dopamine but not SP synaptosomal release. This suggests that protein kinase C activation potentiates the intrasynaptosomal stimulus-secretion coupling mechanism in dopaminergic but not SP-containing nerve terminals in these brain regions.

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10.10 GONADOTROPIN RELEASING HORMONE ASSOCIATED PEPTIDE (GAP) AND LUTEINIZING HORMONE RELEASING HORMONE (LHRH) ARE SECRETED AS INTACT PEPTIDES FROM MEDIAN EMINENCE IN RESPONSE TO DIFFERENT SECRETAGOGUES IN VITRO. W. C. Wetsel, M. M. Valenca*, M. D. Culler* and A. Negro-Vilar. Reprod. Neuroendo. Sect., Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

The sequence for the rat proLHRH has been deduced and found to contain the decapeptide, LHRH, and a 56 amino acid peptide called GAP (PNAS 83:179, 1986). Both LHRH and GAP have been localized in nerve terminals of the median eminence (ME; Nature 316:542, 1985). This location may have functional significance since recent reports have ascribed biological activity to GAP and an N-terminal fragment of GAP (Nature 316:511, 1985; JBC 261:16990, 1986). We have used various agents known to activate different intracellular messenger pathways in LHRH neurons to determine whether GAP is released in vitro from nerve terminals in the ME. In addition, we have used high performance liquid chromatography (HPLC) to characterize all LHRH and GAP release products. Adult, male, Sprague-Dawley rats were decapitated, the ME was dissected, and the tissue fragments were incubated in a Krebs-Ringer bicarbonate glucose buffer. Following a 30 min pre-incubation period, fragments were incubated for additional 30 min periods in the presence of fresh media, either alone or containing prostaglandin E2 (PGE_2), phorbol ester (PDBu), or the depolarizing agent, [K^+]. At the end of the experiment, LHRH and GAP were quantitated by radioimmunoassay. Serial dilutions of a pool of media gave a displacement curve which was parallel to that of the GAP_{1-56} standard. Both GAP and LHRH were secreted into the media under basal conditions. PGE_2 was found to significantly enhance the release of both peptides. PDBu and [K^+] stimulated the highest levels of secretion which were 8- and 4-fold higher than under basal conditions for LHRH and GAP, respectively. In a separate experiment, media that were collected under basal or [K^+]-stimulated conditions were injected separately onto an HPLC apparatus. Peptides were separated according to molecular weight (MW) on a Toyo-Soda G-2000SW column while absorbance was monitored at 280 nm. A single peak of immunoreactive LHRH eluted at approximately 1300 MW, at the same position as synthetic LHRH. Two peaks of GAP immunoreactivity were found. A small peak eluted in the void volume. The predominant peak eluted at 6500 MW, the same position as synthetic GAP_{1-56}. These data indicate that GAP is secreted from median eminence nerve terminals under basal conditions, that GAP is released in response to the same stimuli which stimulate secretion of LHRH, and that the secreted forms of LHRH and GAP appear to be the decapeptide and GAP_{1-56}. These results emphasize the importance of using both in vitro and HPLC methodologies to evaluate the changes which may occur in LHRH prohormone processing and secretion.

10.11 **REPETITIVE TRANSIENT RISES OF CYTOPLASMIC FREE CALCIUM IONS IN A SUB-POPULATION OF LHRH-STIMULATED PITUITARY GONADOTROPES.** D.A. Leong*, D.M. Benedek*, C.E. Lyons*, Jr., G.L. Mandell*, J.A. Sullivan*, M.O. Thorner. Department of Internal Medicine, University of Virginia, Charlottesville, VA 22908.

The precise role of cytosolic calcium ions in the pituitary gonadotrope remains to be established. We have recently combined the new fura-2/AM method for calcium ion measurements using digital imaging microscopy with immunologic techniques that permit quantitation of hormone release from single living cells. This permits the identification of secretory gonadotropes in mixed-cell pituitary preparations (reverse hemolytic plaque assay) together with real time measurements of calcium ions in single cells (fura-2/AM). This approach solves persistent problems associated with (i) mixed-cell populations: secretory gonadotropes in rat pituitary cell preparations comprise just 3-4% of all pituitary cells (ii) population averaging: temporally organised events such as calcium transients can be unambiguously resolved only in single cells. We have previously shown that high doses of LHRH (10.0 nM) rapidly triggers (1-2 sec) calcium mobilization from internal stores in all secretory gonadotropes lasting for 3-5 min. The initial phase of the calcium response is common. However, the calcium profile is strikingly different among individual secretory gonadotropes when stimulation is maintained for a further 45 min at a saturating LHRH dose. Three categories of response are evident: thus calcium levels remain sustained at high levels of calcium (Type I), or plateau to a level above baseline (Type II), or immediately return to baseline (Type III). The second phase of the calcium response is associated with calcium influx through calcium channels, since the response is completely abolished when extracellular calcium is withdrawn. The same repertoire of calcium responses was still obtained in the majority of gonadotropes treated with much lower doses of LHRH (0.001 and 0.01 nM). We report here on a new calcium response profile of repetitive calcium transients triggered in a small sub-population (<10%) of gonadotropes at low (but not high) LHRH doses. Resting levels of calcium ions were stable in this gonadotropes subset prior to stimulation. LHRH activated a striking series of repetitive calcium transients maintained, in some instances, for the entire stimulus period (50 min). There is a common pulse frequency of 2-3 sec (n=7). For each gonadotrope, calcium transients were generated as exact replicas when recordings were analyzed at 100 msec intervals. These findings demonstrate a new level of complexity regulating cytosolic calcium ions levels in the gonadotrope. This phenomenom adds to the accumulating evidence that the mechanism of signal transduction includes information transfer coded in the temporal organization of calcium transients.

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10.12 PARTICIPATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS IN THE REGULATION OF FOLLICLE STIMULATING HORMONE SECRETION IN A PRIMARY PITUITARY CELL CULTURE SYSTEM. G.A.Shangold*, M.Blotner*, E.Y.Lee*, & R.J.Miller, (Spon: R. Dinerstein), Dept. Pharmacol. and Physiol. Sciences and Dept. Obstetrics and Gynecology., Univ. of Chicago, Chicago, Il. 60637.

Calcium channels are clearly important in the control of luteinizing hormone (LH) release by pituitary cells, but to date little information exists concerning their role in the secretion of follicle stimulating hormone (FSH) by these same gonadotroph cells. The present investigation was undertaken to examine the involvement of voltage-sensitive calcium channels (VSCCs) in the stimulation of FSH release by gonadotropin-releasing hormone (GnRH) in primary pituitary cell cultures. Female, 35-day old, S/D rats were sacrificed to obtain pituitaries, from which adenohypophyses were dissected and dispersed in 0.25% trypsin for 20 minutes. After filtration through organza cloth and centrifugation at 225 g for 10 min., cells were plated at 500,000/well in Medium 199 with 10% horse and 2.5% fetal calf serum in 16 mm, 24-well plates. After 2 days, cells were washed 3 times in serum-free media and incubated for 2 hours with test drugs in HEPES buffer. After removal of media aliquots for FSH assay, cells were lysed with 0.2% SDS and protein content deter mined. Cell depolarization with 50 mM K^+ resulted in an increase in basal (5 mM K^+) FSH secretion. GnRH (100 nM) produced a significantly greater release of FSH than did 50 mM K^+. Cd^{++} (100 μM) completely prevented the depolarization-induced increase, and partially blocked the GnRH-mediated increase in FSH secretion. Depolarization-induced FSH stimulation was further enhanced by the dihydropyridine (DHP) VSCC agonist BAY K 8644 (1 μM). The enhancement of FSH secretion caused by 50 mM K^+ with or without BAY K was blocked by the VSCC antagonist nitrendipine (1 μM). Basal (5 mM K^+) secretion was enhanced to a level equal to that brought about by 50 mM K^+ via Na^+ channel activation with 30 μM veratridine. However, neither the K^+- nor GnRH-induced rise in FSH release was inhibited by Na^+ channel blockade with 5 μM tetrodotoxin (TTX). We also examined the effects of the putative regulator of gonadotropin secretion, Neuropeptide Y (NPY), in this cell culture system. As reported for LH secretion (Crowley et al., Endocr. 120:941,1987), NPY (100 nM) had little effect by itself on basal or depolarization-induced FSH release, but did enhance the GnRH-mediated increase in FSH secretion. These results suggest that activation of DHP-sensitive calcium channels can initiate the secretion of FSH from gonadotrophs. VSCCs may also play a role in the stimulation of FSH release by GnRH.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION I

11.1 EXPRESSION AND CELL TYPE-SPECIFIC PROCESSING OF RAT PRODYNORPHIN. L. Devi*, J. Douglass* and E. Herbert* (Spon: E. Lewis). Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, Oregon 97201.

The post-translational maturation of a complex precursor polyprotein, prodynorphin, was studied by transformation of a mouse pituitary cell line ($AtT\text{-}20_{D16v}$) and a rat pituitary cell line (GH_4-C1) with a recombinant plasmid encoding rat prodynorphin. This plasmid was constructed by inserting rat preprodynorphin cDNA downstream from the mouse metallothionein promoter and was introduced by co-transformation with the G418-selectable plasmid pKOneo. Stable transformants were isolated and analysed for the presence of the prodynorphin precursor. Chromatography of the cell extract from the stably transformed cell line AtT-20/ZRD19 on Sephadex G-75 revealed the presence of low molecular weight [Leu]enkephalin containing peptides (<2kd) as well as of larger proteins (up to 30kd). Analysis of the low molecular weight peptides on reverse phase HPLC showed the presence of dynorphin A, dynorphin B, ß-neo-endorphin and dynorphin A-(1-8) in addition to authentic [Leu]enkephalin.

In contrast, chromatography of the cell extract from stably transformed cell line GH_4-C1/ZRD13 on Sephadex G-75 revealed the presence of two prominant high molecular weight [Leu]enkephalin containing peaks and a very small low molecular weight peak representing [Leu]enkephalin pentapeptide.

These results suggest that while AtT-20 cells contain enzymes that efficiently process at the "Lys-Arg", Arg-Arg", and single arginine residues of prodynorphin, GH_4-C1 cells do not. The accurate and efficient processing of prodynorphin in AtT-20 cells and not in GH_4-C1 cells suggest that this method of DNA-mediated gene transfer will provide important information regarding post-translational control of mechanisms regulating the expression of neuropeptides and hormones.

11.2 DOPAMINERGIC AND CHOLINERGIC REGULATION OF STRIATAL PROENKEPHALIN mRNA CONTENT. I. Mocchetti, R. Dal Toso, A. Ritter and E. Costa. FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Medical Center, Washington, D.C. 20007

Northern blot analysis for the determination of proenkephalin (PE) mRNA in combination with radioimmunoassay for PE and enkephalin (ENK) showed that striatal dopaminergic terminals exert a tonic inhibition on striatal intrinsic neurons that store ENK. This effect seems to be mediated by dopaminergic receptor of the D-1 type since a prolonged treatment with the D-1 receptor antagonist, SCH 23390, increases ENK synthesis and processing. However, the effect of dopamine on ENK stores may be indirect since dopamine also tonically inhibits cholinergic neurons which, in turn, may regulate the synthesis of ENK in striatal neurons. Such a possibility is suggested by several independent lines of investigations: cholinergic agonists, such as diisopropylfluorophosphate, increases Met-ENK content in striatum while cholinergic antagonists, such as scopolamine, decreases it (Hong et al., Psychopharmacol. 1980). We confirmed this decrease and found it to be associated with a decrease in PE mRNA; furthermore, scopolamine blocks the increase in ENK and PE mRNA striatal content elicited by haloperidol. Primary cultures of striatal neurons were used to study how a signal transduction at synaptic neurotransmitter receptors modulates mRNA content of postsynaptic neurons. A 3 hr treatment with 10 uM of SKF 38393 a specific D-1 agonist, decreases PE mRNA content of these neurons in primary culture supporting the hypothesis that D-1 receptors modulate the PE mRNA content. The culture exposure to carbachol (50 uM) for 12 hr increases PE mRNA content. This effect is already present after 3 hr of exposure. These results suggest that dopaminergic and cholinergic receptors might be either located in enkephalin containing neurons or trans synaptically linked to these cells. Thus, it appears that in striatal cultures the activation of cholinergic or D-1 receptors can affect in opposite direction the content of PE mRNA.

11.3 CHARACTERIZATION AND SECRETION OF PRO-OPIOMELANOCORTIN-CONVERTING ENZYME ACTIVITY BY DISPERSED BOVINE INTERMEDIATE LOBE PITUITARY CELLS. Maria G. Castro* and Y. Peng Loh. Lab. of Neurochemistry & Neuroimmunology, NICHD, NIH, Bethesda, MD 20892

Pro-opiomelanocortin (POMC) is synthesized by the anterior and intermediate lobe (IL) of the pituitary. This prohormone is processed within the secretory vesicles (SV) to yield active hormones by cleavages at paired basic amino acid residues, the most common being Lys-Arg pairs. An enzyme that cleaves this prohormone specifically at the paired basic residues has been characterized and purified from bovine pituitary IL secretory vesicles. This enzyme, named pro-opiomelanocortin converting enzyme (PCE) is a M_r 70,000 glycoprotein (Y.P. Loh et al., Journal of Biological Chemistry, Vol. 260: 12, pp. 7194-7205, 1985), and has a pH optimum of between 4 and 5. One of the criteria for showing that this pro-hormone converting enzyme is physiologically relevant, is to demonstrate that the enzyme is secreted with the POMC derived peptide products in a co-ordinately regulated manner. Hence, the secretion of PCE together with α-MSH from bovine IL dispersed pituitary cells was investigated.

The dispersed bovine IL pituitary cells were incubated in medium with and without 8-Bromo-cyclic-AMP (cAMP: 3×10^{-3}M). The medium was collected and assayed for secretion of PCE and α-MSH. α-MSH was measured by radioimmunoassay and PCE activity was assayed in pH = 4.0 buffer at 37°C using ^{3}[H] phenylalanine labeled mouse POMC or ^{3}[H] arginine-labeled frog POMC as substrates. The processed products formed were identified by immunoprecipitation with ACTH and β-endorphin antisera, and by co-migration with known markers on acid-urea polyacrylamide gels. With cAMP treatment, secretion of PCE activity and α-MSH was 162.4 ± 3.4% and 142 ± 11% respectively, when compared to the controls (without cAMP). The secreted PCE was shown to cleave POMC primarily to 21-23K ACTH, 13K ACTH, 4.5K ACTH, 16K NH_2-terminal glycopeptide (K = 1000 daltons), β-lipotropin and β-endorphin peptides, similar to those synthesized by the mouse and frog IL in situ. This PCE activity was further characterized to have a pH optimum of between 4 and 5 and an inhibitor profile similar to that of the purified bovine IL PCE. These data provide additional support for the presence of PCE in bovine IL secretory vesicles and a role of this enzyme in the processing of POMC "in vivo".

11.4 CHARACTERIZATION OF PEPTIDES DERIVED FROM PRO-OPIOMELANOCORTIN (POMC) IN A NELSON'S SYNDROME PITUITARY TUMOR. C.W. Wilkinson, S.A. Galt*, T.S. Roberts* and S.R. Plymate*. Geriatric Res., Educ. & Clin. Ctr., American Lake VA Med. Ctr., Tacoma, WA 98493; Dept. of Clin. Invest., Madigan Army Med. Ctr., Tacoma, WA 98431; and Univ. of Washington, Seattle, WA 98195.

Several studies have suggested that pituitary adenomas removed from patients with Cushing's disease or Nelson's syndrome may contain greater proportions of more highly-processed forms of POMC-derived peptides than do normal human anterior pituitaries. By means of high-pressure liquid chromatography (HPLC) and radioimmunoassays (RIA) we have characterized specific molecular forms of POMC-derived peptides in tumor tissue from a 46-year old woman diagnosed as having Nelson's syndrome. The patient had been adrenalectomized bilaterally 16 years previously following a diagnosis of Cushing's disease and had been maintained on replacement doses of glucocorticoids. At the time of transsphenoidal surgery, skin pigmentation was excessive, and plasma concentration of immunoreactive ACTH was greater than 1700 pg/ml.

A portion of the tumor (64 mg wet weight) was frozen on solid CO_2 immediately after removal and was extracted by boiling in 1 N acetic acid, sonicating, and centrifuging for 30 min at 13,000 g. The supernatant was applied to ODS-silica cartridges, washed, and eluted with 80% acetonitrile (ACN), 20% aqueous 0.1% trifluoroacetic acid (TFA). The extract was vacuum-dried and resuspended in 0.1% TFA and applied to a 5 micron, C_{18} reverse phase HPLC column. Separations were attained with a gradient from 30% to 40% ACN against water while maintaining a constant TFA concentration of 0.1%. Eluate fractions were collected and assayed for immunoreactive beta-endorphin, gamma-endorphin, alpha-MSH, and ACTH.

Of the low molecular weight immunoreactive beta-endorphin that was extracted, approximately 80% co-eluted with unacetylated human beta-endorphin 1-31; small proportions co-eluted with forms of beta-endorphin 1-27 and 1-26. Virtually all of the gamma-endorphin immunoreactivity co-eluted with unmodified gamma endorphin (beta-endorphin 1-17). Immunoreactive alpha-MSH eluted in three peaks that appear to correspond to native alpha-MSH (32%), the des-acetyl form (18%), and the C-terminally glycine-extended form (50%). Less than 0.1% of the immunoreactive ACTH co-eluted with human ACTH 1-39; the remainder eluted in a broad peak coincident with corticotropin-like intermediate lobe peptide (CLIP).

POMC-derived peptides in this Nelson's syndrome tumor are not characteristic of those in normal human anterior pituitary.

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11.5 REGULATION OF CARBOXYPEPTIDASE H BY PRODUCT INHIBITION. Vivian Y.H. Hook and Edmund F. LaGamma. Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814 and Dept. of Pediatrics and Neurobiology & Behavior, SUNY at Stony Brook, N.Y.

Carboxypeptidase H(CPH) is one of several enzymes required for processing of peptide hormone precursors. In this study, regulation of CPH by its peptide products through a product and feedback inhibition mechanism was investigated. CPH activity in bovine adrenal medulla chromaffin granules and rat adrenal medulla homogenate was inhibited by the peptides (Met)- and (Leu)enkephalin, vasopressin, oxytocin, LHRH, substance P, and TRH, with oxytocin and ACTH 1-14 having the least effect, at concentrations of 2-20 mM. Inhibition by amidated peptide products (vasopressin, oxytocin, LHRH, substance P, and TRH) show that the final products of the precursor processing pathway can regulate CPH. In contrast, bovine serum albumin did not inhibit the enzyme. These levels of peptides are similar to known intragranular peptide concentrations, indicating that product and feedback inhibition of CPH may play a role in the control of neuropeptide synthesis. The kinetics of CPH peptide inhibition was characterized with enkephalin peptides, the most abundant neuroactive peptide products of CPH present in adrenal medulla. CPH in bovine and rat adrenal medulla showed identical kinetics. In addition, soluble and membrane bound forms of CPH from bovine chromaffin granules showed similar kinetics. The approximate K_I values for the proenkephalin-derived peptides (Met)enkephalin, (Leu)-enkephalin,(Met)enkephalin-Arg^6-Gly^7-Leu^8, and (Met)enkephalin-Arg^6Phe^7 were 12.0, 6.5, 7, and 5mM, respectively, with competitive inhibition kinetics. It is of interest that the K_I for (Met)enkephalin was significantly greater than those for the other enkephalin peptides. This may reflect the effects of higher intragranular concentration of (Met)enkephalin compared to (Leu)enkephalin, (Met)enkephalin-Arg^6-Gly^7-Leu^8, and (Met) enkephalin-Arg^6-Phe^7 since one proenkephalin molecule contains 4 copies of (Met)enkephalin and only one copy of each of the other enkephalin peptides. Thus, the products from one multivalent precursor molecule may equivalently inhibit CPH activity. Product inhibition of CPH and perhaps other processing enzymes may serve to limit the maximum peptide concentration within the secretory vesicle. These data support the hypothesis that precursor processing enzymes may play a significant role in the regulation of peptide production in neuroendocrine cells.

11.6 REGULATION OF TRH LEVELS UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS: mRNA LEVELS AND PEPTIDASE ACTIVITIES. L. Covarrubias*[1], R.M. Uribe*, G. Ponce*, J.L. Charli*, and P. Joseph-Bravo* (SPON: A.M. Colome). [1]CINVESTAV (IPN) and Centro de Investigaciones sobre Ingeniería Genética y Biotecnología (UNAM), A.P. 70479, México, D.F. 04510, México.

A clear view of neuronal phenotype establishment not only requires our understanding of those molecular events which occur during development and differentiation within a cell, but also, of the molecular events due to cell interaction and extracellular factor response in the adult animal. On the other hand, homeostasis control involves a precise regulation of the amount of active components in extracellular fluid as well as their biosynthesis in the specific cell producers. Our interest has been focused on the understanding of these important phenomena. Thyrotropin Releasing Hormone (TRH) metabolism is a good study model since our actual knowledge and experimental tools will allow us to attack both cell phenotype and animal homeostasis establishment. As other neuropeptides, it is synthesized from a protein precursor an is degraded in vitro by two solubles (prolylendopeptidase and pyroglutamateamino peptidase I (PGAI)), one membrane bound (PGAII) and one serum enzyme (thyroliberinase).

We studied what might be two limiting stages of metabolism: TRH mRNA levels and TRH degradation. TRH mRNA levels and degrading enzymes' activities were measured under a variety of physiological conditions: hypo- and hyper-thyroidism, circadian cycle and ontogenic development. mRNA was quantified by Northern blot analysis using nick translated rat hypothalamic TRH cDNA kindly donated by R. Goodman (Lechan et al., Science, 231:159, 1986). TRH-mRNA increased 8x from day 5 to 30; during the circadian cycle it fluctuated 2x between the valley and the peak and in hypothyroid rats, a significant increase was also observed.

Degrading enzymes were also affected. Thyroliberinase activity was increased in the hyperthyroid rat. A 3-5 fold increase in PGAII was observed in the adenohypophysis of hyperthyroid rats. This was tissue specific and enzyme specific (no change for PGAI). Opposite changes were seen in the hypothyroid animal. These data suggest biosynthesis and degradation as regulatory events of TRH metabolism. (Supported in part by grants from CONACyT # ICSAXNA 030915 and # ICCBXNA 02144 and Fondo R. Zevada)

11.7 BIOSYNTHESIS OF MATURE THYROTROPIN RELEASING HORMONE BY AtT-20 CELLS TRANSFECTED WITH A CDNA ENCODING PREPROTRH.
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The structure of rat hypothalamic preproTRH has been deduced by sequencing of preproTRH complementary DNA (cDNA) and consists of a 255 aa sequence containing 5 TRH progenitor sequences , Gln-His-Pro-Gly, demarcated by paired basic aa sequences. To study the post-translational processing and packaging of the precursor for thyrotropin releasing hormone (preproTRH) we have transfected AtT-20 cells with an expression vector containing preproTRH cDNA .

Cotransfection of AtT-20 cells, a mouse pituitary cell line with a neomycin resistance plasmid and an expression vector consisting of the CMV IE promotor (provided by Dr. John J. Kopchick) fused to preproTRH cDNA resulted in several clones of G418-resistant cells containing preproTRH mRNA by Northern blotting. These clones were shown both to contain immunoreactive TRH, (pGlu-His-Pro-NH_2, iTRH) and release iTRH into culture media. Untransfected AtT-20 cells do not contain detectable iTRH or preproTRH mRNA. One of these clones, aKS6.19, was studied further. Cells were extracted with 1M acetic acid/HCl pH 2,boiled and the extract lyophilized for TRH assay using a RIA which only recognizes fully processed TRH. After purification on solid-phase cartridges extracts were also assayed for immunoreactive pCC (ipCC), using an antiserum directed against the sequence Cys-Lys-Arg-Gln-His-Pro-Gly-Lys-Arg-Cys, which recognizes incompletely processed TRH progenitor sequences, and immunoreactive pYT (ipYT), directed against the N-terminal sequence 52-75 preproTRH. The estimated cell contents of iTRH, ipCC and ipYT were 6.88, 1.77 and 2.59 pmol equivalents synthetic standard/mg cell protein respectively. Sephadex G50 chromatography of cell extracts revealed qualitatively similar profiles of ipYT and ipCC to those seen in extracts of rat brain: namely two peaks of ipYT of MW ~3Kd and 7Kd and one of ipCC of ~6Kd. Release of iTRH by aKS6.19 cells was increased by potassium depolarization and by stimulation with dibutyryl cAMP which releases endogenous ACTH from AtT-20 cells (basal TRH release 273.3±5.67 pg/well/hr; 50mM K^+ 492.3±13.68; 5μM Bt_2 cAMP 399.3±26.4. Mean±SEM, n=3). Parallel increases in ipYT release were also observed.

We conclude that a cell line which can post-translationally process endogenous proopiomelanocortin to mature ACTH in secretory granules is also capable of synthesizing mature TRH when transfected with the cDNA for preproTRH. This suggests that the processing mechanisms for post-translational processing of peptide neuromodulators and hormones are not specific for particular peptide precursors. These transfected cell lines provide systems for further studies on the post-translational processing of proTRH.

11.8 HYPOTHYROIDISM INCREASES proTRH mRNA AND IMMUNOREACTIVE proTRH IN THE RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN).
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Recent studies from our laboratories demonstrate that hypothyroidism increases proTRH mRNA in extracts of the PVN, the origin of the TRH-tuberoinfundibular system. To further characterize this response, we have performed semi-quantitative in situ hybridization histochemistry and immunocytochemistry to detect proTRH mRNA and TRH prohormone in hypothyroid rats.

Male, Sprague-Dawley rats were studied after three weeks of hypothyroidism, induced by an initial i.p. injection of propylthiouracil (0.01 mg/g) followed by p.o. methimazole (0.02%) in their drinking water. Hypothyroidism was confirmed by measurement of serum TSH. The brains were fixed by intracardiac perfusion with 4% paraformaldehyde and the region containing the PVN sectioned coronally or horizontally on a vibratome. 20um sections were adhered to glass slides and hybridized with a ^{35}S-labeled proTRH cRNA probe transcribed from the rat proTRH cDNA. The emulsion-coated slides were developed after 5 d of exposure and visualized by darkfield microscopy. The density of silver grains accumulating over each hybridized neuron in the PVN was assessed by computer image analysis, normalized to the mean density per cell over hybridizing neurons in the lateral hypothalamus. Data were expressed as the ratio of individual PVN cell density to mean lateral hypothalamic density versus number of cells. Immunocytochemistry was performed on 50 um free floating sections by the ABC technique using antiserum #351 (1:1000) which recognizes intact or partially processed proTRH.

In control animals, hybridization was seen in the medial and anterior parvocellular divisions of the PVN with a tendency for more intense hybridization in the medial division. The majority of medial parvocellular neurons had ratios of density between 0 and 2 (median: 0.9). In contrast, medial parvocellular neurons in hypothyroid animals showed increased hybridization, with a density ratio range of 0-10 (median: 4.3). Immunocytochemistry showed a marked increase in the accumulation of immunoreactive proTRH in medial but not anterior parvocellular neurons in hypothyroid, but not control animals.

We propose that the simultaneous increase of proTRH mRNA and immunoreactive proTRH in medial parvocellular neurons of the PVN suggest that hypothyroidism induces both transcription and translation of the TRH prohormone. The selective response of medial, but not anterior, parvocellular neurons to hypothyroidism provides further evidence that these cells may be under different regulatory control.

11.9 **PROCESSING OF THE APLYSIA EGG LAYING HORMONE PRECURSOR.**
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The characteristic egg laying behavior of Aplysia californica is thought to be mediated by two clusters of neurosecretory cells called the bag cells. These neurons, which sit on top of the abdominal ganglion, fire preceeding each egg laying episode and release a battery of neuropeptides into the sheath surrounding the ganglion as well as the hemolymph. At least four of these peptides (γ, β, α-BCP and ELH) have distinct biological activities and are produced through proteolytic cleavage of a single 28 kd prohormone. DNA sequence analysis has revealed that the ELH preprohormone contains a signal sequence, eight dibasic putative cleavage sites and numerous single arginine residues. Two system HPLC analysis of crude bag cell homogenates followed by amino acid compositions of the resulting pure peptides has shown that at least seven of the eight dibasic residues, the signal sequence and one single arginine residue are used as proteolytic cleavage sites.

The processing pathway of the ELH precursor is presently under investigation. Intermediates in the processing pathway have been purified by HPLC and are being characterized chemically by amino acid analysis, Edman degradation and tryptic mapping. A series of synthetic peptides from various regions of the precursor have been used to generate antisera. These antibodies are being used for immunoprecipitation studies of the intermediates in the processing pathway as well as for immuno EM investigations of the subcellular localization of the antigens. Pulse chase studies using tritiated amino acids (Phenylalanine, Leucine and Isoleucine) have shown that the amino terminal 2/3 of the precursor is processed more slowly than the carboxyl terminal 1/3 of the molecule; this results in a relatively long lived (apparently 18 kd) intermediate which contains γ, β, and possibly α-BCP as well as several shorter lived, smaller intermediates which all contain ELH. The pathway and kinetics of ELH precursor processing may influence the function of its bioactive peptides in transmission.

11.10 HETEROGENEITY AMONG ACIDIC PEPTIDES ASSOCIATED WITH NEUROSECRETORY VESICLES IN THE BAG CELLS.
S. Arch, M. Mendelson* and M. Deal.* Biological Laboratories, Reed College, Portland, OR 97202.

Use of ^3H-Leu for biosynthetic labeling of bag cell neuropeptides results in the resolution of two major peaks of radiolabel when the contents of a neurosecretory vesicle (NSV) fraction are analyzed by isoelectric focusing. These are the acidic peptide (AP) and the egg-laying hormone (ELH). We have been interested to determine which other peptides are also associated with the NSV fraction. On the basis of the reported nucleotide sequence, we chose to label bag cells with ^3H-Phe since neither the ELH nor the putative AP sequences is illustrated as containing a Phe residue. The IEF profile of Phe-labeled NSV differs in several respects from that obtained with Leu labeling. However, although there is relatively little labeling at the pI 9.3 position occupied by ELH, the pI 4.8 position associated with AP is strongly labeled. To characterize the Phe-labeled acidic species further, we used the pI 4.8 band from IEF as the starting material for SDS-PAGE separation. This selective 2-D approach resolved the Phe-labeled material into two closely spaced peaks of radiolabel at M_r's of 5.6 kD and 4.5 kD. The 5.6 kD peptide is predominant in this case whereas with Leu-labeled material, the 4.5 kD peptide is the major band. If both these peptides are derived from the neuropeptide precursor, the larger is likely to arise from the excision of the α- and γ-BCP's from a common intermediate. We have called this AP-39 to distinguish it from the 27 residue C-terminal AP sequence (AP-27). On the grounds of its level of labeling with the two amino acids, we are led to assume that the 4.5 kD band represents AP-27 and that this peptide contains Phe. Although both AP's are found in an NSV fraction, the presence of membranous contaminants in the fraction preclude the assumption that they are both within the NSV. We, therefore, determined their solubility in response to a range of hyposmolal shocks. The outcome of these experiments disclosed that they show similar solubilities to hyposmolal challenge, thereby indicating that they are enclosed within organelles with the same lability, presumably NSV. The implication from these observations is that the BCP's may be loaded into NSV in an intermediate form and that they are either cosequestered with AP-27 or occupy different NSV's of the same osmotic fragility.

11.11 EXPRESSION OF NEUROPEPTIDE Y mRNA AND PEPTIDE IN MEGAKARYOCYTES: ENHANCED LEVELS IN CERTAIN AUTOIMMUNE MICE. H. Persson*, A. Ericsson*, D. Larhammar, M. Schalling*[1], K.R. McIntyre*[2], J.M. Lundberg*[3], K. Seroogy[1] and T. Hökfelt*[1]. (SPON: B. Green). Depts. Medical Genetics and Immunology[2], Uppsala University, Uppsala, Sweden and Depts. Histology[1] and Pharmacology[3], Karolinska Institute, Stockholm, Sweden

Neuropeptide tyrosine (NPY) is a potent vasoconstrictor agent with a wide distribution in the central and peripheral nervous systems. We demonstrate that high levels of rat NPY mRNA are also found in peripheral blood cells, bone marrow, lung and spleen. Radioimmunoassay revealed high levels of NPY peptide in these tissues. In mice, splenic NPY mRNA and immunoreactive peptide levels differed extensively between strains and were greatly elevated in several strains (NZB, NZBxW and BXSB) that develop a disease resembling human systemic lupus erythematosus. Like the rat, the NZB mouse showed a high content on NPY mRNA in peripheral blood cells and bone marrow. Immunohistochemical staining revealed NPY-like immunoreactivity in large cells morphologically identifiable as megakaryocytes in rat bone marrow and the spleen of the NZB strain. Expression of NPY mRNA in megakaryocytes was confirmed by in situ hybridization. These results demonstrate a synthesis of NPY in megakaryocytes, implying that NPY can be released from platelets and function as a vasoconstrictor during blood vessel damage. In addition, the elevation of splenic NPY in certain autoimmune mouse strains adds to the list of abnormalities associated with these strains.

RETINA I

12.1 RECTIFYING TRANSMISSION AT THE ROD OUTPUT SYNAPSE OF TIGER SALAMANDER RETINA. M. Wilson[1], S. Borges[1], D. Attwell*[2] and S. Wu[3]. [1]Department of Zoology, University of California, Davis, CA 95616, [2]Department of Physiology, University of London, WC1E 6BT, England, [3]Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

Photoreceptors respond to light with a graded hyperpolarization up to 25 mV in amplitude. It has been conventionally assumed that all of this response range is transmitted through the photoreceptor output synapse to second order neurons in the visual system. We have examined the voltage input-output relation of rod to horizontal cell transmission using three different approaches based on simultaneous intracellular recordings from pairs of rods and horizontal cells. All three approaches reveal strong rectification in which only signals coded within 5 mV of the rod dark potential are effectively transmitted to postsynaptic horizontal cells. The gain is high for very small hyperpolarizations (7.2 ± 4.9, mean ± S.D., n = 6), but is much larger for larger rod hyperpolarizations. The whole input-output relation can be approximated by an exponential curve, whose slope decreases e-fold for each 2.1 mV of rod hyperpolarization (2.10 ± 0.44, n = 6). The limitation of signal transmission to the first 5 mV of the rod response is consistent with the reported voltage-dependence of the calcium current in rods (Bader, C.R., Bertrand, D. & Schwartz, E.A., J.Physiol.Lond.,331:253,1982), if it is assumed that calcium influx promotes transmitter release.

What accounts for this apparent paradox that rods have a 25 mV response range, while signals greater than 5 mV in amplitude are clipped ? We propose that the properties of the rod synapse are related to two other poorly related aspects of early visual processing viz. the mixing of signals that occurs by virtue of the strong electrical coupling of adjacent rods, and by virtue of the weak electrical coupling of adjacent rods and cones. We argue that the effect of both kinds of photoreceptor coupling is to offset the limitations imposed by the transmission properties of the rod synapse. We predict that the strengths of both sorts of coupling are quantitatively linked to the relationship between the rod response range and the synapse operating range. Consideration of the strength of coupling for phototreceptors in the salamander retina shows that these predictions are upheld.

12.2 **THE EFFECT OF L-GLUTAMATE ON THE ANOMALOUS RECTIFIER POTASSIUM CONDUCTANCE OF ISOLATED HORIZONTAL CELLS FROM THE WHITE PERCH RETINA.** I. Perlman*, A.G. Knapp and J.E. Dowling. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Isolated horizontal cells from the white perch retina contain at least three kinds of voltage-dependent potassium channels (E.M. Lasater, J. Neurophysiol. 55, 499, 1986) including the so-called anomalous (or inward) rectifier (AR) channels that open upon hyperpolarization of the membrane. L-glutamate, a putative neurotransmitter between cones and horizontal cells, may inhibit the inward rectifier (A. Kaneko & M. Tachibana, J. Physiol. **388**, 169, 1985). This effect produces a net outward current which opposes current flowing inward through glutamate-gated channels, and thus reduces the apparent glutamate current at hyperpolarized potentials. We have used whole-cell recording techniques to study the effect of L-glutamate on cone horizontal cells isolated from the white perch retina. In every cell studied, the I-V curve recorded in standard Ringer's showed a pronounced inward current at potentials below about -70 mV. This current was identified as I_{AR} based on its voltage-dependence, its variation with changes in external potassium concentration, and its blockade by cesium ions. Pressure-ejection of L-glutamate (100 - 200 µM) evoked an inward current that reversed at about +5 mV and reached a maximum at -60 to -70 mV. At more negative potentials, the inward current decreased and in many cases a net outward current was observed. This effect was due to a reduction in I_{AR}, because in the presence of external Cs^+ application of glutamate elicited an inward current that increased linerly as the holding potential was made more negative. To our surprise, however, pressure ejection of Ringer's alone also appeared to reduce I_{AR}. Moreover, when the recording chamber was perfused rapidly with Ringer's, I_{AR} was again reduced, and the I-V relation for glutamate became similar to that seen in the presence of Cs^+. To explain these observations, we suggest that, in the absence of perfusion, potassium accumulates in an unstirred layer around the cells and enhances I_{AR}. Pressure ejection lowers the concentration of postassium around the cells, thus reducing I_{AR}, and perfusion prevents the local accumulation of potassium. We find no evidence that L-glutamate itself directly affects the anomalous rectifier.

12.3 **PROPERTIES AND DOPAMINE SENSITIVITY OF A VOLTAGE-DEPENDENT SODIUM CONDUCTANCE IN ISOLATED HORIZONTAL CELLS FROM THE WHITE PERCH RETINA.** A.G. Knapp, T. Roe* and J.E. Dowling. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Isolated horizontal cells from the white perch retina contain a voltage-dependent, TTX-sensitive sodium conductance, I_{Na} (E.M. Lasater, J. Neurophysiol. **55**, 499, 1986). The function of this conductance is unclear, as horizontal cells do not exhibit sodium-dependent action potentials in vivo. It has been suggested that I_{Na} may enable horizontal cells to depolarize rapidly in response to the offset of illumination; sodium influx may also affect the release of GABA from these neurons (S. Yazulla & J. Kleinschmidt, Brain Res. **263**, 63, 1983).

We have used whole-cell voltage clamp techniques to examine additional properties of I_{Na} in cone horizontal cells isolated from the white perch retina. Under conditions that minimize current flow through other ionic conductances, I_{Na} appears as a transient inward current in response to step depolarizations beyond -50 mV, reaches a maximum amplitude near 0 mV and has an extrapolated reversal potential near +40 mV. Both the activation and the inactivation of g_{Na}, the conductance underlying I_{Na}, were found to be strongly voltage-dependent, with half-maximal values at approximately -25 mV and -60 mV, respectively. Double-pulse experiments demonstrated that the time-constant of inactivation was also voltage-dependent. These characteristics are very similar to those of I_{Na} in other types of neurons.

In the intact perch retina, dopamine (DA) is released from interplexiform cells onto cone horizontal cells, where it acts through its intracellular second messenger cAMP to modulate several aspects of neuronal function. We have found that pressure ejection of DA onto isolated cone horizontal cells consistently reduced I_{Na}. In 65% of cells tested, DA reduced I_{Na} by more than 20%, and in some cases it was possible to abolish I_{Na} altogether. The suppression of I_{Na} required relatively high concentrations of DA ($\geq$ 400 μM) and occured more rapidly (maximal effect within 10 sec) and lasted a shorter time (recovery within 90 sec) than those effects of DA known to be mediated by cAMP. Moreover, the reduction in I_{Na} could not be blocked by antagonists of DA, nor could it be mimicked by agents that promote the accumulation of cAMP in horizontal cells. Thus, this action of DA appears to be a nonspecific one, perhaps involving a direct interaction between DA and the sodium channel molecules or adjacent portions of the horizontal cell membrane. Because of the relatively large doses of DA required to inhibit I_{Na}, we doubt that this phenomenon has any physiological significance. It does, however, constitute a potential pitfall in experiments where high concentrations of DA are applied to neural tissue.

12.4 DOPAMINE MIMICS LIGHT-ADAPTATION IN HORIZONTAL CELLS OF THE XENOPUS RETINA. P. Witkovsky, S. Stone, J. Besharse*. Dept. Ophthal., New York Univ. Med. Ctr., NY, NY 10016 and Dept. Anat. & Cell Biol., Emory Univ. Sch. Med. Atlanta GA 30322

The axon-bearing horizontal cell of the Xenopus retina receives direct synaptic inputs from green-sensitive rods and red-sensitive cones. Intracellular recordings from horizontal cells showed that rod- and cone- initiated signals could be distinguished on the bases of waveform, operating range of light intensity and spectral sensitivity. The balance of cone vs. rod input normally is dictated by the daily light/dark cycle. We found, however, that even after prolonged dark-adaptation, addition of 2-10 μM dopamine to the superfusate evoked changes usually associated with light adaptation: rod input was suppressed, cone input became larger and faster. In addition, the horizontal cell depolarized by 9.1 ± 3.8 mV. Dopamine- induced effects took 10-20 min to appear; more than 30 min in dopamine-free Ringer was required to restore the dark-adapted condition. Multiple cycles of dopamine-induced "light-adaptation" followed by dark-adaptation in dopamine-free Ringer could be induced in single horizontal cells. Intracellular recordings from rods demonstrated that rod threshold and V_{max} were not altered by exposure to dopamine.

Dopamine effects on the horizontal cell were imitated by the D_2 agonist LY 171555 at 1 μM. The D_1 agonist SKF 38393 mimicked light-adaptation at 10-20 μM. Its action was blocked by an equimolar concentration of the D_1 antagonist, SCH 23390. Dopamine effects were not blocked either by SCH 23390 or the D_2 antagonist spiroperidol alone, but in combination the two antagonists suppressed completely the light adaptive action of dopamine. The light-evoked responses of horizontal cells were not modified by nor-epinephrine, epinephrine or serotonin (each at 10 μM). These pharmacological data indicate that dopamine works through a combination of D_1 and D_2 dopamine receptors.

Dopamine actions in distal retina that might play a role in the present results are: (1) cone retinomotor movements (Pierce & Besharse), (2) horizontal cell uncoupling (Piccolino et al.), and (3) augmented conductance change induced in the horizontal cell membrane by the putative cone transmitter, glutamate (Knapp & Dowling). The first of these depends on a D_2 receptor and takes 15-30 min to complete. Cone elongation under light-adapted conditions can be elicited by spiroperidol. We found that 5-20 μM spiroperidol slowed the time course and reduced the amplitude of cone-initiated signals. The second and third mechanisms both are mediated by a D_1 receptor. We observed that exposure to dopamine narrowed the receptive field profile of the horizontal cell. Since the length constant $\lambda = (R_m/R_s)^{1/2}$, this result could be expected from either mechanism 2 (increase in R_s) or mechanism 3 (decrease in R_m), or both. The depolarization evoked by dopamine would be expected of mechanism 3 but not of 2.

The available evidence indicates that, in the Xenopus retina, dopamine synthesis and release occur at a greater rate in daytime than at night. Thus, our data suggest that dopamine plays an important role in the daily cycle of light and dark adaptation.

12.5 cAMP AND cGMP DECREASE THE CONDUCTANCE OF ELECTRICAL SYNAPSES BETWEEN HORIZONTAL CELLS OF THE CATFISH RETINA S.H. DeVries* and E.A. Schwartz* (SPON: J. Art) Dept. of Pharm. & Physiol. Sci., The University of Chicago, Chicago IL 60637

Cyclic nucleotides are claimed to regulate the conductance of gap junctions in several tissues. Their effect depends upon the tissue studied. An increase in cAMP may play a role both in the glucagon-induced increase in coupling observed in cultured rat hepatocytes[1] and the dopamine-induced decrease in coupling between teleost retinal horizontal cells.[2] These opposing actions of cAMP suggest an underlying mechanistic diversity. We have investigated the mechanism whereby coupling is regulated in horizontal cells of the teleost. Retinas of the channel catfish (*Ictalurus punctatus*) were dissociated and cultured. Electrical synapses often formed when processes from neighboring cone horizontal cells were closely apposed. Trans-synaptic conductance was measured by controlling the voltage of each cell in a pair with either two switching voltage clamps or two patch pipette voltage clamps. Micropipettes were filled with (in M) KAc, 3; KCl, 1. Patch pipettes contained (in mM) K-aspartate, 65; Na-aspartate, 10; CsCl, 10; HEPES, 10; EGTA, 10; $CaCl_2$, 1; pH 7.0. Synaptic conductances of 10-100 nS were observed with either technique and frequently maintained for more than one hour without significant rundown. Coupling was decreased more than 90% following brief exposures (100 ms) to dopamine (100 μM) or the D1 agonist, fenoldopam (10 μM). Uncoupling occured within 30-60 sec; recoupling required 10-20 min. Neither dopamine nor fenoldopam elicited a membrane current. Applications of IBMX (100 μM) or forskolin (40 μM) transiently reduced coupling by the same amount as dopamine. These experiments indicate that dopamine may uncouple by raising the intracellular concentration of a cyclic nucleotide. Cyclic nucleotides were introduced into one cell of a pair via a third patch pipette. First the conductance between a pair was measured with two pipettes. Then, the membrane occluding the tip of the third pipette was ruptured allowing cAMP to diffuse into the cell. The abrupt introduction of 1-0.1 mM cAMP decreased junctional conductance 50-80% in less than 1 min. Thereafter, a reduced conductance was maintained. Upon withdrawing the third pipette from the cell surface, full coupling resumed in less than 2 min. In similar experiments, 0.1 mM cGMP reversibly reduced junctional conductance 10-40% and 1-0.1 mM 8-Br cAMP irreversibly uncoupled. 5'-AMP (1 mM) had no effect on junctional conductance.

[1]Saez, J.C., Spray, D.C., Nairn, A.C., Hertzberg, E., Greengard, P. & Bennett, M.V.L. *Proc. Nat. Acad. Sci.* (1986) **83**, 2473-2477.

[2]Lasater, E.M. & Dowling, J.E. *Proc. Nat. Acad. Sci.* (1985) **82**, 3025-3029.

12.6 **TREATMENTS WHICH UNCOUPLE TELEOST RETINAL HORIZONTAL CELLS PROMOTE PHOSPHORYLATION OF A MEMBRANE PROTEIN.** D.G. McMahon, and J.E. Dowling, The Biological Laboratories, Harvard University, Cambridge, MA. 02138.

The neuromodulator dopamine, released from interplexiform cells in the teleost retina, has been shown to have profound effects on the physiology of cone horizontal cells. For example, treatment of white perch (Roccus americana) retinas with dopamine or prolonged darkness reduces the electrical coupling between cone horizontal cells. In isolated pairs of horizontal cells dopamine, and its intracellular second messenger cAMP, uncouple these cells by reducing the junctional conductance, presumably by modifying the gap junctional channels. Since cAMP usually acts through protein phosphorylation we have sought to identify the phosphoprotein targets of this pathway as a first step in understanding the biochemical mechanism by which dopamine and cAMP modulate junctional conductance.

Protein phosphorylation in the perch retina was examined under three conditions which uncouple horizontal cells. Addition of cAMP (1-100μM) to retinal homogenates consistently induced phosphorylation of a protein with an apparent molecular weight of 31Kda on SDS-PAGE. Phosphoproteins of 15Kda, 21Kda, 47Kda, and 70Kda were less consistently observed. Incubation of isolated retinas with dopamine (100μM) resulted in an increase in the phosphorylation of 31Kda and 35Kda proteins. These effects were blocked by the dopamine antagonist haloperidol (100μM). Treatment of retinas with prolonged darkness increased the phosphorylation of the 31Kda protein but decreased that of the 35Kda protein compared with retinas maintained in dim room light.

Two results suggest that the 31Kda protein, which is present in plasma membrane fractions, is of horizontal cell origin. Homogenates enriched for horizontal cells show increased amounts of the 31Kda phosphoprotein compared with homogenates enriched for retinal cells other than horizontal cells. In addition, application of the β-adrenergic agonist isoproterenol (100μM), which increases cAMP levels in the retina but not in horizontal cells, does not phosphorylate the 31Kda protein.

Taken together these results suggest that the 31Kda protein resides in the horizontal cell plasma membrane, and is a target of dopamine's neuromodulatory effects. This protein may be a gap junctional protein, or a protein involved in the regulation of gap junctions. Future experiments will focus on more precise identification of the cellular origin of this protein and its role in modifying electrical coupling.

12.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF CYCLIC AMP IN THE TELEOST RETINA: EFFECTS OF DOPAMINE AND PROLONGED DARKNESS. L.H.Y. Young* and J.E. Dowling. (SPON: A. Adolph) Mass. Eye and Ear Infirmary, Boston, MA 02114 and The Biological Laboratories, Harvard University, Cambridge, MA 02138.

In the fish retina, dopaminergic interplexiform cells serve as centrifugal neurons; they receive input from amacrine cells in the inner plexiform layer and they synapse onto cone horizontal cells in the outer plexiform layer. Horizontal cells are inhibitory interneurons that mediate the antagonistic surround responses of bipolar cells and cone photoreceptors. Many studies have indicated that the lateral inhibitory effects in the outer plexiform layer are modulated by the interplexiform cells and dopamine; however, these studies do not provide any information concerning the mechanisms underlying the effects of dopamine on horizontal cells. In 1981, using enriched horizontal cell fractions prepared from Ficoll sedimentation gradients, Van Buskirk and Dowling provided evidence that dopamine receptors on horizontal cells are linked to adenylate cyclase.

The present study provides information on the immunohistochemical localization of cyclic AMP in the white perch retina. Using a goat anti-cyclic AMP antiserum (gift from Dr. D. O'Brien) with an indirect immunofluorescent staining technique, staining was localized mostly to the outer nuclear layer, proximal inner nuclear layer and ganglion cells in retinas dark-adapted for 30 min. In the distal inner nuclear layer where the cone horizontal cells are located, only faint staining was noted in these short term dark-adapted retinas. Immunofluorescent staining of retinas preincubated for 10 min with 2 mM IBMX followed by a 15 min incubation with 200 uM dopamine showed a marked increase in staining in the distal inner nuclear layer, i.e. in the region of the horizontal cells. Similarly, though to a lesser extent, there was increased staining in this layer following prolonged (> 2 hours) dark adaptation. The staining was blocked by preabsorbing the antiserum with cyclic AMP (Sigma) indicating that the staining was specific to cyclic AMP. These experiments directly localize cyclic AMP to the region of horizontal cells in the fish retina and they provide further evidence that both dopamine and prolonged darkness promote the accumulation of cyclic AMP in the horizontal cells.

12.8 **MODULATION OF CONE INPUTS TO HORIZONTAL CELLS IN THE TIGER SALAMANDER RETINA.** Xiong-Li Yang * and Samuel M. Wu (SPON: W. Strittmatter). Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

We have investigated the effects of illumination on the synaptic inputs from photoreceptors to horizontal cells (HCs) in the tiger salamander (Ambystoma tigrinum) retina after prolonged dark adaptation (at least 2 hours). Under dark-adapted conditions, HCs receive inputs from both rods and cones. The HC response to 500 nm test flash was predominantly mediated by the rods and that to 700 nm flash was predominantly mediated by the cones. In the presence of dim background illumination, the HC response to 700 nm flash was larger and faster than that recorded under dark-adapted condition. This response enhancement occurred when the background light preceded the test flash by 50 msec and the response amplitude reached its maximum level 2-5 seconds after the onset of the background illumination. The response amplitude in HCs increased with the degree of hyperpolarization in rods. These results suggest that cone signals in HCs are probably suppressed by the rods in darkness, and background illumination reduces this suppression and results in an enhancement of cone signals in the HCs. In addition to the response enhancement resulted from background illumination, HC response to 700 nm flash became larger and faster after the cessation of prolonged light exposure. In contrast to the short-term response enhancement resulted from dim background illumination, this long-term response enhancement in HC required exposure of brighter light for at least 10 minutes to reach its maximum amplitude. It is likely that a cone-mediated mechanism in the tiger salamander retina is responsible for the long-term enhancement of the cone signals in the HCs.

This work is supported by grants from the National Institutes of Health (EY04446) and from the Retina Research Foundation (Houston).

12.9 GREEN-LIGHT MODULATES THE COUPLING BETWEEN NON-UNIVARIANT MONOPHASIC HORIZONTAL CELLS IN CARP (*Cyprinus carpio*) RETINA. M. Kamermans*, B. v. Dijk*, R.C.V.J. Zweypfenning* and H. Spekreijse*. (SPON: European Neuroscience Association) Lab. of Medical Physics, Univ. of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam-Zuidoost, The Netherlands.

In carp retina, two types of monophasic horizontal cells have been described (Dijk B. van, thesis Univ. of Amsterdam, 1985): the Univariant Monophasic Horizontal Cells (UMHC) and the Non-univariant Monophasic Horizontal Cells (NMHC). UMHCs receive input from red-sensitive cones exclusively, while NMHCs receive input from both red-sensitive and green-sensitive cones. Experiments were designed to reveal the nature of the input to the NMHCs from the green-sensitive cones.

Two types of experiments were used:

A) The dependence of the response amplitude on test spotdiameter was compared to the dependence of the response amplitude on the position of a slit projected in the receptive field.

B) The influence of a steady red and of a steady green background on the responses to red and green test flashes were recorded.

The results found can be explained only by assuming that the resistance of the GAP-junctions between the NMHCs was increased by the green-sensitive cone system. At high intensities of a green background, receptive field profiles will be smaller and feedback more pronounced.

Conclusions: A) The receptive field organization in the horizontal cell layer is not static but changes with the adaptation state.

B) The green-sensitive cone system can modulate the coupling resistance of NMHCs.

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12.10 PDA AND BICUCULLINE RELEASE ^{3}H-DOPAMINE IN THE TURTLE RETINA. S.D. Critz and R.E. Marc, Sensory Sciences Ctr., Graduate School Biomedical Sciences, Univ. of Texas, Houston, Texas. 77030.

The circuitry of dopaminergic cells in the retina of the red-eared turtle, Pseudemys scripta elegans, is being studied by observation of the effects of putative neuro-active substances on dopamine release. Eyecups from turtles (lighting regimen: 12 hr. dark/12 hr. light) were removed in dim red light 4 hrs. after light onset, placed in an experimental chamber in darkness, and perfused with turtle bicarbonate buffer (110 mM NaCl, 22 mM $NaHCO_3$, 2.5 mM KCl, 2.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 5 mM glucose, 0.025 mM pargyline, and 0.010 mM ascorbate) until stable ERG's were observed. The eyecup was incubated in ^{3}H-dopamine (0.2 uM) for 12 mins. and perfusion resumed to washout label not taken up by the cells. After 1-1.5 hrs. putative retinal transmitters, their antagonists, and other bioactive substances were added to the perfusate and effects determined by liquid scintillation counting of collected fractions.

The glutamate antagonist PDA, (2,3-piperidine dicarboxylic acid) has a potent effect on ^{3}H-dopamine release. The concentration used, 4 mM, is reported to block the response of hyperpolarizing bipolar cells to light (Slaughter, M.M. and R.F. Miller, Science, 219:1230-32, 1983) and thus is in the physiological range. Furthermore this dose is required to abolish the "d" wave of the ERG. Other glutamate analogs: APB (20 uM) and kainate (100 uM) had no effect on dopamine release.

The GABA antagonist, bicuculline (100 uM) caused release of dopamine in turtle, similar to results reported in goldfish (O'Conner et al., J. Neurosci., 6:1857-65, 1986). However, this release appears to be calcium-independent.

Other substances tested: acetycholine, carbachol, strychnine, GABA, muscimol, nipecotic acid, and exogenous dopamine, showed no effects on dopamine release.

These findings support a proposed tonic inhibition of dopaminergic cells by GABA (Marshburn, P.B. and P.M. Iuvone, Brain Res., 214:335-47, 1981), and suggest an excitatory input on certain GABA amacrine cells.

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12.11 PROLONGED DEPOLARIZING RESPONSES IN TURTLE CONES EVOKED BY STIMULATION OF THE RECEPTIVE FIELD SURROUND.
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Intracellular recordings were made from red- and green-sensitive cone photoreceptors in the retina of the turtle (Psuedymys scripta elegans) , maintained via a recirculating, modified tissue culture medium. Depolarizing influences arising from the surround of the cone's receptive field were isolated and studied in quantitative detail by stimulating the retina with annular flashes in the presence or absence of steady central spots. Two prominent types of responses were observed: 1) a prolonged depolarization which apparently has not been reported heretofore and 2) depolarizing spikes. The spikes, resembling those described by Piccolino and Gerschenfeld [Proc. Roy. Soc. Lond. B 206, 1980], were 200-300 msec in duration and not strictly all-or-none. With increasing annulus intensity, the amplitude of the spike response increased abruptly over a narrow intensity range. At somewhat higher intensities, the spike was followed by, and apparently triggered, a prolonged depolarization of 15-20 mv in amplitude.

In response to either short (0.4 sec) or long (20 sec) flashes, the prolonged depolarization was maintained at a level above the cone's resting membrane potential for some 5-10 sec and then abruptly returned to the level which prevailed prior to the flash. Injection of a brief depolarizing current pulse (0.01 to 0.1 nA) evoked a prolonged depolarization virtually identical to that evoked by light, thus showing that the response is intrinsic to the cone cell. Whether evoked by light or current, the prolonged depolarization markedly attenuates the cone's response to an incremental light flash and also has a remarkably long refractory period of 1-2 min during which neither current nor light can reinstate a full-blown depolarization.

These and additional findings have implications for the role of surround antagonism in photoreceptor mechanisms of light adaptation and color opponency.

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12.12 THE ROLE OF HORIZONTAL CELL COUPLING FOR SUPPRESSIVE ROD-CONE INTERACTION.
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Diffuse, rod-stimulating background fields markedly enhance cone-mediated responses to spatially focal, rapidly flickering stimuli. We have called this effect suppressive rod-cone interaction (SRCI) and have documented it by means of intracellular recording from cones and many second and third order neurons, and by means of ERG and psychophysical procedures. Last year at these meetings, we reported that SRCI is blocked by pharmacological agents which block photic responses of horizontal cells (HCs). In the present study, we examined the influence of HC electrical coupling on SRCI by applying the analysis of Lamb (1976). In these experiments, we either recorded intracellularly from an amphibian cone or second order neuron, or we used psychophysical procedures in human subjects: we assessed the influence of the size, shape, and retinal position of both stimuli upon the extent of SRCI. We assume that the background field acts by decreasing a tonic rod-inhibitory dark signal which is mediated by horizontal cells (HCs), and the spatial limitations for SRCI reflect the space constant of the electrically coupled HC network. Regardless of species and specific experimental procedures, our analysis suggests that SRCI is determined by a mechanism with a space constant of 400-550 um. In amphibian neurons, this value is often larger than that directly determined with a cone-stimulating slit in a recorded neuron: but this range agrees with that reported for HC axon terminals, the cellular process most likely to feedback onto cones.

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12.13 MOLECULAR CHARACTERIZATION OF GENES REQUIRED FOR DROSOPHILA PHOTOTRANSDUCTION.
C. Montell,* C. Zuker* and G. Rubin. Biochemistry Dept., Univerity of California, Berkeley, CA 94720.

The fruitfly, *Drosophila melanogaster*, is an excellent system in which to both study the roles of previously identified photoreceptor cell proteins and to isolate and characterize the roles of genes and proteins important in phototransduction that have not yet been identified in any metazoan system. There are two important advantages of studying phototransduction in the fruitly. 1) Many of the relevant genes have already been genetically identified. 2) DNA sequences can be stably and efficiently introduced into the genome by P-element mediated germline transformation. Therefore, it is possible to identify the DNA sequence encoding a genetically defined locus by complementing the mutant phenotype following introduction of the gene into the genome by germline transformation. We are interested in identifying the DNA sequences encoding these phototransduction genes and characterizing the roles of the coresponding proteins.

Using the above approach, we have identified two genes, *trp* (Montell, C. et al., *Science*, 230:1040, 1985) and *ninaC*, by complementing the mutant phenotypes. Both genes are expressed beginning late in development and encode proteins localized specifically to the photoreceptor cells. The *ninaC* proteins share significant homologies to two types of proteins which define two nonoverlapping domains. One domain shares amino acid homology to protein kinases and the other to all of the globular head region of the myosin heavy chain. The *trp* protein is not homologous to previously sequenced proteins but displays a number of very striking internal repeats. Continuing studies directed at identifying the specific functions of these proteins will be described.

Using a different approach, we have identified two opsin genes, rh3 and rh4, expressed specifically in the R7 ultraviolet sensitive subset of photoreceptor cells (Zuker, C. et al., *J. Neurosci.*, in press; Montell, C. et al., *J. Neurosci.*, in press). Thus, a total of four related Drosophila opsin genes have now been isolated.

13.1 A DIRECT CONNECTION BETWEEN THE BASAL GANGLIA AND THE NUCLEUS BASALIS: A TRACT-TRACING STUDY IN HUMAN POSTMORTEM BRAIN. S.N. Haber. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Previous studies based on tracing techniques and histochemical methods have suggested a direct connection betwen the nucleus accumbens and the nucleus basalis (Haber, Neurosci. Abstr., 10:7, 1984; Grove et al, Brain Res. 367:379, 1986). Recently, using immunohistochemical methods to identify enkephalin-containing striatal efferent fibers projecting to the ventral pallidum and acetylcholinesterase staining to identify neurons of the basal nucleus, the overlap of these systems were studied in the human and nonhuman primate forebrain. The results indicate that the two systems tend, anatomically, to avoid each other, with the exception of a particular subcommissural region in which a precise overlap occurs (Haber, PNAS, 84:1408). The study presented here reports on the use of the tracer molecule, wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) in postmortem human basal forebrain to study a direct connection between the basal ganglia and the nucleus basalis.

Blocks of human basal ganglia, approximately 5 mm thick, were removed at autopsy and injected with 0.5-1.0 ul of WGA-HRP, (20% in tris buffer, pH 8.6) using a glass pipette with a 30 um inside diameter tip. Injections were directed into the nucleus accumbens. Blocks were incubated for 24 hr. in calcium-low phosphate buffer, pH 7.2 at 37°C. The tissue was fixed by immersion in 2% paraformaldehyde, 1.25% gluteraldehyde in phosphate buffer and cryoprotected in phosphate buffer with increasing concentrations of sucrose (10%, 20%, 30%). Each block was cut (50um) on a freezing microtome and sections were stained with the TMB method of Mesulam (J. Histochem. Cytochem. 26:106), which were then stabilized with a solution containing 0.05% DAB, 1% colbalt 1% chloride, 1% nickel ammonium sulfate and 0.3% H_2O_2. Several compartments were then counterstained for acetylcholinesterase using the Genser-Jensen and Blackstad method.

Injections into the nucleus accumbens showed fibers leaving the injection site and forming bundles as they traveled into the region of the basal forebrain. Individual fibers could be followed as far as 7 mm. In sections countered stained for acetylcholinesterase, HRP fibers could be seen to traverse individual cholinesterase-positive neurons. These results suggest at the light microscopic level, a direct interaction between the nucleus accumbens and the cholinergic neurons of the basal forebrain may exist in the human forebrain.

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13.2 CELLULAR LOCALIZATION OF CALBINDIN-D_{28K} IN RAT AND MONKEY BASAL GANGLIA. N. Aronin, S. Christakos*, and M. DiFiglia. Univ. Mass. Med. Sch., Worcester, MA 01655, New Jersey Med. Sch., Newark, NJ 07103, and Mass. General Hosp., Boston, MA 02114

Calbindin is a 28,000 M.W. protein found in high concentrations in the mammalian kidney and brain. Regulation of renal calbindin-D_{28K} content is 1,25$(OH)_2$-Vitamin D dependent. Brain and kidney calbindins-D_{28K} are physicochemically identical by peptide mapping and chromatography. Function of calbindin-D_{28K} is unknown. We examined the immunocytochemical localization of calbindin-D_{28K} in the rat and monkey basal ganglia. Anti-calbindin-D_{28K} antiserum was generated in rabbit against purified calbindin from rat kidney. This antiserum crossreacts equally with brain and kidney calbindin-D_{28K}. Brain sections from paraformaldehyde/glutaraldehyde perfused rats(n=7) and monkeys(n=3) were incubated 24-72h with primary antiserum (1:1000-1:1600 dilution), processed with the biotin-avidin method, and treated with diaminobenzidine in H_2O_2. Specific cell labeling was absent with omission of primary antiserum and with primary antiserum preadsorbed with purified calbindin-D_{28K}.

In the neostriatum at the light microscopic level, numerous medium sized cells and their emerging processes were darkly stained. Nuclear staining often appeared darker than cytoplasmic labeling. The caudate neuropil exhibited a dense distribution of immunoreactive processes and punctate elements. Regions of the neostriatum with sparse labeling were also observed. In both species at the ultrastructural level, reaction product was distributed primarily in somata with unindented nuclei and in spiny dendrites. Particularly prominent was the dense immunoreactivity for calbindin-D_{28K} in numerous dendritic spines throughout the neuropil. Axon terminals were also labeled but infrequently. Immunoreactive terminals were filled with round and ovoid vesicles and formed symmetric synapses with unlabeled spines. At the light microscopic level, the globus pallidus exhibited immunoreactive fibers, which in some locations ensheathed unlabeled pallidal dendrites. Large darkly labeled cells were found in the internal and external laminae of the monkey globus pallidus and were continuous with large labeled neurons of the basal nucleus (Meynert). Immunoreactive terminals synapsed with unlabeled pallidal somata and dendrites.

In conclusion, these findings indicate that calbindin-D_{28K} is localized to medium sized spiny neurons in the neostriatum. Its distribution within the basal ganglia is consistent with a striato-pallidal projection of calbindin-D_{28K} containing cells. The prominent immunocytochemical localization of calbindin-D_{28K} immunoreactivity in dendritic spines, which receive the majority of synaptic inputs in the neostriatum, indicates that this protein may play a role in post-synaptic events.

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13.3 THE DISTRIBUTION AND STRUCTURE OF LABELED NEURONS AND AXON TERMINALS IN IMMUNOCYTOCHEMICALLY DEFINED STRIATAL, PERICOMMISSURAL AND SUBCOMMISSURAL FOREBRAIN REGIONS IN THE RAT FOLLOWING INJECTIONS OF WGA-HRP IN THE MIDBRAIN VENTRAL TEGMENTAL AREA: LM OF THE TMB PRODUCT AND EM/GOLGI-EM OF ITS DAB-CO++ STABILIZED COUNTERPART. D.S. Zahm. Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

The mesencephalic ventral tegmental area of pentobarbital anesthetized, male S-D rats weighing 225-250g was injected iontophoretically (1-2μA, 7s on - 7s off, 10-20 min) or by pressure (10-15nl, 1%) with HRP conjugated to wheat germ agglutinin (WGA). After 24-48 hours the animals were sacrificed under anesthesia and vibratome sections (50μm) through the forebrain were cut and exposed either to TMB and H_2O_2 in acetate buffer at pH 3.5 (Mesulam, 1978) or to those reactants at pH 5.0 and subsequently to a DAB-Co++-H_2O_2 solution at pH 7.4 (Rye et al., 1984). The former sections were mounted for LM. The latter were further processed for immunocytochemistry (IR) using antisera against substance P (SP) or neurotensin (NT) with DAB as chromagen, or, following osmication, were processed for Golgi according to the method of Freund and Somogyi (1982) or between glass slides as recommended by Izzo et al. (1987), or were embedded for EM without further processing. The distribution of labeled terminals corresponded to published reports (e.g. Beckstead et al., 1979). Retrogradely labeled neurons were most numerous in medial nucleus accumbens, and in a broad subcommissural forebrain area extending from posteromedial parts of the olfactory tubercle through the lateral hypothalamus. The subcommissural group of labeled neurons was centered where ventral pallidum (VP), the lateral division of the bed necleus of stria terminalis (BST) and the lateral preoptic area abut. That tegmentopetal neurons were numerous in each of these structures was confirmed by defining the boundaries of VP using SP-IR and those of the BST with NT-IR in sections containing retrogradely labeled cells or in adjacent sections. Golgi impregnation showed that retrogradely labeled, Gogli-impregnated neurons in the n. accumbens were medium spiny neurons. At this writing double labeled neurons have not been achieved in the subcommissural region, but LM and conventional EM indicate that labeled neurons and proximal dendrites are medium sized (15-20μm), spindle-shaped, and synapse-poor, in contrast to the synapse-rich ventral pallidal neurons, whose territory they invade. To avoid confusing the local collaterals of retrogradely labeled neurons with anterogradely labeled terminals following these injections, the latter, to date, have been studied only in ventromedial caudate-putamen, where labeled neurons were absent. Labeled terminals in this region contacted dendrite shafts with symmetrical specializations. Support: NS-23805 and the American Parkinson Diseáse Association.

13.4 NEUROCHEMICAL COMPARTMENTALIZATION OF THE SUBSTANTIA NIGRA PARS COMPACTA IN THE PRIMATE. J.JIMENEZ-CASTELLANOS* AND A.M.GRAYBIEL. Dept. Brain and Cognitive Sci., Mass.Inst.Tech.,Cambridge, MA 02139

Cross-sections through the squirrel monkey's substantia nigra pars compacta (SNpc) were stained for acetylcholinesterase (AChE) activity, for tyrosine hydroxylase (TH)-like immunoreactivity, for Nissl substance, or for tetramethylbenzidine (TMB) reaction product following injection of WGA-HRP into the caudate nucleus or putamen (with or without accompanying fluoro-gold injections). Both with AChE and with TH histochemistries, discrete compartmentalization of the SNpc was observed. There were striking instances in which the histochemical compartments corresponded to cytoarchitectonic specializations seen in Nissl and TMB-stained sections.

In AChE-stained sections at mid-nigral levels, finger-like zones of low AChE activity appear in the SN in an otherwise AChE positive neuropil. Some zones of heightened AChE staining are also present. The AChE-poor fingers extend ventrally from a horizontal tier which is darkly stained but itself is divided into a lateral pale part and a medial darker part. Patches of lower and higher AChE staining occur within both parts. In TH-immunostained sections, this horizontal tier of the SNpc is sharply divided into a medial half with densely packed TH-positive cells and neuropil, and a lateral half in which TH-positive cell bodies and neuropil are more sparsely distributed. Both the medial and the lateral halves have prominent TH-positive ventral extensions, some but not all corresponding to AChE-poor fingers. The horizontal tier of the SNpc is readily identifiable in Nissl-stained sections. Its medial half forms a discrete band of densely packed neurons broken up into clusters of more darkly and less darkly stained neurons. The lateral half contains more scattered neurons. Ventrally-extending fingers are not as prominent as in the histochemical preparations. Dorsal to the SNpc proper is a tier of pale TH-immunostaining corresponding to the substantia nigra pars mixta (SNpm). The SNpm shows scarcely any staining. Cell group A8, extending caudally from this zone, has stronger AChE activity.

In the retrograde experiments, SNpm was labeled only after a tracer injection in the ventral caudoputamen. In SNpc there were alternating clusters of labeled neurons in the horizontal tier following injections into either caudate nucleus or putamen (cf. Parent et al. '83) and there were instances in which these were aligned with the lighter or darker patches, respectively, of the Nissl preparations. TMB-labeled neurons and axons also appeared in ventrally extending fingers. Cells in the AChE-poor fingers were labeled both after single putamen injections (3 cases) and single caudate nucleus injections (1 case) but no doubly-labeled neurons were found after combined caudate nucleus-putamen injection. Supported by the Seaver Institute, the McKnight Foundation and NSF BNS 8319547.

13.5 HALOPERIDOL-SENSITIVE SIGMA RECEPTORS IN THE SUBSTANTIA NIGRA PARS COMPACTA: AUTORADIOGRAPHIC EVIDENCE FOR SPECIFIC ANATOMICAL LOCALIZATION of [3H] DTG BINDING SITES. A.M. Graybiel, E. Weber, M-J. Besson and K. Karuzis* (SPON: H. Newman-Gage). Dept. Brain and Cog. Sciences, Mass.Inst.Tech., Cambridge, MA 02139

Findings in anterograde tracer experiments suggest that the dopamine-containing cell groups of the cat's midbrain are divided up according to their differential efferent projections to the striosomal and extrastriosomal matrix subdivisions of the striatum (Jimenez-Castellanos and Graybiel '85, '86). The TH-rich densocellular zone of the substantia nigra pars compacta (SNpc) projects strongly to striosomes. Cell group A8 and the cell-sparse zone of the SNpc project more strongly to the extracellular matrix. We report here autoradiographic evidence suggesting that the striosome-projecting densocellular zone of SNpc is the main nigral locus of binding sites labeled with the sigma selective ligand [3H]-1,3 di-o-tolylguanidine ([3H]DTG).

Autoradiographic experiments were carried out on transverse sections cut from unfixed rapidly frozen blocks taken from the cat brainstem under conditions of deep barbiturate anesthesia. [3H]DTG binding was performed at room temperature for 45 min in 2nM [3H]DTG (specific activity 52 Ci/mmol). Non-specific binding was determined by incubation in the presence of a 10^{-5} M haloperidol. Autoradiographic [3H]DTG binding patterns were studied after 1-2 month exposure times and were compared with patterns of TH immunostaining, of AChE staining, and of [3H]SCH23390 and [3H]naloxone binding visible in adjacent or nearly adjacent sections and, for AChE, in some of the same sections stained after autoradiographic exposures were complete.

[3H]DTG binding was generally low in the pars reticulata of the substantia nigra (SNpr), in cell groups A10 and A8 and in the cell-sparse zone of SNpc. By contrast, dense [3H]DTG binding appeared in a restricted caudomedial band of SNpc that corresponded to the TH-rich, AChE poor densocellular zone of SNpc observed in adjacent sections. Binding was nil in control sections incubated in the presence of 10^{-5} M haloperidol. Very different binding patterns were found following incubations with the D1-selective ligand [3H]SCH 23390 and with the opiate receptor-selective ligand [3H]naloxone. In neither case was the densocellular zone of SNpc preferentially labeled.

This anatomical localization of [3H]DTG binding raises the possibility that haloperidol-sensitive sigma receptors might modulate dopamine release in the SNpc and that such effects might be targeted particularly toward the striosomal system. If so, such specificity could be important in understanding the neural basis of sigma-active antipsychotic and psychotomimetic drugs. Supported by the Seaver Institute, the McKnight Foundation and NSF BNS 8319547.

13.6 A CHOLINERGIC PROJECTION FROM THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS TO THE SUBSTANTIA NIGRA IN THE RAT: A LIGHT AND ELECTRON MICROSCOPIC IMMUNOHISTOCHEMICAL STUDY. M.Beninato*, and R.F. Spencer. Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298-0001.

Putative cholinergic afferents of the rat substantia nigra (SN) were identified by double-labeling with retrograde transport of horseradish peroxidase (HRP) combined with the immunocytochemical localization of choline acetyltransferase (ChAT). The cholinergic projection to the SN originates from neurons located predominantly in the ipsilateral and rostral portions of the pedunculopontine tegmental nucleus (PPN). Other cholinergic neurons that were also retrogradely-labeled with HRP were located in the caudal PPN and lateral dorsal tegmental nucleus (LDTN). A non-cholinergic projection from the PPN was also identified. These findings indicate that the PPN, and to a lesser extent, LDTN are extrinsic sources of acetylcholine to the basal ganglia (cf. N.J. Woolf and L.L. Butcher, Brain Res. Bull., 16:603-637, 1987).

ChAT-immunoreactive axons and synaptic endings were demonstrated in the SN by light and electron microscopy. The distribution of ChAT-immunoreactivity in the was compared to that of acetylcholinesterase (AChE), demonstrated histochemically, and tyrosine hydroxylase (TH) immunoreactivity. Both AChE and TH-immunoreactivity were confined to the pars compacta of the SN (SNc) and SNc dendrites extending into the pars reticulata (SNr). The distribution of ChAT immunoreactivity in the SN as demonstrated by light microscopy revealed a modest network of ChAT-immunoreactive beaded axons in the SNc, as defined by AChE and TH localization, in comparison to a relatively sparse distribution to the SNr. These axonal profiles were most dense in the middle of the rostral-caudal extent of the SNc and appeared to be concentrated in the middle third of the medial-lateral extent. These findings are consistent with those recently described in the ferret (Z. Henderson and S.A. Greenfield, Neurosci. Lett., 73:109-113, 1987). Electron microscopic examination of ChAT-immunoreactive profiles in the SN was confined to the SNc. Unmyelinated small diameter (0.25 μm) ChAT-immunoreactive axons were observed interspersed among numerous other non-immunoreactive axons. ChAT-immunoreactive synaptic endings in juxtaposition to small caliber (0.5 μm) non-immunoreactive dendrites contained numerous spheriodal synaptic vesicles and occasional mitochondria. Synaptic contact zones were characterized by an accumulation of synaptic vesicles along the presynaptic membrane, and a prominent postsynaptic densification producing an asymmetrical pre-/postsynaptic membrane profile typical of excitatory synapses.

These findings provide direct evidence for a cholinergic innervation of the SN, and suggest that this input may have an excitatory effect on neuronal elements in the SNc.

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13.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR TO DISTINCT SUBSETS OF MIDBRAIN DOPAMINE NEURONS. A.Y. Deutch, M. Goldstein, J. Holliday*, R.H. Roth and E. Hawrot. Dept. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510, and NYU Sch. of Med., New York, NY 10016

Presynaptic regulation of striatal dopamine (DA) release via the nicotinic acetylcholine receptor (NAChR) has been extensively characterized. Implicit in the concept of NAChR presynaptic regulation of DA release from striatal afferents originating in the midbrain is the premise that the NAChR is synthesized within DA neurons of the substantia nigra and axonally transported to the terminal. In situ hybridization histochemistry studies have revealed that the α-3 gene encoding the neuronal α subunit of the putative presynaptic NAChR is present in the ventral tegmental area (VTA) and the substantia nigra, pars compacta (SNpc; Goldman et al., Cell 48:965, 1987). We now report the immunohistochemical localization of the NAChR to distinct subsets of midbrain DA neurons. Monoclonal antibodies (mAb) were generated against purifed Torpedo NAChR. Binding competition studies revealed that one mAb designated 35.74 interacts with the main immunogenic region of the NAChR. Anatomical localization of the NAChR in rat brain was undertaken using this antibody. mAb 35.74 staining was heterogeneously distributed in rat brain and paralleled the reported distribution of (3H)-nicotine binding sites, including the deep layers of the cerebral cortex, anteroventral thalamic nucleus, dentate gyrus, medial habenula and its efferent targets (interpeduncular nucleus and median raphe), and a continuum of cells across the ventral mesencephalon corresponding in location to the A8 (retrorubral field, RRF), A9 (SN), and A10 (VTA) DA cell groups. In some regions, including the ventrolateral medial habenula and the ventral midbrain, intense cytoplasmic staining of neurons and processes was observed. Ventral mesencephalic neurons stained with mAb 35.74 corresponded in morphology to the tyrosine hydroxylase (TH)-positive neurons of the A8-A10 cell groups. 6-hydroxydopamine lesions completely abolished mAb 35.74 staining of SNpc DA neurons. Further immunohistochemical studies revealed that while most of the SNpc DA neurons stained for the NAChR, certain other midbrain DA neurons did not. DA neurons in both the medial aspects of the VTA (the nuc. interfascicularis, rostral linear and central linear nuclei, and dorsal extension of TH-positive neurons to the cerebral aqueduct) and the ventral diffuse portion of the A8 cell group were not stained with mAb 35.74. Thus, the NAChR appears to be synthesized in discrete subsets of midbrain DA neurons. These data suggest that ACh may regulate the functional activity of distinct subsets of midbrain DA neurons via interactions with nicotinic AChR, and may provide clues to the sites where nicotine may exert its behavior rewarding properties. Supported in part by MH14092, GM32629, APDA, MDA, TSA, and the AHA.

13.8 SYNAPTOLOGY OF IMMUNOCYTOCHEMICALLY-IDENTIFIED GABAERGIC AND ENKEPHALINERGIC TERMINALS IN MONKEY SUBSTANTIA NIGRA. Gay R. Holstein and Pedro Pasik. Depts. of Neurology and Anatomy, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Observations of immunocytochemically-identified GABAergic elements in the substantia nigra (SN) from 2 adolescent (1 M. mulatta and 1 M. fascicularis) and one 4-week-old monkey (M. mulatta) were performed on 50 μm vibratome sections which were incubated in a GABA antiserum and processed with the PAP technique. A fourth monkey (M. fascicularis) was studied by a preembedding double label immunocytochemical method where the same procedure as above was combined with an anti-leu enkephalin (ENK) ascites fluid and the avidin-biotin complex technique with avidin-ferritin conjugate as the marker. Similar results were obtained in all subjects treated alike, and no immunostaining was observed in control sections.

Using serial section electron microscopy, a differential distribution of GABAergic inputs to SN cell bodies was apparent. GABAergic boutons occasionally were observed to contact GABA-immunoreactive somata, primarily forming symmetric synapses, whereas such input to GABA-negative cell bodies was rare. A preliminary analysis of the double labeled material revealed that some of the myelinated and unmyelinated GABA-positive fibers were leu-ENK-immunoreactive as well. Boutons which were labeled for both of these substances were also present. These elements contained densely packed vesicles and formed synaptic contacts with dendrites of SN neurons. In addition, some axonal and dendritic profiles showed the GABA immunostain only.

The observations using single label immunocytochemistry, together with our previous report on the pattern of dendritic innervation by GABA-immunoreactive boutons (Neurosci. Lett., 1986) suggest that the GABAergic input to GABA-positive elements in SN is sparse and more uniformly distributed to dendrites and somata. In contrast, GABA-negative elements throughout SN receive a more dense GABAergic input, which is restricted to the dendritic field. The results of the double label procedure indicate that at least a subpopulation of the GABA-positive boutons in SN, namely those profiles with small dark mitochondria and densely packed vesicles, may also contain leu-ENK, and form synapses with neuronal elements in this structure. These boutons resemble the extrinsic afferent terminals which take origin in the neostriatum, pallidum and nucleus accumbens septi.

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13.9 NADPH-DIAPHORASE POSITIVE NEURONS IN THE STRIATUM CONTAIN THE MESSENGER RNA (mRNA) FOR SOMATOSTATIN BUT NOT FOR GLUTAMIC ACID DECARBOXYLASE (GAD): AN IN SITU HYBRIDIZATION STUDY.
M.-F. Chesselet and Elaine Robbins* The Medical College of Pennsylvania, 3200 Henry Ave., Philadelphia, PA 19129.

The presence of a high level of NADPH-diaphorase activity characterizes a subset of striatal neurons resistant to a variety of neuronal injuries; in particular, these neurons are spared in local injections of quinolinic acid and in the striatum of patients with Huntington's Disease. Striatal NADPH-diaphorase positive neurons contain somatostatin-like immunoreactivity, suggesting that they are medium aspiny interneurons with an indented nucleus, characteristics described for somatostatin-positive cells. Striatal neurons exhibiting similar density and morphological features as the somatostatinergic neurons have recently been shown to express significantly higher levels of GABA and GAD like immunoreactivities as well as of GAD mRNA than the more numerous GABA-ergic striatal efferent neurons. It is unclear, however, whether somatostatin and GABA coexist in these neurons or whether this subset of striatal GABA-ergic cells form a distinct neuronal population.

In the present study, we sought to confirm that the neuropeptide somatostatin can be synthesized in the NADPH-diaphorase positive neurons of the striatum by demonstrating the presence of the mRNA for preprosomatostatin in these neurons by in situ hybridization cytochemistry. In addition, we have examined whether these neurons also express high levels of GAD mRNA. Frozen sections of rat brain were fixed in paraformaldehyde, stained for NADPH-diaphorase activity, acetylated, dehydrated, and hybridized with ^{35}S-labelled RNA probes transcribed from cDNA coding for preprosomatostatin (R. Goodman, Tuft U.) and GAD (A. Tobin, UCLA). After post-hybridization washes and treatment with RNAse, the sections were dehydrated and processed for autoradiography using Kodak emulsion. An intense labelling was observed with the preprosomatostatin RNA probe in most NADPH-diaphorase positive neurons in the striatum. In contrast, striatal neurons labelled with GAD RNA did not express NADPH-diaphorase activity. The results suggest that NADPH-diaphorase neurons in the striatum produce somatostatin but not GAD, the enzyme necessary for the formation of GABA. In light of the available immunohistochemical data, this suggests that neurons engaged in intense GAD and GABA synthesis constitute a population of medium sized striatal interneurons distinct from previously described peptidergic interneurons. Supported by the Hereditary Disease Foundation, MH and BSN.

13.10 DOPAMINE RECEPTOR-MEDIATED REGULATION OF DOPAMINE TURNOVER IN RAT FETAL SUBSTANTIA NIGRA TRANSPLANTS.
R. Meloni*, N. Hayman* and K. Gale.(SPON: D.Stoff). Dept. of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007.

Rats receiving solid grafts of fetal substantia nigra placed into a cortical well over one striatum (previously denervated by 6-hydroxydopamine) and their non-transplanted (6-hydroxydopamine lesion and cortical well only) and non-lesioned (cortical well only) controls, were used to assess (by HPLC) levels of dopamine (DA) and its metabolites (DOPAC and HVA) in different regions of the striatum following haloperidol (HAL) (1 mg/kg ip 60 min before sacrifice). The transplant recipient rats, when tested 4 mo after transplantation, showed a 50-100% reduction in turning behavior to both apomorphine and amphetamine, as compared to the pre-transplant baseline, while the lesioned rats without transplant had a slightly elevated turning behavior.

The DA and DOPAC concentrations in the regions of host striata that were well-innervated by the grafts were 8-10 fold higher than those of the non-innervated regions of the same striata, where DA was less than 1% of normal.

Significant (*) effects of HAL on DOPAC/DA and HVA/DA ratios were as follows:

	STRIATUM ON TRANSPLANT SIDE		STRIATUM ON NON-OPERATED SIDE	
	DOPAC/DA	HVA/DA	DOPAC/DA	HVA/DA
SALINE	.54	.18	.26	.05
HALOPERIDOL	.98*	.32*	.61*	.19*
% INCREASE	81%	78%	134%	257%

These data indicate that although DA turnover is higher in the terminals of the grafts than in the non-lesioned striata, it is nevertheless subject to increase in response to DA receptor blockade by HAL. In addition, in HAL-treated rats the **transplant itself** showed a significant (*) increase (by 100%) in both the DOPAC/DA and HVA/DA ratios: from 0.21 to 0.43* and from 0.05 to 0.10* respectively, indicating that DA turnover **intrinsic** to the transplant is also subject to regulation by DA receptors. The proportional increases induced by HAL in metabolite/DA ratios associated with the transplant and its projections were not as great as those observed in the intact striata, indicating either that 1) the impact of DA receptor blockade on transplant DA turnover is less than that on the intact nigrostriatal circuitry, or 2) the apparent rate of accumulation of metabolites may be altered by differences in acid transport in the vicinity of the transplant.

Our data suggest that the transplant is not acting only as a "reservoir" releasing DA at a constant rate, but that feedback control of transplant DA utilization exists. In the event that **host** neurons may partecipate in this feedback regulation, this could allow for a dynamically active transplant, responsive to changes in host neural activity.

13.11 ELECTROPHYSIOLOGICAL EVIDENCE FOR THE FORMATION OF A CORTICOSTRIATAL PATHWAY IN NEOSTRIATAL TISSUE GRAFTS.
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Extracellular and intracellular recordings were used to study responses recorded from neurons within neostriatal grafts in rats. Grafts were made using cell suspensions from embryos of gestational age between 15 and 18 days. The neostriatum of young adult recipients of the same strain was damaged unilaterally by injection of kainic acid or ibotenic acid to form a lesion approximately 1 mm in diameter. Between 3 and 6 days later an injection of suspended embryonic neostriatal tissue was made stereotaxically into the same position. Recording experiments were performed between 6 weeks and 6 months of surgery. The grafts produced in this way were very reliable in their placement in the neostriatum, making it possible to stereotaxically place recording electrodes into the graft. Stimulating electrodes were placed in the medial agranular field of the frontal cortex, which projects very heavily to the area of neostriatum into which the graft was placed. Animals were anesthetized with urethane and ketamine.

Recordings from the intact neostriatum show a characteristic pattern of initial excitation, inhibition, and rebound excitation following stimulation of the cerebral cortex. Recordings taken from within the graft showed this same pattern of excitation and inhibition. The initial excitatory phase of the response, which has previously been shown to be due to the monosynaptic excitatory effect of corticostriatal afferents, was reliably observed in neurons within the graft, and was normal in amplitude and duration. The inhibitory phase of the response, which is believed to result primarily from the removal of a powerful tonic excitatory synaptic input from the cortex, was also present in the graft.

Intracellular injection of HRP was used to localize neurons from which the recordings were made. Neurons stained in this way were observed to be located within the graft.

These findings indicate that neostriatal tissue grafts can receive an innervation from the cerebral cortex that is comparable to that of the normal neostriatum.

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13.12 ONE-YEAR SURVEY OF C.N.S. RECEPTOR ADAPTIVE RESPONSIVENESS IN WISTAR RATS BEARING UNILATERAL NIGRAL 6-OHDA LESIONS.
A. C. Rossi, G. Achilli*, C. Caccia*, M. Carpentieri*, M. A. Cervini*, R. Maj*, L. Pegrassi*and M. Buonamici*
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The time-course of the modifications of the main neurotransmission systems in male Wistar rats bearing unilateral nigral 6-OHDA lesions (Ungerstedt, U.et al.,Brain Res.,24: 485, 1970) is reported. The more relevant times for this analysis were chosen basing on the evolution of dopamine-agonist-induced rotational behavior (apomorphine: 0.5 mg/kg s.c.). Rotational behavior contralateral to the lesion does consistently appear one month after the lesion, increasing in intensity up to one year. Binding assays were performed with the following ligands: ^{3}H-SCH 23390, ^{3}H-spiperone and ^{3}H-QNB respectively for D-1, D-2 and muscarinic receptors in caudate-putamen (CP); ^{3}H-prazosin for α-1, ^{3}H-yohimbine for α-2 and ^{3}H-dihydroalprenolol for β receptors in sensory-motor cortex (SMC); ^{3}H-serotonin for S-1 receptors in hippocampus (HP). Dopamine (DA) and DOPAC, norepinephrine (NE), serotonin (5-HT) and 5-HIAA were simultaneously determined using reverse-phase HPLC with electrochemical detection. The results are expressed as % variations of the lesioned cerebral side as compared with the intact one. Only B_{max} values are reported no variations in K_D values being evident.

Area (receptors and transmitters)		% of contralateral values (= 100%) 1 month	3 months	12 months
CP	D-1	no change	+ 40	+ 30
	D-2	+ 15	+ 20	+ 15
	muscarinic	no change	no change	no change
	DA	- 97	- 94	- 95
	DOPAC	-100	- 80	- 80
SMC	α-1	+ 40	+ 24	+ 30
	α-2	no change	no change	no change
	β	+ 66	+ 20	-
	NE	- 90	- 80	- 85
HP	S-1	no change	no change	no change
	5-HIAA	no change	no change	no change

Our results indicate that this focal lesion leads to long-lasting and quite complex modifications of the considered cathecolaminergic systems in CP and SMC of the lesioned cerebral side which are consistent with findings reported for human brain tissues from parkinsonian patients (Cash, R., et al.,Brain Res.,322: 269, 1984).

13.13 STRAIN-DEPENDENT DIFFERENTIAL EFFECTS OF MONOAMINE OXIDASE INHIBITION ON DOPAMINERGIC NIGROSTRIATAL FUNCTIONING IN MICE. R. Bose* and C. Pinsky* (SPON: L. Wilson). Department of Pharmacology and Therapeutics, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3.

Invertebrates lacking active monoamine oxidases (MAO) metabolize biogenic amines by formation of gamma-glutamyl conjugates. These neuronally-inactive conjugates are present also in MAO-containing mammalian neurons. MAO inhibition speculatively could result in an increased degree of gamma-glutamylation of monoamines, thereby diverting glutamyl moieties from biosynthesis of glutathione and rendering the neurons vulnerable to free radical damage. We have examined the acute effects of clorgyline and pargyline, two MAO inhibitors of differential selectivity, in two strains of mice on dopamine-mediated vertical climbing activity (VCA). Also examined were the total glutathione and lipid peroxidation in striatum, cerebral cortex and cerebellum of these mice.

The selective MAO-A inhibitor clorgyline significantly decreased VCA in Swiss-Webster mice ($p \leq 0.01$) while significantly increasing VCA in the Belknap strain ($p \leq 0.05$). The much less selective agent, pargyline, did not alter VCA in Swiss-Webster but decreased VCA numerically in Belknap mice. In rodents, both serotonin and dopamine have been shown to be metabolized by MAO-A. Serotonin, being the preferred substrate for this enzyme, would be better protected and the resulting relative increase in serotoninergic tone would inhibit, by feedback, dopaminergic neurotransmission leading to the reduced VCA seen in Swiss-Webster mice. The Belknap strain, however, appears to differ in showing increased VCA following MAO-A inhibition, possibly due to preferential protection of dopamine or to a lack of serotoninergic inhibitory feedback in the basal ganglia. The reduced lipid peroxidation seen in the striatum of mice treated with MAO inhibitors may be a reflection of reduced H_2O_2 formation consequent on MAO inhibition. Total glutathione content was not significantly altered in the brain regions studied here. Glutamylation or other glutathione conjugate formation therefore does not seem likely as an alternative metabolic pathway for monoamines after acute treatment with MAO inhibitors in these two strains of mice. Further studies with repeated administrations of MAO inhibitors, corresponding to clinically effective regimens, are being carried out.

This study was supported by the United Parkinson Foundation (Chicago), Medical Research Council of Canada and the Centre on Aging of the University of Manitoba.

MONOAMINES AND BEHAVIOR I

14.1 ASPARTAME WITH CARBOHYDRATE FAILS TO AFFECT FOOD INTAKE, APPETITE MOTIVATION, MOOD, LEARNING AND BEHAVIOR IN CHILDREN. S. Saravis*. R. Schachar*. S. Zlotkin*. L. Leiter*, and G.H. Anderson. Hospital for Sick Children and Dept. of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada.

Aspartame (the methyl ester of phenylalanine and aspartate) is a sweetener in wide use in foods and drinks available to children. The objective of the present study was to test the hypothesis that aspartame combined with carbohydrate will induce neurobehavioral responses in children. A double-blind crossover design was used to examine the effects of a challenge of aspartame ingested with carbohydrate on food intake (energy and macronutrient selection) at lunch time, on subjective feelings of hunger and mood, on performance on a conditional associative learning task, and on selected measures of motor behavior and social interaction.

Ten female and ten male subjects (C.A.: 9.8 +/- 0.8 years) participated in two morning sessions. After an overnight fast, they were given a standardized breakfast at 08:30 and the treatment at 10:30. The treatment consisted of an ice slurry of strawberry Kool-Aid with either the test dose of aspartame (34 mg/kg) or the equivalent sweetness as sodium cyclamate and amino acids as alanine. Both slurries contained polycose to provide each child with 1.75 g/kg of carbohydrate. The dependent variables were measured from 25 to 115 minutes after the slurries were consumed.

No treatment effects were found for any of the dependent measures. Significant time-dependent effects were found for subjective feelings of hunger and for activity level. It is concluded that in comparison with sodium cyclamate, aspartame in doses of 34 mg/kg combined with carbohydrate does not affect short-term energy or macronutrient intake, subjective feelings of hunger or mood, or learning and behavior, in normal 9 and 10 year old children.

This research was supported by the International Life Sciences Institute-Nutrition Foundation, Washington, D.C.

14.2 ELECTROPHYSIOLOGICAL STUDIES OF THE FUNCTIONAL ROLE OF SINGLE, IDENTIFIED SEROTONERGIC NEURONS IN THE LOBSTER VENTRAL NERVE CORD. *P.M. Ma*, B.S. Beltz and E.A. Kravitz,* Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

The injection of certain amines and peptides into the systemic circulation of the lobster has profound modulatory effects on posture and behaviour. Serotonin produces a long-lasting postural change characterized by an over-all flexion of the abdomen, walking legs and claws---a posture reminiscent of the stance taken by dominant lobsters. Several lines of evidence suggest that this results from serotonin promoting the read-out of central motor programs resulting in postural flexion. Direct recording from abdominal postural muscles in intact animals during the "serotonin posture" shows that there is an increase in excitatory junctional potentials (ejps) in flexor muscles and a decrease in ejps in extensor muscles. Recordings from motor nerve roots or from motoneurons in isolated nerve cord preparations superfused with serotonin show that the amine increases the rates of firing of flexor excitatory and extensor inhibitory motoneurons while it decreases the rates of firing of flexor inhibitory and extensor excitatory motoneurons. As a next stage in examining the role of serotonin in postural control in lobsters, immunocytochemical methods were used to search for individual serotonergic cells that might be involved in these circuitries. Within the abdominal and thoracic nerve cord of the lobster, there are several sets of morphologically identifiable serotonergic neurons. One of these sets contains seven pairs of homologous, rostrally projecting neurons, one pair each in each of the six abdominal and the last thoracic ganglia. Among these cells, the pairs in the last thoracic and the first abdominal ganglia are particularly large (roughly 100 um in diameter), and can be identified and impaled reliably for intracellular stimulation and recording. Intracellular recordings show several unique physiological features of these serotonergic cells. Unlike most neuronal somata in the central nervous system of the lobster, large (up to 60 mV), fast action potentials can be recorded from these cells. Most of these cells are active spontaneously, firing action potentials at a steady rate of about 1 Hz. The serotonergic cells receive inputs from at least two major sources: sensory and command inputs. The former are mostly excitatory. The latter are more diverse: flexor command fibres usually excite, while extensor command fibres usually inhibit the serotonergic neurons. In isolated nerve cord preparations, direct intracellular activation of the serotonergic cells has no effect on motor output in recordings from motor nerve roots or from postural muscle fibres. Facilitating interactions are seen, however, with simultaneous activation of the serotonergic cells and flexor command fibres. When the serotonergic cell is activated along with the flexor command, the functional efficacy of the command fibres is enhanced. When the serotonergic cell is inhibited while stimulating the command fibres the ability of the latter to activate motoneurons is reduced. Serotonin may act, therefore, by facilitating transmission between flexor command fibres and their target motoneurons, thereby potentiating the read-out of motor programs resulting in postural flexion. If so, one role of serotonergic neurons may be to act as "gain-setting" elements in postural circuitries. (Supported by MDA and NIH)

14.3 DOPAMINERGIC (D2) MEDIATION OF RAT 22 kHz POSTEJACULATORY ULTRASONIC VOCALIZATION. R. Cagiano*, R.J. Barfield, N.R. White*, E.T. Pleim* & V. Cuomo* (SPON: T.R. McGuire). Dept. of Biol. Sci., Rutgers University, New Brunswick, N.J. 08903 USA and Institute of Pharmacology, University of Bari, 70124 BARI, Italy.

The role of ultrasonic vocalization in the sexual behavior of rats has been studied extensively. There are two types of calls: 1) a 50 kHz mating call emitted by both male and female and 2) a 22 kHz postejaculatory vocalization (PEV) produced only by the male. The 50 kHz calls are associated with sexual arousal in both sexes and are accompanied by an alert, aroused "theta" EEG pattern. The 22 kHz PEV, on the other hand, is associated with the period of sexual refractoriness following ejaculation. During the PEV the rats characteristically display a sleep-like spindling EEG pattern and are behaviorally suppressed.

The neurochemical control of ultrasonic vocalizations is complex and may involve several neurotransmitters. The research reported here was designed to investigate the role of dopamine receptor subtypes in the regulation of ultrasonic vocalization and masculine copulatory behavior. Intact sexually experienced male Long-Evans rats were treated with either saline or selective dopamine D1 (SKF 38393; Smith Kline & French) and dopamine D2 (LY 171555; Eli Lilly) receptor agonists, 15 min before a 30 min mating encounter with sexually receptive ovariectomized female rats injected with estradiol benzoate and progesterone 48 and 4 h before the test session, respectively. A significant decrease in the number of intromissions required for the first ejaculation was found in animals treated with SKF 38393 10 mg/kg/ip and with LY 171555 at doses ranging from 0.01 to 0.5 mg/kg/sc. Rats treated with LY 171555 at the highest dose displayed no postejaculatory vocalization. This phenomenon was found to be dose-dependent because the vocalization reappeared as doses were reduced. Raclopride, a new selective dopamine D2 receptor antagonist (0.5 mg/kg/sc), given 30 min before the test session, antagonized the suppressive effects of LY 171555 on postejaculatory vocalization whereas SCH 23390 (a dopamine D1 receptor antagonist; Schering), at the dose of 0.01 mg/kg/i.p., did not. Finally, raclopride given alone at the same dose drastically suppressed all behavioral activity. Results to date suggest that the dopamine D2 receptor plays an important role in the regulation of the 22 kHz postejaculatory vocalization, and that it has little or no involvement with other aspects of masculine copulatory behavior or 50 kHz mating calls.

14.4 INVOLVEMENT OF THE AMYGDALA IN STIMULUS-REWARD ASSOCIATION : INTERACTION WITH THE VENTRAL STRIATUM. M.Cador*, B.J. Everitt*+ and T.W.Robbins*. Depts. of Experimental Psychology and +Anatomy , University of Cambridge, Cambridge, England. (Spon: C. Dourish)

The nucleus accumbens as part of the ventral striatal complex, has a pivotal neuroanatomical position between the limbic system and the basal ganglia which has led to the proposal that it is an "interface between motivation and action".The dopamine (DA) agonist d-amphetamine (AMPH) injected into the nucleus accumbens enhances the control over behaviour exerted by conditioned reinforcers (CR). This effect is blocked by DA depletion from the ventral striatum effected by intra-accumbens 6-hydroxydopamine (Taylor & Robbins, Psychopharm., 90,390,1986). The ventral striatum receives projections from the basolateral nucleus of the amygdala, an area which has been implicated in stimulus-reward association. In the present study, we examine the involvement of the amygdala in the potentiation of responding for conditioned reinforcers following intra-accumbens AMPH injections.Thirsty rats were trained to associate a light (CR) with water and then implanted with permanent guide cannulae aimed at the nucleus accumbens. Half of these rats received excitotoxic lesion of the amygdala (AMY) by infusions of N-methyl-d-aspartate (0.12 M in 0.3 ul phosphate buffer) into the basolateral region whereas the other half received control infusions of the vehicle. In the test phase, water was no longer presented but responding on one of two novel levers produced the light (CR lever), whereas responding on the other lever (NCR lever) had no effect. The two groups received four counterbalanced intra-accumbens infusions of AMPH (3,10,30 ug/1ul) or vehicle over four test days. Intra-accumbens AMPH infusions dose-dependently increased responding on the CR lever but had no significant effect on responding on the NCR lever. Compared with controls, the AMY group exhibited a significant, selective reduction in responding on the CR lever, with no change on the NCR lever, irrespective of drug or control treatment. Control experiments showed that the AMY group were not hypodipsic and exhibited similar levels of locomotor activity compared to the vehicle group.The hyperactivity response following either i.p. or intra-accumbens AMPH was also unchanged. These results indicate a role for the amygdala in mediating the effects of stimulus-reward associations on behaviour, via DA-dependent mechanisms of the ventral striatum.

14.5 DIFFERENTIAL EFFECTS ON SPONTANEOUS MOTOR BEHAVIOR, FEEDING, AND ORAL STEREOTYPY FOLLOWING AMPHETAMINE MICROINJECTION INTO ANATOMICALLY DEFINED SUBREGIONS OF RAT STRIATUM. A.E. Kelley and A. Gauthier* Department of Psychology, Harvard University, Cambridge, MA 02138

The neurotransmitter dopamine (DA) has been implicated in a wide range of motor and motivational behaviors. Many of these behaviors are thought to be mediated by the striatum (including nucleus accumbens and caudate-putamen), the major projection target of mid-brain DA neurons. Although cytoarchitectually consistent throughout, lesion and anatomical studies of the striatum's afferent connections have suggested functional heterogeneity. However, there have been few studies of discrete DA stimulation of striatal subregions. This study analyzed the behavioral effects of amphetamine injections into striatal sites chosen on the basis of differential afferent fiber systems. These sites were the nucleus accumbens, which receives its primary input from hippocampus,the ventrolateral striatum, which is heavily innervated by amygdala and perirhinal cortex, and dorsolateral striatum, which is afferented by sensori-motor cortex. Three groups of rats were implanted with cannulae aimed at these sites. They were maintained with food and water ad libitum throughout the experiment. On test days, rats received either saline, 0.1µg, 1.0µg, or 5.0µg in 0.5 µl every two days. Rats were returned to the home cage immediately after infusion, where their behavior was recorded over a 40 min. period by observation and an automated event recorder. The session was also videotaped. Behaviors measured were locomotion (center crossings), rearing (frequency and duration), feeding and drinking (duration,bouts and intake). Two further categories were analyzed from videotape following the experiment: stereotyped biting and oral behavior (combined scores of feeding and biting). When injected into nucleus accumbens, amphetamine produced a dose-dependent increase in locomotion and rearing, and a decrease in average duration of rears (total duration/frequency). There were no effects on feeding or drinking. In contrast, amphetamine injected into ventrolateral striatum enhanced feeding, and at higher doses an intense, stereotyped biting of the forepaws was observed. Combined scores of oral behavior were dose-dependently increased by amphetamine in this site. Locomotor and rearing scores were decreased in the first part of the session, indicated by significant dose x time interactions. Amphetamine injected into dorsolateral striatum produced no measurable effects on the behaviors recorded. These findings provide evidence for differential behavioral effects of amphetamine within subareas of "limbic-afferented" striatum, which are in addition distinguishable from effects within "non-limbic" striatum. A specific ventrolateral site may be linked to the motor circuitry underlying feeding and oral behavior.

14.6 **NEUROCHEMICAL CONSEQUENCES OF CONDITIONED CIRCLING IN LOCALIZED REGIONS OF THE STRIATUM.** C. Szostak*, A. Jakubovic, A.G. Phillips and H.C. Fibiger. Div. Neurol. Sci., Dept. Psychiatry, Univ. British Columbia, Vancouver, B.C. Canada V6T 1W5.

High rates of circling established and maintained by operant procedures have been associated with increased dopamine (DA) utilization within the striatum (STR) (J. Neurosci. 6, 1986). The observation that conditioned circling is associated with bilateral increases in DA utilization is in contrast with the hypothesis that circling is dependent upon a hemispheric imbalance in striatal DA activity. Given that the STR is not a homogenous structure in terms of its neuroanatomical connections, neurochemistry or behavioral functions, the detection of behaviorally-induced neurochemical asymmetries may be facilitated by fine-grained dissections of the STR. Accordingly, the effects of conditioned circling on concentrations of DA and its metabolites in 10 discrete sub-regions of the STR were determined.

Spraque-Dawley rats were trained to circle in their preferred direction for water according to a Fixed Ratio 2 schedule of reinforcement. The neurochemical consequences of conditioned circling were determined upon attainment of stable levels of responding. The STR was sectioned into 3 consecutive, 1 mm thick coronal slices. The first 2 slices were both dissected into 4 quadrants (dorsolateral, dorsomedial, ventrolateral, ventromedial) while the tail of the STR (3rd slice) was divided into dorsomedial and ventrolateral subregions. DA metabolism, as determined by metabolite concentrations and metabolite to DA ratios, was enhanced bilaterally within the anterior dorsolateral and dorsomedial STR, relative to non-circling, water-deprived control rats. No other neurochemical effects were detected.

The present results confirm that conditioned circling is associated with a bilateral augmentation in DA utilization. No evidence of behaviorally-induced neurochemical asymmetries was obtained even though rats were trained to circle in their preferred direction, a procedure which should enhance the likelihood of detecting asymmetrical effects. The present results are consistent with the suggestion by Joyce, Davis and van Hartesveldt (Eur. J. Pharmacol. 72, 1981) that the dorsal STR critically underlies rotational behavior.

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14.7 HYPERRESPONSIVITY OF LOCUS COERULEUS IS ASSOCIATED WITH STRESS-INDUCED BEHAVIORAL DEPRESSION. J.M. Weiss, P.E. Simson, J.A. Knight, & C.D. Kilts. Dept. of Psychiatry, Duke University Medical Center, Durham, NC 27710.

Depression of active behavior occurs in rats after they have been exposed to highly stressful, uncontrollable events. Evidence from various sources indicates that this behavioral effect is mediated by a large fall in the concentration of norepinephrine (NE) in the locus coeruleus (LC) region of the brain, resulting in decreased availability of NE for release in that brain region and, consequently, an understimulation of alpha-2 receptors that normally respond to this transmitter. Since LC alpha-2 receptors inhibit firing of LC cells, reduced stimulation of these receptors should disinhibit LC cells. Therefore, firing (depolarization) of LC cells should be elevated after animals have been exposed to stressful conditions that depress active behavior.

A recent observation increases our ability to assess the influence of alpha-2 receptors on LC activity. Simson and Weiss (J. Neurosci., in press) found that LC cells considerably increase their firing to excitatory stimuli when alpha-2 receptors are blocked; in fact, the response of LC cells to excitatory stimuli is a much more sensitive index of alpha-2 receptor blockade in the LC than is a change in baseline firing rate. Therefore, responsivity of LC cells to stimulation should be particularly indicative of whether a "functional alpha-2 blockade" is present in the LC after exposure to stressful conditions that depress active behavior.

As in previous experiments, animals exposed to uncontrollable shock (N=11) showed less active behavior in a 15 min. swim test given 1.5 hours after shock. Using an Activity Score [AS] computed by subtracting seconds of floating from seconds of struggling, shocked animals showed less activity (mean AS = -306.4 ± 67.0) than did animals given no shock (N=7; mean AS= -91.2 ± 24.9; $p<.01$) Immediately after the swim test, animals were anesthetized with chloral hydrate and LC activity recorded. Baseline LC activity was elevated in shocked animals (mean firing rate 2.0 ± 0.4 per sec) in comparison to unshocked animals (1.2 ± 0.1), although this difference did not reach statistical significance ($t=1.7$, $df=16$). However, responsivity to paw compression (PC), an excitatory stimulus to the LC, was markedly, and significantly, elevated in shocked animals. In response to PC, average firing rate was 7.4 ± 0.7 per second in shocked animals and 4.4 ± 0.5 in animals given no shock ($p<.001$).

The results described above indicate that LC cells of stressed animals showing depression of active behavior are hyperresponsive to an excitatory input. That increased activity of the LC is involved in stress-induced behavioral depression was also supported by correlational findings. For the individual animals in this study, we correlated (a) the average increase in response of LC cells from baseline produced by PC and (b) Activity Score in the swim test. The correlation was $r=-.70$ ($p<.001$).

In summary, responsivity of LC units to excitatory stimulation was found to be elevated in stress-induced behavioral depression, and the degree of LC responsivity observed in individual animals correlated with the magnitude of depression seen in these animals.

14.8 PHENYLALANINE AND SPONTANEOUS BEHAVIOR IN THE RAT: ANALYSIS USING COMPUTER PATTERN RECOGNITION. P.J. Mullenix, M.S. Tassinari* and W.J. Kernan*. Toxicology Dept., Forsyth Dental Center, Boston, MA 02115 and Veterinary Diagnostic Lab., Iowa State University, Ames, IA 50011.

The effects of phenylalanine on the spontaneous motor behavior of the rodent have not been clearly defined. Increased, decreased and unaffected locomotor activity have all been reported (Thurmond et al., Pharmacol. Biochem. Behav. 12:525-532, 1980; Snodgrass, J. Pharm. Pharmacol. 26:931-936, 1974; Gibson et al., Psychopharmacology 76:118-121, 1982). The extent to which differences in behavioral methodology contribute to this confusion is unknown. Therefore, a new computer pattern recognition system (Kernan et al., Pharmacol. Biochem. Behav. 27:in press, 1987), which automatically classifies behavioral acts and determines the structure of motor output, was used to analyze the acute effects of phenylalanine in rats.

Fifteen male Sprague-Dawley rats (250-300g) were dosed orally with 281 mg/kg L-phenylalanine (PHE) suspended in a vehicle comprised of .5% w/v methylcellulose, .1% v/v polysorbate and distilled water. An additional fifteen male rats received the vehicle only. Both treated and control rats were fasted overnight prior to gavage. One hour subsequent to treatment, a control and an experimental rat were placed simultaneously on opposite sides of a divided, Plexiglas, novel environment which they explored for 15 minutes. All tests were conducted during the animals' diurnal period between 9:00 A.M. and 12:00 Noon. Movements were monitored from horizontal and vertical views using two video cameras. Behavior sampling rate was at 1 frame per second, and the frames were transfered to computers for analysis in real time. The behaviors recorded and the parameters quantified were more extensive than previously possible with other techniques. The frequency, duration and distribution in time of 16 different motor acts, as well as the velocity of movement, horizontal and vertical movements, circling and time spent in any sub-area of the test chamber, were determined for each rat. Immediately following the behavioral test, blood samples were obtained by cardiac puncture, and plasma PHE concentration was determined using HPLC.

The concentration of PHE in the plasma of control rats was 117.3 ± 9.8 µmol/l (SE, N=6), and PHE concentration in the plasma of treated animals was 246.4 ± 37.9 µmol/l (SE, N=6). Despite the relatively high concentration of PHE in the plasma of the treated rats, no significant signs of abnormal spontaneous behavior could be related to PHE treatment. Among the parameters tested, no pattern or trend characteristic of hyperactivity, an overt sign of CNS stimulation, was observed.

14.9 LATERAL DIFFERENCES IN MONOAMINE CONTENT OF DISCRETE BRAIN NUCLEI IN AMPHETAMINE-SENSITIZED RATS. J.M. Rivet*, G.J. LaHoste*°, P. Mormède* and M. Le Moal INSERM U-259, rue Camille Saint Saëns 33077 Bordeaux Cedex, France;°Dept. Psychobiol., Irvine University, Irvine, CA, USA.

Recent studies suggest that the preferred direction of rotation shown by rats following peripheral administration of amphetamine is due to an endogenous asymmetry of striatal dopamine (DA). This has been confirmed in female but not male rats. In addition, sensitization to amphetamine has been shown to result in enhanced release of DA following amphetamine challenge. In order to pursue these phenomena further, we measured monoamines and their metabolites in discrete brain nuclei in amphetamine-sensitized male and female rats which has been categorized as left- or right-turning (LT or RT). d-Amphetamine sulfate was administered (1.2 mg/kg in males; 0.85 mg/kg in females) three times at 10-day intervals and rotation was observed. Four to six weeks following the last injection, rats were placed in a novel environment for 15 minutes and decapitated. Brains were quickly removed and frozen at -80°C. Punches of discrete nuclei from the left and right hemispheres were taken from cryostat sections. Monoamine levels were measured by HPLC-EC.

Neurochemical differences were found in the lateral striatum of male rats according to their behavioral lateralization, DA and 5HT being higher in left turners. No such difference was found in females or in the medial striatum. Furthermore transmitter levels were equally distributed in both sides of these brain regions so that there was no neurochemical correlates of behavioral asymmetry. This is in contrast to what has been previously reported for female rats when amphetamine was administered prior to sacrifice.

Hypothalamic areas were also investigated for neurochemical asymmetries. The arcuate nucleus of both male and female rats showed a striking asymmetry in DA distribution, the left side having the highest DA concentration. However, the DOPAC/DA ratio, an index of DA turnover was the same in both sides. The paraventricular nucleus of male rats showed a difference in indole distribution, 5HT and 5HIAA levels being higher in the right side. However there was no relationship between neurochemical and behavioral lateralization. Little is known about functional significance of such differences.

14.10 CSF NOREPINEPHRINE AND MHPG, BUT NOT CSF HVA, IS UNDER STATE DEPENDENT CONTROL IN SCHIZOPHRENIA. D.P. van Kammen, W.B. van Kammen*, J. Peters*, M. Linnoila*, J. Yao*, and N. Nugent*. VA Medical Center, Highland Drive, Pittsburgh, PA 15206

In spite of the considerable evidence of dopaminergic involvement in schizophrenia, disturbances in the noradrenergic system have repeatedly and consistently been reported. In this study we examined our previous findings of increased CSF NE in schizophrenic patients. We have reported relationships between psychosis and CSF NE and MHPG, that could not always be replicated. In this study we controlled for changes in clinical state by comparing CSF monoamines in drugfree patients who were stable with those that relapsed. After we obtained informed consent, we studied 29 schizophrenic patients (DSM III) (age 20-50 yrs.) who were chronically treated with haloperidol (20 mg/day), received an LP according to standard procedures. After placebo replacement 14 patients relapsed and 15 did not in an 8 week placebo replacement trial. At the time of meeting relapse criteria patients received a second LP. Psychosis ratings (Bunney-Hamburg scale) of the day before and the hours of sleep of the night before the LP's were recorded. CSF NE, MHPG, HVA and 5HIAA were measured with HPLC.

RESULTS: Haloperidol treated patients, who would relapse later, had higher CSF NE levels (0.742±0.33 vs. 0.425±0.27 pg/ml, $p<.01$), but CSF HVA, MHPG and 5HIAA, sleep and psychosis ratings were identical compared to those patients who would not relapse after haloperidol withdrawal. After haloperidol withdrawal CSF NE, (0.875±.478 vs. 0.419±.26 $p<.007$), CSF MHPG (47.5±16.8 vs. 34.8±9.1, $p<.03$), psychosis (10.3±1.7 vs. 4.2±1.7, $p<.0001$) were significantly higher in the patients who relapsed than in the patients who did not. Relapsing patients slept considerably less the night before the LP (5.0±2.2 vs. 7.3±0.6 hours, $p<.003$). Similarly, correlations between these variables among the relapsers showed CSF NE to intercorrelate highly with sleep, and psychosis (r's.from -.55 and -.59 to .52 and .74). The relationship between CSF NE and MHPG were identical in both groups ($r=.50$, $p<.05$).

DISCUSSION: Our data indicate that increased NE is already present in haloperidol treated schizphrenic patients who will relapse after haloperidol withdrawal and that NE activity is further increased around the time of relapse. This increase in NE may contribute to psychotic worsening rather than be the result of the stress of psychosis exacerbation. Longitudinal sleep and behavioral, and weekly plasma data will be presented at the meeting as well.

14.11 DOPAMINE AND NOREPINEPHRINE ALTER SENSORY PHYSIOLOGY IN SCHIZOPHRENIA AND MANIA BY DIFFERENT MECHANISMS. L. Adler, G. Gerhardt, R. Franks*, N. Baker*, H. Nagamoto*, C. Drebing* and R. Freedman. Depts. of Psychiatry and Pharmacology, Univ. Colo. Hlth. Sci. Ctr., Denver, CO 80262.

Hyperactivity to sensory stimulation is a prominent characteristic of both schizophrenia and mania. Neurophysiological recordings show a common deficit in an inhibitory gating mechanism which regulates sensitivity to repeated auditory stimuli. Dopamine and norepinephrine are hypothesized to have major roles in these illnesses, but how they might cause aberrant sensory processing has not yet been elucidated. In this study, gating of auditory evoked potentials (as measured by the P50 auditory evoked potential conditioning-testing paradigm) during treatment of both illnesses was correlated with plasma dopamine and norepinephrine metabolites. Schizophrenics were found to have a fixed deficit in inhibitory auditory sensory gating, which is a familial trait unchanged by medication. During acute illness, they have an additional transient hypersensitivity to stimuli, manifested as smaller evoked potential waves, which is mediated by dopamine. Plasma HVA in treated schizophrenics decreased significaantly from baseline (18.9 $\pm$ 4.7 to 13.1 $\pm$ 3.2 ng/ml s.d., $p < 0.01$, by paired t-test). Manics have only the deficit in inhibitory gating, which is transient and mediated by norepinephrine. Plasma MHPG in manics decreased from 5.6 $\pm$ 1.3 to 3.4 $\pm$ 0.05 ng/ml s.d.($p < 0.01$ by paired t-test) with a corresponding improvement in auditory sensory gating. Thus, the common neurophysiological deficit in the two psychoses arises from different biochemical abnormalities, which may explain similarities in acute symptoms and differences in lifetime course.

14.12 EVIDENCE THAT BETA-ADRENERGIC ACTIVITY INHIBITS RATHER THAN FACILITATES LORDOSIS BEHAVIOR IN THE FEMALE RAT. S.D. Mendelson and B.B. Gorzalka. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C. V6T 1Y7, Canada.

Observations of inhibition of lordosis behavior following central (Foreman and Moss, Pharmacol. Biochem. Behav., 9:235, 1978) and peripheral (Fernandez-Guasti et al., Pharmacol. Biochem. Behav. 22:279, 1985) administration of racemic propranolol have led to the hypothesis that activation of central beta-adrenergic receptors facilitates lordosis behavior in female rats. In the present study both (-) propranolol (9 mg/kg) and (+) propranolol (9 mg/kg) were found to inhibit lordosis when administered peripherally to ovariectomized rats that had received estrogen and progesterone. Of the two optical isomers of propranolol, only the (-) form is known to be active at beta-adrenergic receptors, whereas both (-) and (+) isomers produce stabilization of neural membranes (Fitzgerald, Clin. Pharmacol. Ther. , 10:292, 1969). These data suggest that the stabilization of neural membranes rather than beta-adrenergic blockade was responsible for the lordosis-inhibiting effects of propranolol. Consistent with this possibility are our findings that the centrally active beta-adrenergic antagonist metoprolol (Cowen et al., Br. J. Pharmacol. , 76:265, 1982) (0-75 mg/kg) had no effect on lordosis behavior, whereas the membrane stabilizing drug lidocaine (30 mg/kg) inhibited lordosis responding. Peripheral administration of the centrally active beta-adrenergic agonist salbutamol (6-20 mg/kg) was found to inhibit lordosis, and this effect was reversed by the co-administration of metoprolol (20 mg/kg). These data suggest that stimulation of central beta-adrenergic receptors may inhibit rather than facilitate lordosis.

GENE STRUCTURE AND FUNCTION I

15.1 cDNA CLONING OF A SEROTONIN 5-HT_{1C} RECEPTOR USING ELECTROPHYSIOLOGICAL ASSAYS OF mRNA INJECTED XENOPUS OOCYTES

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We have designed a strategy for the cloning of neurotransmitter receptor and ion channel cDNAs which is based on electrophysiological assays of mRNA injected Xenopus oocytes. This procedure circumvents the purification of these membrane proteins which is hindered by their low abundance and their hydrophobic nature. It involves methods for RNA fractionation by high resolution gel electrophoresis, directional cDNA cloning in a single-stranded vector, and screening of the cDNA library by voltage-clamp measurements of currents induced by serotonin in mRNA injected oocytes.

We used this approach to isolate a serotonin receptor cDNA clone from mouse choroid plexus papilloma. The clone was identified by hybrid depletion and hybrid selection procedures. The receptor expressed in oocytes injected with hybrid selected RNA is fully functional, indicating that it is composed of a single subunit encoded by a 5 kb RNA. The pharmacology of the hybrid selected receptor confirms that we have successfully cloned a 5-HT_{1C} receptor cDNA.

15.2 ANALYSES OF CLONED SEROTONIN 5-HT_{1C} RECEPTOR cDNAs

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Serotonin (5-HT) induces conductances in Xenopus oocytes injected with rat brain mRNA. Although there are at least six subtypes of serotonin binding sites in the mammalian central and peripheral nervous systems, we have shown that only the 5-HT_{1C} receptor is functionally expressed in mRNA injected oocytes. This receptor opens an endogenous Ca dependent Cl channel in the oocyte membrane via inositol trisphosphate (IP_3) release. We have shown that 5-HT_{1C} receptors mediate an increase in phosphoinositide turnover in intact rodent and pig choroid plexi. The richest source of 5-HT_{1C} binding sites is the choroid plexus while other regions of the brain, for instance, hippocampus, cortex and striatum, express lower densities of these receptors. Using serotonin-induced currents in mRNA injected oocytes as a bioassay, we have isolated a cDNA clone for the serotonin 5-HT_{1C} receptor.

We will present preliminary sequence data for a 5-HT_{1C} receptor and compare computer analyses of our data to other receptors and transmembrane proteins. Using Northern blot analysis, we have determined the abundance and size distribution of serotonin 5-HT_{1C} receptor mRNA in various brain regions and in several peripheral tissues which receive serotonergic innervation. Since these receptors are present in all mammalian species examined, we have analyzed the homology between 5-HT_{1C} receptor genes in rat, mouse, pig and human by Southern blotting.

15.3 **MUTATIONAL ANALYSIS OF A CYCLIC AMP-RESPONSIVE ENHANCER ELEMENT IN THE HUMAN VIP GENE.** J.S. Fink, M. Verhave*, T. Tsukada*, G. Mandel* and R.H. Goodman*. Division of Molecular Medicine, New England Medical Center, Boston, MA 02111 and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Transcription of the human VIP (hVIP) gene is regulated by cyclic AMP (cAMP). We have previously identified a 17 base pair (bp) region within the 5'-flanking sequences of the hVIP gene that is important for transcriptional regulation by cAMP. These sequences are capable of conferring cAMP-responsiveness to a heterologous viral promotor. In this study we sought to determine (1) if the VIP cAMP regulatory element (CRE) has characteristics of an "enhancer", i.e. the ability to confer cAMP-regulation to a heterologous promotor in an orientation and distance-independent manner, and (2) the critical nucleotides necessary for activity of the VIP CRE.

Fusion genes containing portions of the 5'-flanking region of the hVIP gene linked to the Rous sarcoma virus (RSV) promotor and the chloramphenicol acetyltransferase (CAT) reporter gene were introduced into PC12 or C6 glioma cells and tested for responsiveness to forskolin, an activator of adenylate cyclase. The VIP CRE conferred cAMP regulation when introduced in either orientation, or when placed 300 bp upstream from the RSV promotor or 3' to the CAT coding sequences. S1 nuclease mapping demonstrated that the VIP CRE faithfully regulated transcription from the heterologous RSV promotor. Using synthetic oligonucleotides containing base changes within the VIP CRE we determined that mutation in the 3' or 5' ends of the VIP CRE resulted in a 40-75% loss of cAMP responsiveness. Mutation of bases in a middle region of the VIP CRE (nucleotides -78 to -80 from the VIP transcription initiation site) preserved cAMP responsiveness.

We conclude: (1) the hVIP CRE is a regulatable enhancer, such as is present in the interferon, phosphoenolpyruvate carboxykinase, proenkephalin and c-fos genes, and (2) sequences contained within the VIP CRE form 2 functional domains which are necessary for full activity.

15.4 TRANSCRIPTIONAL CONTROL OF THE RAT CORTICOTROPIN RELEASING HORMONE GENE. A.F. Seasholtz*, R.C. Thompson*, E. Herbert*, and J.O. Douglass* (SPON: R. Nishi). Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

The rat corticotropin releasing hormone (CRH) gene has been isolated and characterized by DNA sequence analysis (Thompson, R.C., Seasholtz, A.F., and Herbert, E., Mol. Endo., 1:363,. 1987). RNA blot analysis demonstrates the expression of the CRH gene in numerous regions of the rat brain as well as the spinal cord, adrenal gland, pituitary, and testis. The rat CRH gene exhibits a structural organization similar to that of the human gene (Shibahara, S. et al., EMBO 2:775, 1983), having two exons separated by an intervening sequence. Analysis of the nucleotide sequence homology between the human and rat CRH genes reveals several highly conserved regions (greater than 90% homology) including the CRH peptide-encoding sequence and the 5' flanking sequence. Studies with a variety of other genes have shown that DNA sequences 5' to the mRNA cap site often contain transcriptional control sequences including enhancer (or silencer) elements, tissue-specific elements, or DNA elements responsible for regulation by cAMP, phorbol esters, or glucocorticoids. The 335 bp of DNA immediately 5' to the putative mRNA cap sites for the human and rat CRH genes show 94% homology, suggesting that this region may contain regulatory elements which have been conserved through evolution. In order to test for transcriptional control elements, 1.4 kb of 5' flanking DNA from the rat CRH gene has been fused to the coding sequence of the E. coli chloramphenicol acetyltransferase (CAT) gene to create the plasmid CRHCAT. In this fusion gene construct, the amount of CAT enzyme activity reflects the amount of protein produced under the control of the linked CRH regulatory region. The CRHCAT plasmid DNA has been transfected into a variety of cell lines including PC-12 (rat pheochromocytoma), AtT-20 (mouse anterior pituitary), and CV-1 (monkey kidney) cells. We have been able to demonstrate regulation of CAT gene expression by cAMP analogues and glucocorticoids, suggesting the presence of these regulatory elements in the 5' flanking sequence of the rat CRH gene. The exact locations of these transcriptional control elements are being determined using deletion analysis.

15.5 REGULATION OF EXPRESSION OF A NEURON SPECIFIC GENE: NEUROPEPTIDE Y

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Neuropeptide Y (NPY) is a 36 amino acid amidated peptide that is produced exclusively by neural tissue. It is present in abundance in mammalian brain and in a subset of sympathetic neurons. The peptide meets some of the criteria required for neurotransmitter status. It is a potent vasoconstrictor peripherally and has been implicated in the central control of blood pressure, circadian rhythms and feeding behavior.

The PC 12 clonal cell line has been used extensively as a model of neuronal differentiation. Nerve growth factor (NGF) induces these cells to undergo changes to neuronal morphology. We have used this cell line to investigate the molecular mechanisms involved in the regulation of expression of the NPY gene. In particular the effect of NGF on the levels of NPY has been determined by Northern blot hybridization analysis. Low levels of NPY mRNA were identified in resting naive PC 12 cells which was indistinguisable from NPY mRNA extracted from rat brain. Levels were induced five fold by the addition of NGF to the medium at a dose of 30ng/ml, whereas lower doses had no effect. The effect of NGF on NPY mRNA was rapid in onset and reversible, preceeding the NGF induced changes in morphology by several days. A smaller twofold rise in NPY mRNA was observed in cells treated with epidermal growth factor but no effect was found following treatment with fibroblast growth factor, bradykinin or dexamethasone. These results indicate that NPY gene expression is regulated in PC 12 cells at the level of NPY mRNA.

To identify the cis and trans factors that regulate NPY gene expression, the NPY gene was cloned from a rat genomic library. Structural analysis revealed 4 exons and 3 introns residing in a 15kb fragment of rat genomic DNA. The organisation is very similar to that of pancreatic polypeptide gene and identical with the human gene, such that exons encode functional domains in the mRNA and NPY precursor. The promoter regions of human and rat NPY are highly homologous suggesting important cis elements are present that meidate cell-specific and transcriptional regulation.

15.6 NERVE GROWTH FACTOR GENE EXPRESSION: REGULATION OF TRANSCRIPTION AND mRNA STABILITY. M.Zheng*, P.D.Rennert* and G.Heinrich. Lab for Mol. Endo., Howard Hughes Med. Inst., Dep. Med., Mass. Gen. Hosp., Boston, MA 02114

Nerve growth factor (NGF) is essential for the survival of specific neurons. During development and adulthood NGF production by neuronal targets is limited to minute amounts under basal conditions but can be rapidly and substantially modulated by injury and hormones. Inasmuch as NGF production is limited by cellular levels of NGF mRNA we are focusing on the molecular mechanisms that regulate NGF gene transcription and NGF mRNA stability, the two crucial determinants of NGF mRNA levels and NGF production.

To structurally and functionally identify the cis elements that regulate NGF gene transcription, we cloned and analyzed the promoter and 5' flanking regions of mouse and rat NGF genes, and fused these regions to a reporter gene encoding human growth hormone. Comparison of the nucleotide sequences of rat and mouse NGF genes revealed >90% homology in exon 1 and the promoter region, but <70% further upstream, suggesting that important regulatory elements are close to the promoter. Transcriptional initiation sites were mapped by S1-nuclease protection and reverse transcription. Deletion analysis, gel delay, and footprinting experiments are being carried out to characterize the cis and trans factors that act on the NGF gene.

To determine NGF mRNA stability, transcription was inhibited with actinomycin D in L929 fibroblasts, and slices of mouse salivary gland and rat heart, and NGF mRNA decay measured by Northern blot hybridization. NGF mRNA was unstable in L929 cells and heart (T1/2 > 1h), but stable in SMG (T1/2 > 12h). Dexamethasone rapidly reduced NGF mRNA levels in L929 cells, but had no effect on T1/2. Puromycin had no effect on T1/2 in L929 cells or SMG. The short T1/2 of NGF mRNA in target tissues may account in part for the low tissue levels of NGF. To identify the region(s) of NGF mRNA that determine stability, segments of NGF mRNA were spliced into the stable human growth hormone mRNA. Analysis of the stability of the chimeric mRNAs by transient expression of the chimeric genes in L929 cells will delineate the region(s) of NGF mRNA involved in regulation of its stability.

15.7 **CLONING AND SEQUENCE ANALYSIS OF cDNA FOR RAT CARBOXYPEPTIDASE E (ENKEPHALIN CONVERTASE).** L.D.Fricker Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx NY 10461

Many peptide hormones and neurotransmitters are produced from larger precursors which contain pairs of basic amino acids surrounding the bioactive peptides, and the sequential action of trypsin-like and carboxypeptidase B-like enzymes would liberate the peptides. Carboxypeptidase E (CPE, also designated enkephalin convertase, carboxypeptidase H, and EC 3.4.17.10) is thought to be the carboxypeptidase B-like enzyme involved in the production of many different peptide hormones and neurotransmitters. Two enzymatically active forms of CPE, a soluble and a membrane-bound form, are present in peptidergic secretory granules. The two forms have similar enzymatic and physical properties, as well as an identical N-terminal amino acid sequence. Recently, a cDNA clone encoding CPE has been isolated from a bovine pituitary cDNA library (Fricker,L.D., et al, Nature 323:461, 1986). This cDNA clone was not full length, and no information regarding the precursor structure of the enzyme was provided from the nucleotide sequence. In order to obtain a full length clone, the bovine cDNA was used to screen rat brain cDNA libraries. A clone which appears to code for the complete CPE protein was isolated and sequenced. This initial translation product contains an additional 42 amino acids on the N-terminus of the active forms of CPE. Of this N-terminal peptide, the first 25 amino acids resemble a signal peptide, and there are several potential signal peptide cleavage sites in positions 17-25. The remainder of this N-terminal peptide contains several charged amino acids, and does not fit with the predicted structure of signal peptides. Furthermore, there are five adjacent arginines immediately preceding the N-terminus of the active forms of CPE. This suggests that a trypsin-like endopeptidase is involved in the production of enzymatically active CPE.

The predicted amino acid sequence for rat CPE is 94% homologous with bovine CPE. As with bovine CPE, the rat CPE sequence contains several pairs of basic amino acids. One of these pairs is 20 amino acids from the C-terminus of the initial translation product, and this region is completely homologous with the bovine sequence. The predicted secondary structure of this C-terminal region is an amphipathic α-helix, which may interact with the lipid bilayer. This could contribute to the binding of the enzyme to the membranes. Since the soluble form of CPE is 3000 Da smaller than the membrane-bound form, cleavage of this C-terminal peptide at the pair of basic amino acids near the C-terminus would be consistent with the difference in molecular weight of the two forms. Biochemical studies that address this possibility will be presented.

15.8 THE COMPLETE NUCLEOTIDE SEQUENCE AND STRUCTURE OF THE GENE ENCODING BOVINE ADRENAL PHENYLETHANOLAMINE N-METHYLTRANSFERASE D.K. Batter, S.R. D'Mello*, L.M. Turzai*, H.B. Hughes*, A.E. Gioio*, and B.B. Kaplan Molecular Neurobiology Program, Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Phenylethanolamine N-methyltransferase (PNMT) is the terminal enzyme of the catecholamine biosynthetic pathway, catalyzing the conversion of norepinephrine to epinephrine. Importantly, the expression of the PNMT gene, in part, defines the adrenergic cell phenotype. To determine the molecular genetic mechanisms controlling selection of the catecholamine phenotype, we have initiated studies on the regulation of adrenal PNMT gene expression. In this communication, we report the complete structure and nucleotide sequence of the gene for bovine adrenal PNMT.

A cloned PNMT cDNA probe was used to screen a Charon 28 bovine genomic library. After screening approximately 1.0 x 10^6 pfu, one recombinant phage (λP1) was identified which included the entire PNMT gene within 17 kilobases of genomic DNA. A restriction endonuclease map of λP1 was constructed and exon-containing fragments identified by Southern blot analysis. These fragments were sub-cloned into M13 vectors, and sequenced by the Sanger dideoxy method. Analysis of these fragments, and several more further upstream, indicates that the bovine PNMT gene is 1594 base pairs (bp) in length, consisting of three exons interrupted by two introns. The transcription initiation site was identified by conventional primer extension analysis and a recently published method using T4 DNA polymerase (Hu & Davidson Gene 42:21-29, 1986). Results of both these experiments indicate that transcription is initiated at a 'C' residue, located 12 bp upstream from the ATG translation start site. The 3' untranslated region is 88 bp in length and contains the expected polyadenylation signal (AATAAA). A putative promoter sequence (TATA box) is located 25 bp upstream from the transcription initiation site. Genomic Southern blot analysis indicates that the PNMT gene is probably present in a single copy per haploid genome. Additionally, computer comparison of the nucleotide sequence data with consensus sequences of known regulatory elements revealed possible binding sites for glucocorticoid receptors, and for the Sp1 regulatory protein. Interestingly, whereas the location of putative Sp1 binding sites are restricted to the 5' flanking region of the gene, those for the binding of glucocorticoid receptors appear both in 5' and 3' flanking regions and within the third exon of the PNMT gene. The functional significance of these putative regulatory sequences, with respect to PNMT gene expression, remains to be elucidated. Comparison of the sequence of the bovine PNMT exons to cDNA sequences encoding several biogenic amine-synthesizing enzymes revealed no significant regions of homology.

15.9 BC1 RNA IS ENCODED BY A UNIQUE GENE WHICH IS EXPRESSED HETEROGENEOUSLY IN THE RAT NERVOUS SYSTEM. H. Tiedge*, R. T. Fremeau, A. H. Ahn*, C. Slater*, D. I. Lugo, J. Pintar, J. L. Roberts, and J. Brosius*, Columbia University College of Physicians and Surgeons, 722 W 168th St., New York, NY 10032.

BC1 is a small non-messenger RNA that is subject to developmental and tissue-specific gene expression. This cytoplasmic RNA was initially identified by Sutcliffe and coworkers (PNAS U.S.A. 79: 4942, 1982) based on its ability to hybridize to the 75 nucleotide repetitive ID element. cDNA sequence analysis demonstrated that BC1 RNA is homogeneous and is transcribed from only one gene (DeChiara, T. M., and Brosius, J., PNAS U.S.A. 84: 2624, 1987). BC1 RNA is comprised of three domains: (1) the 5' ID element which includes an internal RNA polymerase III promoter, (2) a central A-rich region, and (3) a 3' unique sequence.

To begin to understand the possible functions of BC1 RNA in the nervous system, we examined its cellular localization in rat brain and selected peripheral tissues by hybridization *in situ*.. The unique part of the molecule was used as a probe. Within the brain, BC1 RNA is expressed heterogeneously. High levels of expression were found in the thalamus, the hypothalamus, the zonal layer of the superior colliculus, the dorsal cortex of the inferior colliculus, the dorsal and medial central gray, the dorsal tegmentum, and the amygdaloid complex. Intermediate levels were detected in certain layers of the neocortex and the hippocampus. Low levels were detectable in the cerebellum and the striatum. Only background labelling was observed overlying the corpus callosum, the anterior commissure, and the choroid plexus. Brain stem levels were generally low except for the area postrema, the nucleus tractus solitarius, and the spinal trigeminal nucleus. In the spinal cord BC1 RNA was found exclusively in the central gray matter. BC1 RNA has also been found to be expressed in peripheral nervous tissues such as the dorsal root ganglia, the superior cervical ganglia, the retina, and the anterior lobe of the pituitary. No specific labelling was observed in liver, kidney, and spleen, or in any section hybridized with a sense-strand probe.

During embryogenesis, BC1 RNA was detectable at a low level as early as day e11.5, mainly in the developing spinal cord. By day e16, significant amounts of BC1 RNA were detectable in a distinct area of the myelencephalon. By postnatal day 8, the pattern of BC1 expression in the brain was very similar to that found in adult.

Investigations of the molecular mechanisms underlying the temporal and spatial regulation of the BC1 gene will be presented.

15.10 MOLECULAR CLONING OF A cDNA ENCODING RABBIT MYELIN P_2 PROTEIN V. Narayanan*, E. Barbosa*, R. Reed* and G. Tennekoon*. Depts. of Neurology and Molecular Biology and Genetics, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205. (SPON: R. Johnson)

Myelin basic protein P_2 causes experimental allergic neuritis (EAN). Although it was initially thought to reside exclusively in the PNS, immunocytochemical studies have shown its presence also in the rabbit CNS. Amino acid sequence of P_2 has shown that P_2 is homologous with other fatty acid binding proteins, with the closest homology being to adipocyte fatty acid binding protein. Although the adipocyte fatty acid binding protein was cloned (Bernlohr 1984), we were unable to use this cloned cDNA (pAL 422) for investigation of expression of myelin P_2, so we proceeded to clone myelin P_2 protein.

Total RNA was isolated from the sciatic nerves of 9 to 10-day-old New Zealand white rabbits followed by selection of polyA$^+$ RNA. In vitro translation of polyA$^+$ RNA using the rabbit reticulocyte system followed by immunoprecipitation with rabbit anti-bovine P_2 antibody gave a single band of M.W. 14,800. The polyA$^+$ RNA was then used to construct cDNA as described by Gubler and Hoffmann, and the library construct in the bacteriophage gt10. From 1 ng of DNA 10^6 recombinants were obtained. An oligonucleotide mixture was synthesized based on the amino acid sequence of rabbit myelin P_2 protein from positions 14 to 22 (N-Glu-Asn-Phe-Asp-Asp-Tyr-Met-Lys-Ala-OH). After purification by HPLC, the oligonucleotides were end-labeled with (^{32}P) -ATP and used to screen the library. From about 15,000 clones, 6 positive clones were identified after plaque purification and re-screening. Cross-hybridization with the probes suggested that all six clones belonged to the same family. One of the phage clones containing a 1.2 kb insert was subcloned into Bluescript vector - pSN 2.9B. Sanger dideoxy sequencing revealed a single open reading frame corresponding to the published P_2 amino acid sequence. RNA blots using the nick translated pSN2.9B recognized a 2 kb mRNA in rabbit sciatic nerve and spinal cord. However, no signal was detected on RNA blots containing total RNA from liver and heart. These tissues are known to contain other fatty acid binding proteins.

The developmental profile of P_2 mRNA levels in sciatic nerves and CNS as well as the full length nucleotide sequence will be presented.

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15.11 MUTATION IN THE GENE FOR MYELIN PROTEOLIPID PROTEIN (PLP) AND DM-20 IN THE DYSMYELINATING *JIMPY* MOUSE. K.-A. Nave, C. Lai*, F.E. Bloom, and R.J. Milner. Research Institute of Scripps Clinic, and Depts. of Neuroscience and Biology, University of California, San Diego, La Jolla, California 92037.

Proteolipids (PLP and DM-20) are the major integral membrane proteins of CNS myelin and are implicated in the formation and maturation of the myelin sheath. We show that the DM-20 protein of myelin derives from an alternatively spliced PLP gene transcript and differs by the internal deletion of 35 amino acids from the 276 residue PLP sequence. DM-20 specific mRNA splicing results from the selection of an alternative 5' splice site located 105 base pairs within the third exon of the PLP gene.

The mutant mouse *jimpy*, which has a developmental defect in the formation of CNS myelin, lacks immunoreactive PLP but has demonstratable expression of PLP mRNA on Northern blot analysis. We have characterized PLP cDNAs obtained from 20 day old *jimpy* mouse brain mRNA and found a deletion of 74 nucleotides in the protein coding region. Additionally, this deletion causes a frameshift of translation and predicts that *jimpy* -PLP has a truncated and altered C-terminus, which is unlikely to be functionally inserted into the myelin membrane. DM-20 mRNA in *jimpy* shows the same deletion, which further demonstrates that both proteins are derived from the same X-linked gene.

Genomic Southern blotting experiments and sequence analysis of the *jimpy* PLP gene, however, show that the 74 nucleotide sequence is not deleted in the mutant gene but corresponds to a separate exon. We suggest that a mutation affecting a consensus splice site of the PLP gene and leading to the loss of the fifth coding exon from both PLP and DM-20 mRNA is the primary defect of the *jimpy* mutant.

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15.12 EXPRESSION OF A HOMEOBOX GENE IN THE CNS OF NORMAL AND TRANSGENIC MICE. J Zakany*, M. Patel*, C. M. Nguyen-Huu* (SPON: C. Noback) Depts. of Urology and Microbiology, College of Physicians and Surgeons, Columbia University, New York, NY, 10032.

Several mouse homeobox containing genes are expressed in specific regions of the developing central nervous system. We have isolated a murine homeobox containing gene localized on chromosome 6 designated Hox-1.3. The longest open reading frame in the cDNA sequence predicts a protein of 269 aminoacid residues with the homeobox located very close to the carboxyl terminus. The sequence of the genomic DNA shows the presence oftwo exons separated by an 0.95 kb intron positioned 20 bp upstream of the homeobox. RN-ase protection experiments indicate that the 5 end of the Hox-1.3 transcript is located at 0.25 kb upstream of the first ATG initiation codon. In the adult, Hox-1.3 transcripts are detected at high levels in the spinal cord and at lower levels in other tissues such as brain, testis, liver and kidney. In the midgestational embryo, the gene is mainly expressed in the lower myelencephalon and the cervical spinal cord.

In order to identify cis-acting regulatory sequences of the Hox-1.3 gene we have generated several lines of transgenic mice containing putative Hox-1.3 promoter/regulatory sequences fused to indicator sequences. The first fusion gene (Hox-1.3-hGh) contains about 3 kb 5 flanking sequences, the first exon, the intron and part of the second exon of Hox-1.3 fused in frame to human growth hormone gene sequences. The second fusion gene (Hox-1.3-lacZ) contains the complete Hox-1.3 gene with the structural gene for E. coli -galactosidase iserted immediately downstream of the initiation codon. The indicator sequences have been arranged to ensure their transcription into a hybrid message and translation into a hybrid Hox-1.3-hGH protein or a functional -galactosidase protein. Thus the expression of the transgenes can be analyzed at the molecular as well as histochemical levels. The results of the comparative analyses of the expression of the transgenes and the endogenous Hox-1.3 gene in the central nervous system will be presented.

15.13 **Interspecies Comparison of the Promoter Region of the Putative Drosophila Sodium Channel.** C. Smith* and L. Salkoff (SPON: L. Salkoff). Dept. Anat. & Neurobio., Washington Univ. Sch. Med., St. Louis, MO 63110.

Unlike the vertebrates, the expression of the voltage-sensitive sodium channel in the invertebrates, such as Drosophila, is limited to neural tissue. Thus, the promoter of the Drosophila sodium channel gene must contain elements that direct its expression uniquely to neurons. We are seeking to identify those promoter elements that specifically address the expression of the voltage-gated sodium channel gene to neurons. The deduced amino acid sequence of a Drosophila melanogaster ion channel, with high homology to the vertebrate sodium channel, has been determined, (Salkoff et al, Soc. Neuro. Sci. Abst., this issue). This deduced sequence encompasses all four homology units, containing all the intramembrane elements assumed to be essential for correct channel function. This includes 24 putative membrane spanning units (6 per homology unit) and 24 presumed gating charges having the same location as the gating charges of the vertebrate sodium channel.

The genomic DNA sequence for these regions as well as the promoter region 5' to the coding regions has been determined. We are conducting an interspecies comparative study of the promoter region of this possible sodium channel gene. Sequences located 5' to the transcriptional start site may be involved with controlling the expression of the gene so that it is expressed in the appropriate tissue type (neuron but not muscle), at the proper time in development and in the correct amount. We are using a second species, D. virilis, in this study to take advantage of the fact that Drosophila DNA drifts at approximately 1% per 1,000,000 years. D. virilis is about 50,000,000 years distant from melanogaster. Consequently, there is a high probability that any highly conserved regions in the promoter sequences of these two species will be of functional significance. This supposition is supported by the findings of Scholnick et al (Science 234:998-1002 (1986)) in their study of the melanogaster dopa decarboxylase promoter. A portion of this work compares the promoter regions of melanogaster and virilis. Many of the conserved sequences were shown to be essential for tissue specific expression and for controlling the level of expression in different tissues.

Our sequence data of the virilis promoter show several areas of very highly conserved DNA; in some instances the homology to the melanogaster sequence exceeds 90%. To begin our analysis of the significance of these conserved regions, we will link the virilis promoter to beta-galactosidase and, after p-element transformation of wild type Drosophila with this construct, we will study beta-galactosidase expression in response to specific alterations made to the conserved sequences in the promoter.

16.1 SEROTONIN FACILITATES A NON-CHOLINERGIC SYNAPSE IN THE BUCCAL MASS OF APLYSIA. D.P. Lotshaw and P.E. Lloyd, Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

Sensitization of defensive reflex behaviors in the marine mollusk Aplysia has been attributed to heterosynaptic facilitation of central sensory neuron to motor neuron synapses. Serotonin (5-HT) can mimic heterosynaptic facilitation at these synapses and 5-HT released from the metacerebral cell (MCC) has also been found to mediate arousal of feeding behavior by potentiating cholinergic buccal muscle contractions (Weiss et al., J. Neurophysiol. 41:181-203). In the present study 5-HT or MCC stimulation was found to facilitate a non-cholinergic neuro-muscular synapse in the buccal mass, demonstrating the existence of peripheral serotonergic heterosynaptic facilitation.

The motor neuron employed was one of the large ventral neurons of the symmetrically paired buccal ganglia. The neuron was located on the rostral border of the ventral cluster between the identified neurons B15 and B8 and was readily characterized by the absence of inhibitory synaptic input from the identified buccal neurons B4 and B5. The motor neuron innervated the most rostral I3 muscle bundles of the buccal mass. The I3 muscle consists of several parallel bundles which encircle and close the jaws upon contraction. Bursts of action potentials at frequencies greater than 15 Hz are required to induce muscle contractions. Contractions were not affected by the cholinergic antagonist hexamethonium. Motor neuron induced muscle contractions exhibited long lasting potentiation and the interval between motor neuron spike bursts was generally in excess of two minutes. Stimulation of the MCC or brief perfusion of 1μM 5-HT over the I3 muscle transiently increased isotonic contractions between 100% and 200% over controls.

Muscle post-synaptic potentials (PSP) were recorded intracellularly in response to brief bursts of motor neuron spikes evoked at 20 to 25 Hz because the PSP exhibited pronounced facilitation whereas single spike PSPs were not detectable. Perfusion of the muscle with 1 μM 5-HT or MCC stimulation induced multiple effects on the muscle membrane and synaptic transmission: the muscle fiber membrane potential usually hyperpolarized by a few mV, the muscle fiber input resistance decreased, the magnitude of the PSP increased, and the relaxation rate of the PSP increased. A post-synaptic site of action may partially explain these serotonergic effects. If the muscle membrane hyperpolarization may be attributed to an increased ion conductance, possibly K^+, the corresponding decreased input resistance would be expected to increase the relaxation rate of the PSP by decreasing the membrane time constant. The magnitude of the muscle fiber hyperpolarization was insufficient to account for the increased PSP amplitude, which was observed to increase by 50% to 100% over controls in response to 1 μM 5-HT or MCC stimulation. It remains to be determined whether the 5-HT induced increase in the PSP reflects an increase in transmitter release or an increase in the sensitivity of the post-synaptic membrane to the transmitter. (Supported by NIH NS23569 and Whitehall Foundation grants).

16.2 **STRUCTURE AND BIOLOGICAL ACTIVITY OF MYOMODULIN, A NOVEL NEUROPEPTIDE PRESENT IN AN IDENTIFIED CHOLINERGIC BUCCAL MOTONEURON OF *APLYSIA*.** **K.R. Weiss, E.C. Cropper, R. Tenenbaum* and I Kupfermann. Cntr. Neurobiol. & Behav., N.Y. State Psychiat. Inst., and Columbia Univ., N.Y., N.Y. 10032**

The accessory radula closer muscle (ARC) has provided a useful model for the study of motor control, specifically modulatory processes involved in the expression of food induced behavioral arousal. Food-arousal is a behavioral state that is expressed in part via modulation of the properties of muscle contractions. ARC contractions are known to be modulated by several substances, including a peptide factor, termed myomodulin, which is present in cholinergic ARC motoneuron B16. To help define the complement of potential modulatory peptides in the muscle we used HPLC to fractionate extracts of ARC muscle, and then tested the various fractions for bioactivity on the muscle. These studies have shown that the ARC contains numerous bioactive substances that either enhance or depress ARC contractions, and we have purified and sequenced a number of these substances. Inhibitory peaks include some unidentified substances as well as FMRFa and a novel peptide termed buccalin (Cropper et al., these abstracts). Two of the potentiating substances consist of SCP_A and SCP_B. Gas phase sequencing of a third potentiating substance established a putative sequence for a novel peptide. It was found that only the amidated replica had chromatographic and biological properties identical to that of the native peptide present in the muscle. The structure of this peptide is Pro-Met-Ser-Met-Leu-Arg-Leu-amide. In additional experiments utilizing 3 stages of HPLC, we found that this peptide precisely coelutes with the ^{35}S labeled peptidic factor myomodulin extracted from B16 neurons. We therefore conclude that the newly sequenced peptide is identical to the previously unsequenced factor-myomodulin, and that this peptide is present in the cholinergic motoneuron B16.

In conclusion, sequencing the SCPs and myomodulin from the ARC muscle and the localization of these peptides to the cholinergic motoneurons for the ARC muscle, coupled with our knowledge of the modulatory role of the serotonergic innervation of this muscle creates an exciting opportunity to study the role of multineuronal convergent modulation in a behavioral context.

16.3 **BUCCALIN: A NOVEL MODULATORY NEUROPEPTIDE COLOCALIZED TO THE SCP-CONTAINING CHOLINERGIC MOTONEURON B15 OF *APLYSIA*.** **E.C. Cropper, R. Tenenbaum*, I. Kupfermann, and K.R. Weiss. Cntr. Neurobiol. & Behav., N.Y. State Psychiat. Inst., and Columbia Univ., N.Y.,N.Y. 10032**

Recently we demonstrated that the cholinergic motor neuron B15 contains the two neuropeptides, SCP_A and SCP_B. These neuropeptides, when exogenously applied, potentiate motor neuron evoked contractions of the ARC, the muscle innervated by B15. B15s labeled with 35-S methionine, and fractionated using RP-HPLC, are however, characterized by a peak of radioactivity in addition to those which coelute with synthetic SCP_A or SCP_B. To determine whether B15 synthesizes a novel neuropeptide the non-SCP peak of radioactivity was added to material extracted from ARC muscles, and the peak was purified using four stages of sequential RP-HPLC, followed by gas phase sequence analysis. A peptide with the proposed structure for the B15 peptide was then commercially synthesized, and its chromatographic properties compared to those of the non-SCP peak of B15 radioactivity. From these experiments we determined that B15 does indeed contain a novel neuropeptide, buccalin, and that its structure is: Gly-Met-Asp-Ser-Leu-Ala-Phe-Ser-Gly-Gly-Leu-amide. To test its bioactivity buccalin was applied to ARC neuromuscular preparations. In contrast to the SCPs, buccalin decreased the size of motor neuron elicited contractions of the ARC. Also unlike the action of the SCPs, the change of contraction size was not associated with a change of relaxation rate, suggesting that buccalin is acting primarily presynaptically. Consistent with this idea we found that buccalin decreased ARC EJPs, but had no effect on the size of muscle contractions produced by direct application of ACh to the ARC.

In summary, we have purified and sequenced buccalin, a novel neuropepide; colocalized it with SCP_A and SCP_B to the cholinergic motor neuron B15, and demonstrated that when exogenously applied to the ARC it decreases ARC contraction size without affecting rate of relaxation. We have thus characterized an experimentally advantageous system for the behaviorally relevant study of peptide cotransmission.

16.4 TRANSPORT OF NEUROPEPTIDES FROM CENTRAL GANGLIA TO MUSCLES INVOLVED IN FEEDING IN *APLYSIA*. Philip E. Lloyd, Dept Pharm Physiol Sci, Univ Chicago, Chicago IL 60637

A number of neuropeptides with modulatory actions on feeding (buccal) muscles have recently been identified and sequenced. These peptides include FMRFamide (Fa), the two small cardioactive peptides (SCPs), myomodulin (Mm; Cropper et al., PNAS In Press), and bucculin (Bn; Cropper et al., these abstracts). These peptides modulate the efficacy of synaptic transmission between motor neurons and buccal muscle. Complements of these peptides are also found in motor neurons along with conventional transmitters. If these peptides are modulatory transmitters, it should be possible to demonstrate that they are transported from their sites of synthesis in the neuronal cell bodies to their presumed release sites in buccal muscle. To determine if such transport occurred, buccal or cerebral ganglia were incubated with ^{35}S-methionine in a small sub-chamber. Intact nerves ran through a vaseline barrier into a second larger sub-chamber containing the buccal mass. The outer chamber contained 100μM cold methionine to inhibit incorporation of leaked labelled methionine. After a 24 h label period, both chamber were chased with saline +1mM methionine for 24 or 48 h. At the end of these periods, individual muscles or muscle groups were dissected, extracted in the presence of cold peptides, and run on RP-HPLC (counter-ion: TFA) and aliquots of the samples counted. Remaining aliquots of peaks were run on a different RP-HPLC system (counter-ion: HFBA) to support tentative peptide identifications. The results can be summarized as follows: i) All 5 peptides were transported from buccal ganglia to buccal muscles; ii) In overall quantities of transport, the peptides were ranked Mm ≥SCPs >Fa >>Bn; iii) Different complements of the peptides were reproducibly transported to different muscles e.g. peptides transported to the ARC muscle were ranked Mm ~SCPs ~Bn >>>Fa; iv) Mm was the predominant peptide transported via the radula nerves; v) No measureable peptide transport from the cerebral ganglia to buccal muscle was observed. However, Mm was the predominant peptide transported from the cerebral to the buccal ganglia suggesting that this peptide may have important central actions. These results indicate that these peptides are all likely to be transmitters and that peptide complements should vary from muscle to muscle. (supported by NIH NS23569 and the Whitehall Foundation)

16.5 **PURIFICATION AND SEQUENCING OF TWO NEUROPEPTIDES CONTAINED IN NEURON R15 OF *APLYSIA*.** I. Kupfermann, H. Bayley*, P.E. LLoyd, R. Tenenbaum*, L. Buck*, E.C. Cropper, and K.R. Weiss. (SPON: L.D. Mitchell) Cntr. Neurobiol. & Behav., N.Y. State Psychiat. Inst., and Columbia Univ., N.Y.,N.Y. 10032

A peptidic factor contained in the neuron R15 increases the water content of *Aplysia* suggesting that peptides released from the cell regulate water balance (Kupfermann & Weiss, 1976). We now report the purification and sequence analysis of two R15 peptides, one of which mimics the bioactivity of the peptidic factor.

Peptides were extracted from 820 dissected cells and separated by two steps of reverse-phase HPLC. The purification yielded a single bioactive peptide (R15 α1) which was analyzed for amino acid content and subjected to sequence analysis, suggesting the structure:

Asp-Val-Ser-Asp-Gly-Ser-Ala-Glu-Arg-Arg-Pro-Tyr-Thr-Arg-

Met-Gly-Ser-Gly-Gly-Leu-Lys-Leu-His-Cys-Gln-Val-His-Pro-

Ala-Asn-Cys-Pro-Gly-Gly-Leu-Met-Val-Thr.

The peptide failed to react with iodoacetate indicating that the two cysteines are connected by a disulfide bridge. To confirm the assigned structure the peptide was synthesized, with a disulfide bridge. The chromatographic properties and bioactivity of the synthetic material were identical to those of the native peptide. Interestingly, the peptide contains the sequence Arg-Arg, a potential site for proteolytic processing.

Several other HPLC peaks were inactive in the bioassay for water uptake. The sequence of one of these peptides (R15 β) is:

Ser-Asp-Leu-Leu-Gly-Ala-Leu-Leu-Ser-Arg-Asn-Ser-Pro-

Ser-Ser-Tyr-Gly-Leu-Pro-Ser-Arg-Asp-Met-Ser-Thr-Ala-Tyr.

Both peptide sequences are encoded within a single, recently sequenced, cDNA (Buck et al.).

16.6 PRIMARY STRUCTURE OF Aplysia ATRIAL GLAND AND BAG CELL PEPTIDES. G.T. Nagle, S.D. Painter, J.E. Blankenship and A. Kurosky* Marine Biomedical Institute and *Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston,TX 77550.

The role of the neuroendocrine bag cells in the initiation of egg laying has been intensively investigated in the marine mollusc Aplysia californica. In vivo and in vitro electrophysiological studies of A. brasiliana bag cells, however, have contributed significantly to our knowledge of the bag-cell system. We therefore purified and chemically characterized the egg-laying hormone (ELH) from the bag cells of A. brasiliana. Amino acid compositional and sequence analyses demonstrated that it is a 36-residue peptide whose sequence is identical to that of A. californica ELH. This is consistent with earlier observations that suggested extensive sequence homologies between the two peptides: (1) bag-cell extracts from either species induce egg laying when injected into the other species; and (2) A. brasiliana genomic DNA hybridizes with an A. californica ELH cDNA probe.

While the bag cell egg-laying hormones of A. brasiliana and A. californica are chemically identical, they differ from three ELH-related peptides expressed in the A. californica atrial gland, an exocrine organ secreting into the oviduct (Nagle et al., J. Biol. Chem. 261:7853, 1986; Rothman et al., J. Biol. Chem. 261:1616, 1986). Unlike bag cell ELH, each of the three atrial gland peptides (A-ELH, [Ala^{27}]A-ELH, and [Gln^{23},Ala^{27}]A-ELH) is disulfide-bonded to an identical 18-residue acidic peptide (A-AP). A small proportion (20%) of the atrial gland peptide complexes are further cleaved in vivo.

In addition to the ELH-related sequences, we have isolated and chemically characterized several other peptide products of ELH-family genes expressed in the atrial gland. These include peptide A, A-AP and a 13-residue peptide whose amino acid composition matches a sequence predicted from genetic analyses of an A gene cDNA clone. We have also isolated peptide B and a 13-residue peptide whose amino acid composition matches a sequence predicted from genetic analyses of a genomic B clone. The two 13-residue peptides define the precise site of signal sequence cleavage in the peptide A and B precursors. Supported by NSF BNS85-17575, NIH 22079, & NIH 11255.

16.7 FRESHLY DEPOSITED EGG CORDONS AND ATRIAL GLAND FACTORS INDUCE COPULATORY BEHAVIOR IN APLYSIA. S.D. Painter, A.R. Gustavson*[1],V.K. Kalman, G.T. Nagle and J.E. Blankenship. The Marine Biomedical Institute, University of Texas Medical Branch, Galveston,TX 77550 and [1]Dept. of Psychology, University of Southern California, Los Angeles,CA 90089.

Egg-laying activity in the marine mollusc Aplysia appears to be regulated by a small family of genes, each of which encodes a polyprotein precursor containing a peptide sequence homologous to egg-laying hormone (ELH). The ELH-family genes are expressed in an organ-specific manner within the animal. Thus, for example, the ELH gene is expressed in the neuroendocrine bag cells of the abdominal ganglion. The peptide products of this gene induce egg release from the ovotestis and have well-defined effects on both neuronal and behavioral activity. In contrast, at least three other genes of the ELH family are expressed in the atrial gland, an exocrine organ secreting into the oviduct of Aplysia. The peptide products of the atrial gland genes have no known physiological function. Our experiments were designed to test the hypothesis that the atrial gland gene products might serve a pheromonal role and coordinate reproductive activity among (rather than within) individuals. Our studies show that there is a shorter latency to copulation when an Aplysia is paired with an animal that is actively laying eggs than when it is paired with a sexually mature but non-laying animal. Moreover, the effect of egg deposition on latency to copulation can be mimicked by the addition of either acidic or neutral extracts of the atrial gland to the bathing medium. In contrast, addition of extracts of other regions of the reproductive tract, including the oviduct, do not influence the latency to copulation. These results suggest that atrial gland products, secreted onto the egg cordon as it passes through the oviduct, may play a pheromonal role and influence reproductive behavior among individuals. Experiments are in progress to determine whether or not the active factor(s) are products of the ELH-family genes expressed in this organ. Supported by NIH Grants NS-22079 and NS-11255, and by NSF Grant BNS 85-17575.

16.8 SYNTHETIC NEUROPEPTIDE EGG-LAYING HORMONE (ELH) OF APLYSIA CALIFORNICA INDUCES NORMAL EGG-LAYING: STRUCTURE-ACTIVITY STUDIES. F. Strumwasser, D. L. Schiller* and S.B.H. Kent*. Marine Biological Laboratory, Woods Hole, MA 02543 and Division of Biology, California Institute of Technology, Pasadena, CA 91125.

ELH in Aplysia is synthesized by the bag cell neurons of the abdominal ganglion and controls egg-laying (EL) and associated behaviors. ELH contains 36 amino-acid residues and has an amidated carboxy-terminal lysine (Chiu et al., '79). The primary structure of the preprohormone for ELH has been deduced from a cDNA clone. The prohormone has 271 amino-acid residues and ten putative cleavage sites (Scheller et al., '83). There is good evidence that a number of peptides, besides ELH, are released during a synaptically initiated electrical discharge of the bag cells. ELH and an acidic peptide (pI 4.8) are released (Stuart et al., '80) as well as two forms of α-bag cell peptide (Sigvardt et al., '86). Other as yet unidentified peptides are also released during the bag cell discharge. These findings raise the possibility that the integrated behavior of EL is due to the action of several peptides rather than ELH alone, although purified ELH induces EL. Our studies were directed at addressing whether synthetic ELH is sufficient to induce normal EL and to determine what portion of the peptide is necessary for EL. ELH and its fragments were synthesized by either manual or automatic stepwise solid phase peptide synthesis on a benzhydrylamine-resin and purified by reverse-phase HPLC on a Vydac C4 column. Purity, as judged by analytical HPLC, was 92-98%. Synthetic ELH 1-36 amide induced normal EL when injected at doses of 70-200 ug in 7/7 animals. Locomotion was inhibited, eggs were extruded in the egg string, the string was properly attached to the substrate and wound, and the weight of egg string produced and the latency to onset of EL were normal. Thus we conclude that ELH is sufficient to induce all the presently known components of EL. Although purified ELH is reported to induce EL at approximately 2.5 nanomoles (11 ug), we used approximately 10 to 100 times this quantity when testing fragments. Removal of either the amino-terminal or carboxy-terminal 7 amino-acid residues resulted in a peptide unable to elicit EL. Thus ELH 8-36 amide (1500 ug), ELH 14-36 amide (1250 ug) and ELH 1-29 amide (1500 ug) did not induce EL. This implies that both relatively intact amino- and carboxy-termini are essential for EL. This fact is particularly interesting in that there is specific charge conservation in the position of residues 6, 8, 12, 17, 21, 22, 24, 28, 30, 32 and 36 when the sequences of A. californica and A. parvula (Nambu and Scheller, '86) are compared. Specific charge conservation is also present in the ELH of the fresh water snail Lymnaea stagnalis except at residues 21, 28 and 36 (Ebberink et al., '85). Subtle changes such as ELH 1-35 amide (200 ug) allow normal EL in A. c. (Supported by NS21046 to F.S. and NSF DMB85-00298 to S.B.H.K.)

16.9 STRUCTURE AND FUNCTION OF A NOVEL NEUROPEPTIDE (CALFLUXIN) GENERATED FROM THE EGG-LAYING HORMONE PRECURSOR OF *LYMNAEA*. R.H.M. Ebberink*, W.J.A.G. Dictus* and J. Joosse* (SPON: European Neuroscience Association). Department of Biology, Vrije Universiteit, Amsterdam, The Netherlands, NL-1007 MC.

Recent studies on the role of Ca^{2+} as an intracellular messenger in the regulation of the secretion process in a female accessory sex gland (albumen gland) of the freshwater snail *Lymnaea stagnalis* have shown that the glandular cells contain in a particular stage of the egg-laying process a relative high concentration of intracellular Ca^{2+}. Subsequent in vitro studies indicated that the increase in intracellular Ca^{2+} concentration is induced by a neuropeptide (calfluxin). This peptide could be extracted from the cerebral commissure, the neurohaemal area of the caudo dorsal cells, which regulate the complex processes of egg-laying and the associated behavior.

Calfluxin has been purified and the structure elucidated (Arg-Val-Asp-Ser-Ala-Asp-Glu-Ser-Asn-Asp-Asp-Gly-Phe-Asp). The chromatographical and biological behavior of the synthetic peptide is identical to that of the native peptide. Calfluxin appears to be one of the peptides generated from the egg-laying hormone precursor of *Lymnaea*. Beside calfluxine at this moment five other peptides of the precursor are of interest: the ovulation hormone (egg-laying hormone), three ß-CDCPs (pentapeptides) and α-CDCP (nonapeptide). Calfluxine is situated between the ß-CDCPs and α-CDCP region of the precursor.

Comparison of the egg-laying hormone precursors of *Lymnaea* and *Aplysia* show significant homology in the egg-laying hormone regions (50%), the aCDCP/aBCP region (70%) and the ß-CDCP/ß-,χ-BCP regions (80-100%). The region converting the ß-CDCP with α-CDCP (the calfluxin region) is much shorter than the counterpart of the ELH precursor. However, the amino acid sequences 4-7 and 8-12 of calfluxin are located uninterrupted on the counterpart of the *Aplysia* precursor.

Signal-respons coupling was studied with calfluxin, and these studies indicate that mobilization of intracellular Ca^{2+} is mediated via the protein kinase C stimulated activation of the Na^+/H^+-exchange, thus leading to an increase of the internal pH. IP_3 may have a role in the mobilization of Ca^{2+} from internal sources, whereas the influx of extracellular Ca^{2+} is mediated by protein kinase C independent Ca^{2+}-channels.

16.10 ACTIVATION OF THE BAG CELLS BY ELH/BCP-IMMUNOREACTIVE NEURONS IN THE RIGHT PLEURAL GANGLION OF APLYSIA CALIFORNICA. R.O. Brown and E. Mayeri. Div. of Neuroscience, Dept. of Physiology, University of California, San Francisco, CA 94143-0444.

The bag cells are 800 neuroendocrine cells in the abdominal ganglion of Aplysia which control egg-laying. They fire in episodic discharges to release multiple neuropeptides, including egg-laying hormone (ELH) and alpha-bag cell peptide (α-BCP), derived from a common precursor. Immunoreactive ELH and α-BCP have also been co-localized to small clusters of 12-24 neurons in the cerebral ganglion and 4-16 neurons in the pleural ganglion. The electrophysiological properties and functions of these head ganglia ELH/BCP cells, and their relationship to the bag cells, have not previously been described.

We identified a cluster of white cells in the right pleural ganglion which resemble the bag cells morphologically and physiologically. Intracellular recordings were made from 18 of these cells in 12 preparations. In 2 preparations they were injected with Lucifer Yellow, and subsequent immunocytochemical processing with α-BCP antiserum confirmed they were ELH/BCP cells.

Intracellular electrical stimulation of ELH/BCP cells in isolated right pleural ganglia caused depolarizing afterpotentials and synchronous afterdischarges (N=4 out of 4 preparations). This indicates that the pleural ELH/BCP cells, like bag cells, posess an intrinsic positive feedback mechanism to produce regenerative discharges.

In combined right pleural plus abdominal ganglia preparations with the right pleuroabdominal connectives intact, activation of pleural ELH/BCP cells by intracellular stimulation caused depolarizations and afterdischarges in the abdominal ganglion bag cells (5/5). Direct stimulation of bag cell discharges in the abdominal ganglion caused depolarizations (2/3) but not discharges (0/3) in the pleural ELH/BCP cells. These results indicate a bidirectional, but asymmetrical, functional connection between the pleural ELH/BCP cells and the bag cells.

α-BCP, and the structurally related ß-BCP and γ-BCP, have autoexcitatory effects on the bag cells, and are candidate neurotransmitters for the spread of activation between pleural ELH/BCP cells and the bag cells.

The ELH/BCP cells in the pleural ganglion are the first identified cells found to activate the bag cells. They, and by extension the cerebral ELH/BCP cells, may comprise a descending neuronal pathway which normally triggers bag cell discharges. In addition to activating the bag cells, neuropeptides released by these ELH/BCP cells might also act directly on neurons in the head ganglia to mediate aspects of egg-laying behavior.

This work was supported by NIH grant NS16490.

16.11 **INACTIVATION OF ALPHA-BAG CELL PEPTIDE IN THE ABDOMINAL GANGLION OF APLYSIA.** B.S. Rothman, G.A. Phares* and T.A. Groves*. Dept. of Biology, San Francisco State University, San Francisco, CA 94132.

We are using the isolated abdominal ganglion of Aplysia to study in detail the inactivation of a single peptide neurotransmitter, alpha-bag cell peptide (α-BCP[1-9]). α-BCP and a group of 6-9 other peptides are synthesized and released by the bag cells, two clusters of 400 homogeneous neuroendocrine cells located in the rostral margin of the ganglion. α-BCP mediates bag cell-induced inhibition of left upper quadrant (LUQ) neurons L2, L3, L4 and L6.

Sigvardt et al. (J. Neurosci. 6: 803) showed that after electrical stimulation of the bag cells, recovery from perfusate of LUQ inhibitory activity depended on a battery of protease inhibitors, and recovery of α BCP[1-9] and its derivative, α-BCP[1-8], strictly depended on the presence of diprotin A, a dipeptidyl-aminopeptidase inhibitor. These findings suggested that both α-BCP[1-9] and α-BCP[1-8] were inactivated by a membrane-bound peptidase located within the extracellular space of the ganglion. Furthermore, since α-BCP[1-8] was also recovered, and this peptide is 30-fold more active than α-BCP[1-9] on LUQ neurons, it seemed likely that α-BCP[1-9] underwent both activation and inactivation by separate peptidases.

To further test these hypotheses, we mimicked release of α-BCP without the concomitant release of the other bag cell peptides by arterially perfusing synthetic α-BCP[1-9] into the ganglion. Specifically, an isolated abdominal ganglion was mounted in a low dead-volume sealed chamber, and continuously perfused with an artificial sea water solution containing α-BCP plus 40 µg/ml of protein carriers. The perfusate was collected on ice and analyzed by a reverse phase-HPLC system that can resolve all α-BCP fragments tested. When α-BCP[1-9] was perfused at concentrations of 1-10 µM, 40% to 80% of intact α-BCP was recovered, and two fragments, α-BCP[3-9] and α-BCP[1-8], were generated respectively at about 10% and 5% the levels of α-BCP[1-9]. These products were identified by comigration with chemically synthesized standards on HPLC under isocratic conditions. Diprotin A inhibited generation of α-BCP[3-9] in a dose dependent manner, with an IC50 of 2 µM, but had little effect on the generation of α-BCP[1-8]. Arterial perfusion of α-BCP[1-8], under conditions identical to those above, generated α-BCP[3-8] and α-BCP[1-7], fragments analogous to those derived from perfusion of α-BCP[1-9].

These results provide additional evidence that α-BCP[1-9] is both activated and inactivated by membrane-bound peptidases. Combined with the findings of Sigvardt et al., the results suggest that inactivation is the dominant process. These processes may provide a means by which α-BCP[1-9] can produce short-lasting but intense actions limited to the abdominal ganglion.

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16.12 THE BAG CELL PEPTIDES ALPHA-BCP AND ELH MODULATE THE EXCITABILITY OF TARGET NEURONS IN THE ABDOMINAL GANGLION OF *APLYSIA* BY CHANGING Ca^{2+}- AND K^+-CURRENTS. Rene F. Jansen* and Earl Mayeri. Department of Physiology, University of California, San Francisco, CA 94143.

The neuropeptides egg-laying hormone (ELH) and alpha-bag cell peptide (alpha-BCP) are putative neurotransmitters derived from a common precursor protein, and are released by the bag cells in the abdominal ganglion of *Aplysia californica*. ELH is the neurotransmitter that mediates the long-lasting excitation of the left lower quadrant (LUQ) neurons, and alpha-BCP mediates inhibition of the left upper quadrant (LUQ) neurons. We here report further on the voltage clamp analysis of the ionic mechanisms of long term regulation of neuronal excitability by ELH and alpha-BCP.

Pressure application of alpha-BCP onto surgically isolated LUQ neurons causes a hyperpolarization which interrupts ongoing pacemaker activity in these neurons. The slow inward current that underlies pacemaker activity is partly blocked by Ca^{2+}-channel blockers and partly by TTX. Experiments indicate that alpha-BCP closes the slowly inactivating Ca^{2+}-channel. In Ba/TEA/4AP-containing seawater, the alpha-BCP evoked current has a U-shaped I-V characteristic that peaks at about 10 mV. It is blocked by adding the Ca-channel blockers Co^{2+}, Cd^{2+} or Ni^{2+} to the medium, and is unaffected by removing external Na^+ or adding TTX. Alpha-BCP additionally causes an increase in K^+-conductance. This K^+-current is blocked completely by external Rb^+ (5 mM), and partially by external Cs^+ (10 mM).

ELH induces hours-long repetitive firing in LLQ neurons without substantially changing the resting membrane potential. The currents evoked by ELH in these neurons include: i) a hyperpolarizing, inwardly rectifying K^+-current that is blocked by Rb^+ (5 mM) and Cs^+ (10 mM), and which has a chord-conductance that shifts with the external $[K^+]$, ii) a small, steady depolarizing current and iii) a depolarizing current activated at membrane potentials above -40 mV.

The second current is Na^+-dependent, present at all membrane potentials tested, and is the only current that remains after prolonged exposure (typically 1hr.) to 0 mM Ca^{2+}/ 10 mM Ni^{2+}-containing medium. The third current is apparently unaffected by adding Ni^{2+} (10 mM) to Ca^{2+}-containing medium (10-15 min.), and is not blocked by 0 mM Na^+ or 100 mM TEA/ 0 mM Na^+; we are currently investigating the nature of this current. The ionic mechanism of action of ELH on the LLQ neurons is apparently different from that of ELH on the buccal motorneuron B_{16} described by Kirk and Scheller (Proc.Natl.Acad.Sci. 83:3017).

We conclude that alpha-BCP and ELH are neurotransmitters that use multiple and apparently distinct ionic mechanisms to modulate the excitability of their respective target neurons.

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16.13 **MODULATION OF IONIC CURRENTS IN THE IDENTIFIED MOTORNEURON B15 OF APLYSIA BY 5-HT, SCPb, AND FMRFamide.** Ronald Taussig* and Richard H. Scheller, Dept of Biol. Sci. Stanford Univ., Stanford, Ca 94305

The accessory radular closure muscle in Aplysia is inervated by two identified buccal ganglion motorneurons, B15 and B16. Previous studies have demonstrated that several neurotransmitters have direct affects on the electrical activities of these neurons. Egg-laying hormone and the biogenic amine serotonin (5-HT) both excite B16, while small cardioactive peptide b (SCPb) and 5-HT depolarize B15. Both motorneurons are inhibited by the amidated tetrapeptide Phe-Met-Arg-Phe-amide (FMRFamide). Using the two electrode voltage clamp technique, we have identified ionic currents in B15 which are modulated by 5-HT, SCPb, and FMRFamide.

5-HT, SCPb, and FMRFamide were bath applied to an axotomized B15 under voltage clamp. In normal saline containing 20 mM Cobalt, both 5-HT (50 uM) and SCPb (10 uM) induced or enhanced a voltage dependent slow inward current. Replacement of sodium with N-Methyl D-Glucamine abolished this response but uncovered two additional currents which were affected by application of either 5-HT or SCPb. Both of these currents were sensitive to varying extracellular potassium concentrations.

Bath application of FMRFamide (5 uM) to B15 modulate currents in opposite directions to those of the excitatory transmitters 5-HT or SCPb. FMRFamide reduced the voltage dependent slow inward carried by sodium, while simultaneusly increasing a current resembling the S-current.

AUDITORY SYSTEM I

17.1 CYTOCHALASIN D SUPPRESSES SOUND EVOKED POTENTIALS IN THE GUINEA PIG COCHLEA. S.E. Barron, R.P. Bobbin, P.S. Guth, and C.H. Norris, (Dept. of Pharmacology, Tulane Medical School and Dept. of Otorhinolaryngology LSU Medical School, New Orleans, LA 70112)

Although actin has been localized in hair cells, evidence for its role in intact cochlear preparations is lacking. The present study was undertaken to test the hypothesis that actin is involved in cochlear function (e.g. the active process) by observing the effects of a potent inhibitor of actin polymerization, cytochalasin D, on evoked cochlear potentials. Perilymphatic spaces of guinea pig cochlea were perfused with Ringer solutions containing either cytochalasin D (10^{-8} to 10^{-5} M) or DMSO (0.00005% to 0.05%) at a rate of 2.5 µl/min for 10 min (Bledsoe et al., Hear. Res. 4:109, 1981). Immediately after each period of perfusion, the compound potential (CAP), N_1 latency, cochlear microphonics (CM), and summating potential (SP) evoked by 10 kHz tone bursts of varying intensity were recorded from an electrode in the basal turn. Cytochalasin D suppressed CAP, N_1 latency and SP in a dose-dependent fashion but had only slight effects on CM. DMSO had no effect. These results provide pharmacological evidence that actin is involved in cochlear function. . . possibly the active mechanical process of the organ of Corti. (Supported by Grants from NSF, NINCDS, the Southern Hearing and Speech Foundation, Kresge Foundation and the LA. Lions Eye Fnd.)

17.2 THE ORIGIN OF PHASE LOCKING IN THE AUDITORY RESPONSE IN THE PIGEON. K.G. Hill*, Jianwu Mo* and G. Stange*. SPON: (Eldon E. Ball). Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra City, ACT 2601, Australia.

Recordings were obtained of spike potentials in single fibres in the VIIIth nerve of the pigeon (Columba livia) in order to study properties of phase locking to a sinusoidal stimulus waveform. In response to closed-field, monaural sound presentation, tuning and intensity functions of spike responses were determined. From measures of spike time to 4 µs accuracy, period histograms were constructed and characterized in terms of vector strength and phase angle of response. Intensity functions also were determined for vector strength and phase angle of response for individual fibres at several frequencies.

Typically, response-frequency profiles showed a single peak, denoting fibre characteristic frequency (CF), bordered at adjacent higher and lower frequencies by areas of suppression of background firing rate. With increasing sound pressure level (SPL) the response peak widened and suppression bands shifted to higher and lower frequencies. Spike rate intensity functions at CF showed monotonic increase, whereas at adjacent lower and higher frequencies, spike rate initially declined then increased with increasing SPL. Vector strength intensity functions monotonically increased at both CF and at adjacent frequencies, starting below excitatory rate threshold or at suppression thresholds, independent of increasing or decreasing spike rate.

At low SPL up to about 50dB, intensity functions for phase angle of response to different frequencies were rather flat. The phase angle of response to frequency below CF, however, was greater than that at CF, indicating relative phase lag, whereas, phase angle for frequency above CF was less than that at CF, indicating relative phase lead. Phase angles of response in a single fibre to three frequencies, at, above and below CF, closely match measurements of group delay in the basilar membrane (BM) velocity response (Gummer, A.W., Smolders, J. and Klinke, R. Hearing Research, in press.

The independance of vector strength intensity functions from spike rate functions imply that phase locking of spikes does not depend on transsynaptic excitation of afferent fibres in accord with hair cell receptor potentials. Close correspondence between phase angle of spike response and group delay of BM mechanical response at CF suggests that the factor responsible for phase locking has a frequency-dependent locus on the BM. It is suggested that this factor is the cochlear microphonic potential.

17.3 THE EFFECTS OF NOISE ON PHASE-LOCKED DISCHARGES OF AMPHIBIAN AUDITORY NERVE FIBERS. P.M. Narins. Dept. of Biology, Univ. of California at Los Angeles, Los Angeles, CA 90024.

Amphibian auditory nerve fibers fire in a phase-locked fashion in response to sinusoidal stimuli of frequencies up to 1.2 kHz at 22°C. For many species of frogs this range includes not only sounds of biological significance (predators, prey, etc.) but communication signal frequencies as well. In this study the degree of phase-locking was quantified for 65 single units in the Puerto Rican coqui, Eleutherodactylus coqui, both in response to tones presented alone, and in the presence of continuous, broadband masking noise of various levels. Detailed studies of the low-frequency, suppressible fibers and the mid-frequency, non-suppressible fibers from the amphibian papilla (a.p.) and high frequency, non-suppressible fibers from the basilar papilla (b.p.) revealed that fibers from all three populations exhibit clear phase-locked responses to pure tones. B.p. units only phase-lock to test frequencies (TFs) below the fiber's characteristic frequency (CF). The phase-vs.-intensity functions for all fibers are complex; they show level-independence near threshold intensities, follow no consistent pattern at middle intensities (10-40 dB>CF-threshold), but at higher intensities another pattern emerges. For TFs>CF, increasing intensity results in an increased phase lead relative to CF, and for TFs<CF raising intensity results in an increased phase lag relative to CF. This pattern is most easily seen for a.p. fibers.

Wideband noise presented at increasing levels progressively reduced the phase-locking of auditory nerve fibers to tones. Specifically, the vector strength (VS, a quantitative measure of phase-locking) falls off with increasing levels of masking noise. The noise level required to produce a fixed reduction in VS was clearly related to the fibers CF-threshold. However, on the average, TFs<CF showed a steeper rate of VS falloff with masking noise level than do TFs>CF. Moreover, TFs<CF showed either phase-independence or a slight phase lag with increased masking noise level whereas those TFs>CF exhibited a pronounced phase lead in the presence of masking noise. These effects were observed for all a.p. fibers tested, suggesting that the underlying mechanisms do not necessarily involve two-tone suppression.

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17.4 DERIVED AUDITORY POTENTIALS IN THE ALLIGATOR LIZARD (Gerrhonotus multicarinatus). T.A. Jones and T.N. Decker*, Depts. of Oral Biology, UNMC and Special Education and Communication Disorders, UNL Lincoln, NE 68583.

In birds and mammals, a vibration of the basilar membrane travels from the base to the apex of the cochlea at a velocity which decreases as a function of distance. The time of its arrival at any particular position will depend on the traveling wave velocities encountered and the distance traversed during travel. Through the use of a high-pass subtractive masking paradigm (Teas et al., 1962 . Don and Eggermont 1978) derived potentials can be recorded that temporally relate the latency of neural activation as a function of place of stimulation on the membrane. Typically in the mammal the relationship shows that as progressively more apical portions of the basilar membrane are masked, the remaining far-field components evoked by the click are shifted later in time. The progressive latency shift is thought to be indicative of the traveling wave on the basilar membrane. This hypothesis has not been explicitly tested in an ear that does not have a traveling wave phenomenon. The auditory system of the alligator lizard (Gerrhonotus multicarinatus) has been shown to have a relatively wide frequency response and neurons show relatively sharp tuning curves between 100 and 5KHz. Yet the ear does not employ smoothly graded mechanical tuning in the periphery to accomplish frequency analysis (Weiss et al., 1978). Therefore, it does not have a continuous preeminent cochlear traveling wave. In the present study derived far-field auditory responses were recorded in the alligator lizard using broad band stimuli (acoustic clicks) and maskers (high pass cutoffs: half octave steps, 250-8000Hz, slopes: -96dB/octave). Clicks were presented at a repetition rate of 10/sec. Auditory responses were obtained using subcutaneous electrodes (vertex-G1, ipsilateral neck=G2, contralateral neck = ground). Electrical signals were led to an amplifier (10,000X; LF=100; HF=10,000) and in turn to a computer and digitized. Threshold was determined and 20dBSL clicks used to elicite responses thereafter. The level of unfiltered wide-band noise sufficient to mask far-field responses was determined. Without changing intensity levels, far-field responses to 20 dBSL clicks were recorded in the presence of high-pass-filtered noise. Maskers were presented in descending order according to frequency cutoff (8000 Hz to 250 Hz). The narrow-band contributions to auditory responses were generated by successively substracting the response waveforms obtained in the presence of noise at each half-octave cutoff. Peak-to-peak amplitudes and latencies of the response onset were measured.

Tonotopic groups making substantial amplitude contributions to the far-field response included those between 500 and 8000 Hz. The progressive shifts in onset latency normally associated with these tonotopic groups in mammals were essentially absent in lizard derived responses from 1000-8000Hz. These findings support the hypothesis that the latency shifts normally associated with tonotopic groups in mammals do reflect the temporal behavior of a traveling wave.

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17.5 EFFECT OF TEMPERATURE ON THE TUNING OF ORTHOPTERAN HAIR CELLS. B.P. Oldfield* (SPON: J.D. Pettigrew). Neuroscience Laboratory, Dept. of Physiology and Pharmacology, Univ. of Qld., St. Lucia, 4067, Qld. Australia.

Despite recent evidence of intrinsic electrical tuning in some vertebrate hair cells (reviewed in 1) such mechanisms are not thought to be responsible for the tuning of hair cells, such as those of mammals with characteristic sound frequencies greater than 1 kHz (2). Orthopteran hair cells, however, are tuned to sound frequencies greater than 1 kHz without the assistance of peripheral mechanical filters (3,4,5). The nature of the intrinsic tuning mechanism in these auditory receptors is yet to be identified. A primary tool in discriminating between intrinsic and extrinsic tuning mechanisms has been the quantification of the effect of temperature on the tuning of hair cells (6,7). This tool has been applied to the hair cells of the locust *Valanga irregularis* (Orthoptera, Acrididae) where I show that the tuning mechanism is even more temperature-dependent than that of vertebrate hair cells known to be intrinsically-tuned.

The auditory organs of locusts contain up to 70 auditory receptors and are tuned to sound frequencies between 1 and 18 kHz. By isolating the auditory organ and recording from receptors with glass microelectrodes I have shown that the characteristic sound frequency of individual hair cells undergoes a reversible increase for temperature increases between 1 and 7°C. The average change in characteristic frequency (n=16) was 0.14 octaves/°C, which corresponds to a thermal Q_{10} of 2.7. This value exceeds that expected from temperature-dependent changes in the mechanical properties of a simple mechanical resonator. The extreme temperature-dependence of the tuning properties thus supports other evidence that these hair cells are tuned intrinsically, and not by virtue of the mechanical properties of peripheral filters. This finding confirms that auditory receptors can be tuned to sound frequencies in excess of 1 kHz as a result of intrinsic, possibly electromechanical, properties.

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17.6 COCHLEAR PERFUSION TO STUDY NEUROCHEMICAL MECHANISMS AT A MULTITRANSMITTER SYNAPSE. D.W. Hoffman, K.L. Jones* and A.E. Stinnett* (SPON: E.J. Walsh). Neurochemistry Lab, SIU Sch. of Med., Springfield, IL 62708

We are using the chinchilla cochlea as a model system to study neurochemical interactions of co-localized transmitters. The cochlea is innervated by brainstem neurons which terminate under both inner and outer hair cells. Those which innervate eighth nerve dendrites under inner hair cells have been shown to contain enkephalins (Fex and Altschuler, PNAS 78:1255, 1981) dynorphins (Hoffman et al., Hearing Res., 17:47, 1985) and acetylcholine (ACh) (Norris and Guth, Acta Otolaryngol., 77:318, 1974). We use this preparation as an in situ perfusion chamber to study neurochemical interactions of these co-localized transmitters.

The auditory bulla is approached ventrolaterally to avoid disruption of the ossicular chain and tympanic membrane. The bulla is opened, and a hole is drilled into the scala tympani of the cochlea near the base, using a fine pick and root canal file. Glass capillary tubing drawn out to a fine tip is cemented into the hole with cyanoacrylate ester. PE-10 tubing connects the pipette to a microliter syringe driven by a syringe pump, which can provide flow rates of as low as 1 microliter/min. A second (efflux) pipette is placed near the first, but in the scala vestibuli, to provide complete perfusion of the perilymphatic space. We use this preparation to study neurotransmitter release, and transmitter interactions in this natural perfusion chamber.

Met- and leu-enkephalin, dynorphin B and ACh are all released by high potassium or veratridine. Peptides are measured by HPLC-RIA, and ACh by radioenzymatic assay. These results expand the data on the neurotransmitter/neuromodulator role of these neuropeptides in the inner ear, and confirm the earlier identification of ACh in inner ear perfusates by bioassay. We are now seeking to determine the effects of neuropeptides and ACh and each other's release processes.

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17.7 ISOLATION OF NEUROACTIVE SUBSTANCES FROM HAIR CELL EXTRACTS. W. F. Sewell and E. A. Mroz *. Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Departments of Otolaryngology and Physiology, and the Program in Neuroscience, Harvard Medical School, Boston, MA. 02114.

Hair cells, the sensory cells of the inner ear, release a yet-unidentified neurotransmitter to excite primary afferent neurons. Because this neurotransmitter does not appear to be one of the known neurotransmitter candidates, our strategy for its identification is to extract and purify substances from hair cell tissue that can excite afferent fibers innervating hair cells.

We have previously reported (Assoc. Res. Otolaryngol. Abstr. 9: 114, 1986) that aqueous extracts of goldfish (*Carassius auratus*) inner ears excite afferent fibers innervating the hair cells of the lateral line organ of *Xenopus laevis*. Gel-filtration chromatography (Sephadex G-10) of such extracts yields two peaks of excitatory activity, one eluting with low-molecular-weight substances (ca. 200) and one eluting with high-molecular-weight substances (> 700). Both peaks are well separated from glutamate and aspartate.

We now report that further purification of the high-molecular-weight peak by gel-filtration chromatography (Sephadex G-25) suggests that it is due to a substance with a molecular weight of about 4000. We have identified calcitonin gene-related peptide (molecular weight of 3800) as a candidate to be the high-molecular-weight active substance, based upon similarities in molecular weights, tissue distributions, and biological activities.

We also report that the low-molecular-weight peak can be resolved into two excitatory components by further purification with cation-exchange chromatography. Each component elutes with a peak of ninhydrin-positive material. The chromatographic behaviors of these components suggest that they are due to zwitterions. The values of pH at which the components elute suggest that one zwitterion has a titratable anionic group with a pK of 2.3 and that the other has a titratable anionic group with a pK of 2.8.

17.8 SOUND-EVOKED EFFERENT EFFECTS ON THE DISCHARGE PROPERTIES OF SINGLE AUDITORY-NERVE FIBERS. E.H. Warren, III* [1,3] and M.C. Liberman* [1,2,3] (SPON: N.Y.S. Kiang). [1] Program in Neuroscience, and [2] Department of Otolaryngology, Harvard Medical School; and [3] Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114.

The mammalian cochlea is supplied with an efferent innervation that originates in the region of the superior olivary complex (Rasmussen, *J. Comp. Neurol.*, 84:141, 1946). The recent observation that these efferent neurons respond to sound, even in barbiturate-anesthetized animals (Liberman and Brown, *Hearing Res.*, 24:17, 1986), suggests that this efferent activity may affect the response properties of auditory-nerve (afferent) fibers as described in many different preparations. Since roughly 1/3 of the efferent neurons projecting to each cochlea respond best to contralateral acoustic stimulation (Liberman and Brown, *ibid.*,), one test for influences of sound-evoked efferent activity on auditory-nerve discharge is to compare the response properties of single afferent fibers with and without contralateral stimuli (Buño, *Exp. Neurol.*, 59:62, 1978).

We find that the sound-driven response of single auditory-nerve fibers in barbiturate-anesthetized cats can be suppressed by contralateral tones at levels as low as 30 dB SPL. Contributions of middle ear muscles are eliminated by cutting their tendons on both sides prior to recording from single units. Ipsilateral stimuli are tone bursts at the characteristic frequency of the afferent fiber, roughly 10 dB above threshold. Discharge rates to this probe tone with and without contralateral tones of different frequencies and levels are compared. The suppression is strongest 1) when the contralateral stimulus is continuous (rather than bursted), 2) when the contralateral tone is near the unit's CF, and 3) among units with CF near 1.5 kHz. All of these characteristics are predictable based on existing knowledge of the properties of olivocochlear efferent neurons.

Contralateral acoustic stimulation is similar to electrical stimulation of the efferent bundle (e.g. Wiederhold and Kiang, *J. Acoust. Soc. Am.*, 48:950, 1970) in many respects. Both show relatively slow onset of suppression (200 msec to maximal effect). For both conditions, the effect on the ipsilateral rate-level function at CF for units with high (> 18 sec $^{-1}$) spontaneous rates (SRs) can reasonably be described as a simple *translation* of the function along the sound-level axis toward higher tone levels, whereas the effects on low-SR units often include a reduction in the saturated discharge rate, as well.

The strongest evidence that contralateral suppresion is mediated by the olivocochlear efferents is that the effect disappears immediately after sectioning the efferent bundle within the inferior vestibular nerve. In all our experiments the completeness of the cuts is confirmed histologically.

The finding that contralateral-sound stimulation can influence cochlear activity suggests a rapid method for assessing the overall physiological state of the olivocochlear efferent system in an animal. Comparisons of gross cochlear potentials with and without a preceding contralateral stimulus can conceivably provide an index of efferent activity which might prove helpful in studies of auditory system function.

17.9 TWO-TONE SUPPRESSION IN INNER HAIR CELL RESPONSE PATTERNS. M.A. Cheatham* and P. Dallos. Auditory Physiology Lab. Northwestern University, Evanston IL 60201.

In an attempt to understand the processing of complex stimuli by the peripheral auditory system, we have measured ac receptor potentials (RP) from individual inner hair cells (IHC) in the guinea pig organ of Corti. The purpose of the experiment was to characterize a phenomenon known as two-tone suppression (2TS) in which the addition of a second sinusoid to a single tone input decreases the response to the latter. Although many experiments have documented the magnitude changes which result from interactions between the two inputs, phase changes have received little attention. Since phase behavior is important to the understanding of underlying mechanisms, we have studied 2TS to ask whether the magnitude and phase changes resulting from the introduction of a suppressor differ from those to be expected by simply reducing stimulus level.

Data obtained from the third turn where characteristic frequencies (CF) range between 800 and 1000 Hz indicate that RPs for single tone stimuli are level dependent. For example, when frequency response functions are obtained to define response areas at several levels, frequency selectivity is enhanced as input level decreases. This occurs because of a strong compressive nonlinearity at CF, which results in the steepening of high and low frequency slopes as stimulus level is decreased. This behavior is reflected in the accompanying phase shifts which go through a lead/lag transition at CF which moves to a slightly higher frequency as input is reduced. Thus, by comparing the magnitude and phase changes occurring during suppression with predictions made on the basis of the well-documented nature of level-dependent responses to single-tone stimuli, it should be possible to determine whether 2TS operates by simply attenuating input to the cell.

To ascertain the magnitude and phase changes resulting from the presence of a high frequency suppressor (1500 Hz), frequency response functions were measured for IHCs. Results indicate that the effects are not predicted by a simple attenuation model. Although the suppressor causes a decrease in the magnitude of the ac RP, the largest deviations are measured at the CF of the cell causing the frequency response functions to become broader. The CF also moves to a lower frequency and the phase goes through a lag/lead transition exactly opposite to the results expected by simply decreasing input to the cell. Consequently, a simple attenuation model does not adequately describe basic features of 2TS in the apex of the cochlea for high-side suppressors.
(Supported by NIH Grant NS08635).

17.10 CHANGES IN CAT EAR CANAL ACOUSTIC DISTORTION-PRODUCT SIGNALS PREDICT NEURAL-RESPONSE THRESHOLD INCREASES AFTER OVEREXPOSURE. M.L. Wiederhold, J.W. Davis*, C.E. Sheridan* and G.A. Schulmeier*. Div. of Otorhinolaryngology, Univ. of Texas Health Science Center, and Audie L. Murphy Memorial Veterans' Hospital, San Antonio, TX 78284.

Previous studies have shown that when two tones were presented simultaneously, distortion-product signals can be observed in responses of auditory-nerve fibers, the cochlear microphonic and in the sound field in the external ear canal. These indicators of nonlinear cochlear mechanics are vulnerable to a number of physiological insults, including noise exposure. With primary-tone stimuli at frequencies f_1 and f_2, a prominent distortion-product (DP) signal at $2f_1 - f_2$ can readily be detected in the sound pressure measured in the cat ear canal. Using a fast-Fourier-transform spectrum analyzer, f_1 levels (L_1) needed to generate DPs at -10 dB SPL ranged from approximately 40 to 70 dB SPL. For all animals in which DPs were seen with low primary-tone levels, there was a sharp dip in the growth function near L_1 = 70 dB SPL, with rapid growth above this dip. Below the dip, the DP level is maximized when L_2 is 10 dB below L_1. Using primary frequencies of 4.0 and 5.2 kHz, the low-level portion of the DP growth function is reduced more (in dB) by acoustic overstimulation than is the portion above the dip. Several measures of change in the DP growth function have been correlated with short-term increases in N_1 threshold (TTS) caused by exposure to either 4 kHz tones or 500 Hz octave bands of noise, over a range of levels and durations. Either reduction of DP level at a fixed level of primary tones (vertical measure) or increase in primary-tone level needed to produce a criterion DP level (horizontal measure) is highly correlated with neural threshold increases. Changes in DP are minimal after exposures which caused neural threshold increases less than 20 dB, but increase 1 to 2 dB for each dB of N_1 threshold increase, beyond 20 dB. Nomarski light-microscopic examination of plastic-embedded surface preparations of the exposed cochleas revealed little disruption of hair-cell stereocilia which could be correlated with either exposure or threshold shift. Prominent vacuolization was seen in afferent-fiber dendrites beneath inner hair cells. The extent of vacuolization along the basilar membrane is related to the amount and frequency range of threshold increases.
(Supported by Veterans Administration Medical Research Funds.)

17.11 THE DEPENDENCE OF SPONTANEOUS ACTIVITY OF LATERAL LINE AFFERENTS ON CALCIUM, MAGNESIUM, AND COBALT. S.L. Guth* and D.G. Drescher (SPON: J. Benjamins). Lab. of Bio-otology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

The afferent neurons of the mechanoreceptive lateral line (and other acousticolateralis organs) have a significant level of activity in the absence of mechanical stimulation. The source of this spontaneous activity has been attributed to the vesicular release of afferent neurotransmitter from the mechanosensory hair cells onto primary afferent dendrites (Flock and Russell, J. Physiol. 257:45, 1976). Such release of neurotransmitter is believed to be a calcium-dependent process which can be perturbed by altering the composition of the solution bathing the synaptic side of skin containing lateral line organs. Concentration-effect curves were determined for the action of Ca^{++}, Mg^{++}, and Co^{++} (0.1 - 8 mM) on lateral line organs of post-metamorphic Xenopus laevis. Spontaneous activity was inversely proportional to the concentration of each divalent cation in the absence of others. High concentrations of divalent cations were capable of suppressing spontaneous activity completely. For any given concentration of divalent cation, spontaneous activity was higher in the presence of Mg^{++} than either Ca^{++} or Co^{++}. Remarkably, the concentration-effect curves for Ca^{++} and the calcium channel blocker, Co^{++}, were very similar. It is difficult to reconcile these results with the hypothesis that spontaneous activity depends entirely upon voltage dependent calcium current and alternative hypotheses are suggested (Drescher and Drescher, Life Sci. 40:1371, 1987).

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17.12 SYNAPTIC MORPHOLOGY AND VESICLE MORPHOMETRY OF HAIR CELLS IN THE TELEOST SACCULAR MACULA. M.J. Drescher*, D.G. Drescher, J.S. Hatfield* and C.M. Seitz*. Lab. of Bio-otology, Wayne State Univ. Sch. of Med., and Dept. of Pathology, Harper Hospital, Detroit, MI 48201.

The hair cells of the teleost saccular macula are of a single type, thought to be similar to vestibular type II hair cells of higher vertebrates. Saccular maculae of the rainbow trout, fixed in 1% glutaraldehyde, 4% formalin, 0.1 M sodium phosphate, pH 7.2, and postfixed in osmium tetroxide, have been examined by electron microscopy and analyzed with Bioquant II (R and M Biometrics).

Numerous clear vesicles were observed in hair cells, particularly below the level of the nucleus. Electron-dense synaptic bodies, similar to those described for the goldfish by Hama and Saito (J. Neurocytol. 6:361, 1977), were present at presynaptic sites and in the infranuclear cytoplasm at a distance from the plasma membrane ("heterotopic" bodies). The synaptic bodies were surrounded by halos of clear vesicles which appeared to be connected to the bodies by thin, radiating filaments. Synaptic bodies occurred singly and in clusters of 6-15 or more, reminiscent of the "ribbon fields" of pinealocytes (Vollrath, L., Z. Zellforsch. 145:171, 1973).

Morphometric analysis yielded diameters from perimeters for three populations of vesicles. Vesicles encircling synaptic bodies in hair cells had diameters of 47.5 ± 0.6 nm, vesicles in hair cells unassociated with synaptic bodies had diameters of 47.3 ± 0.5 nm, while vesicles in presumptive efferent endings on hair cells had diameters of 55.4 ± 0.5 nm (mean ± SEM). Presumptive efferent vesicles were significantly larger than hair cell vesicles (for both populations; $p < 0.001$), while no significant difference in diameter was determined between the two vesicle populations within the hair cells. Similar differences between efferent and hair cell vesicles were observed for glutaraldehyde and osmium tetroxide-based fixatives. Roundness factors ($4\pi Area/Perimeter^2$) were approximately 0.9 for all three populations of vesicles. In summary, the hair cell synaptic vesicles appear to be smaller than the presumptive efferent synaptic vesicles.

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INTERHEMISPHERIC RELATIONS

18.1 AN INVESTIGATION INTO THE EFFECTS OF LEFT AND RIGHT HEMISPHERE REMOVAL ON RELEASE CALLING IN FROGS. R.H. Bauer, C.A. McCandlish* and V.D. Douglas*. Dept. Psych. Mid. Tenn. St. Univ., Murfreesboro, TN 37132.

Over 100 years ago Broca demonstrated that the left hemisphere plays a major role in articulation of speech. Since this initial observation, a wide variety of evidence has supported the finding that language is lateralized in the left hemisphere. Attempts to demonstrate hemispheric dominance in animals were for the most part unsuccessful, and for a time hemispheric dominance was thought to be a major distinction between the human and animal brain. However, Nottebohm (Science, 167:95, 1970) clearly demonstrated that singing in male canaries is lateralized to the left hemisphere. Thus, lateralization is not a major difference between the human and animal brain but, at least for vocalization, lateralization occurs early in evolution. The major purpose of the present study is to examine the possibility that vocalization of a species farther down the evolutionary scale is also lateralized to the left hemisphere. Frogs and toads are the earliest species to exhibit true vocal cords and vocalization is produced by neural control from higher brain areas. Furthermore, calling behavior is known to alter the behavior of other members of the species in socially significant ways. For example, when a male mounts another male or a nongravid female the mounted frog emits a sharp, rapid call until they are released, hence the name release call.

Adult male frogs (Rana pipiens) approximately 8 cm long were housed in two compartment cages with water on one side. Release calling was produced by lightly holding the frog behind the front legs, caudally along the thorax. Baseline release calling was recorded during two 30 sec periods, 5 days a week for 10 weeks. Four groups of six frogs each were formed by matching frogs on the number of croaks in the baseline. These groups were as follows: (a) right telencephalic ablations, (b) left telencephalic ablations, (c) sham operations, and (d) nonoperates. The telencephalon from the olfactory bulb to the dorsal groove was removed by aspiration. Testing resumed 7 days after treatment for 6 weeks postoperatively.

Release calling was not significantly altered by left or right hemispheric ablations, indicating that this behavior is not lateralized in either hemisphere. The mating call, as distinguished from the release call, increases during the breeding season and appears to be involved in territoriality and attraction of females. Calling of this type can be induced by hormone injections. We are currently investigating the effects of hormone injections on the mating call by frogs with left and right hemispheric ablations.

18.2 **SEXUAL DIMORPHISM OF LATERALIZATION IN MICE SELECTIVELY BRED FOR DEGREE OF FUNCTIONAL ASYMMETRY: INTERACTION OF SEX, HANDEDNESS AND GENOTYPE**
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Sex differences in the degree of lateralization, but not in its direction, were reported in C57BL/6J inbred mice tested for handedness (Collins, Science, 187:181-184, 1975). In 'unbiased worlds' female mice were more strongly lateralized than males. To demonstrate that differences in degree of lateralization were heritable according to an autosomal pattern, a selective breeding program was initiated. A genetically heterogeneous, 8-way cross, foundation population was formed and 11 generations of bidirectional selection was practiced for extremes of lateralization. This produced two lines of mice that differed markedly in strength of handedness (Collins, in S. D. Glick (Ed.), Cerebral Lateralization in Nonhuman Species, Academic Press, 41-70,1985). Following a period of random within line mating, selection was reimpressed during S28-S30 generations.

The degree of lateralization according to handedness direction, sex, and line was examined in mice for S7 through S10 ($n = 1749$) and S28-S30 ($n = 1123$). Laterality by Sex by Handedness interactions were found in both data sets ($p = 0.003$ and 0.07). In the strongly lateralized HI line, sinistral females were more lateralized than sinistral males (1.52 vs 1.31 logit transformed PPE score, unweighted means), whereas the pattern was nonsignificantly reversed for dextral mice (1.32 vs 1.35). In the weakly lateralized LO line, sinistral males were more lateralized than sinistral females (0.71 vs 0.64), and dextral females were more lateralized than dextral males (0.66 vs 0.56). The laterality by sex pattern of the HI line was reversed in the LO line. Thus, in lines selected for extremes of lateralization, sex differences in degree of asymmetry interact with the direction of laterality and genotype or evolutionary history.

This selective breeding program, based originally on a sex difference observed in inbred mice, may have altered normal sexual characteristics of the lines. HI line females seem somewhat 'masculinized'. For example, they are aggressive fighters and show greater absolute asymmetries of orbitofrontol cortex and hippocampus. LO males appear somewhat 'de-masculinized'--they are hypogonadal with lowered circulating testosterone level. This suggests that developmental effects of gonadal steroids may play a role in affecting differences in degree of asymmetry as well as sexually dimorphic forms of hemispheric lateralization distinguishing the lines.

18.3 NEOCORTICAL SYMMETRY AND ASYMMETRY IN THE RAT: DIFFERENT PATTERNS OF CALLOSAL CONNECTIONS. G.D. Rosen, A.M. Galaburda, and G.F. Sherman. Neuroanatomical Dyslexia Lab, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Histological analysis of area 17 in the rat has revealed that volume asymmetries of this area reflect side differences in neuronal numbers. Furthermore, brains with symmetrical visual cortices have a greater bilateral number of neurons in this area than brains with asymmetrical visual cortices; asymmetrical visual areas, moreover, show a relative reduction in neuronal numbers on one side, rather than an increase on the other side. We have suggested that asymmetry may reflect the unilateral enhancement of ontogenetic cell loss in the cortex, and symmetry the interference with this process (Galaburda *et al.*, *Neuropsychologia*, 1987, in press). The purpose of this study was to determine whether the suggested preservation of neurons in symmetrical brains is accompanied by preservation of callosal patterns that antedate developmental cortical cell death.

Eight 90 day-old Wistar rats received midsagittal callosal sections. After one week's survival, animals were sacrificed and brains removed, fixed, embedded, sectioned at 30 μm and stained using the Fink-Heimer method for degenerating axon terminals. Adjacent sections were stained for Nissl substance with cresyl violet. Somatosensory area 2 was parceled on the Nissl sections; the Fink-Heimer sections were digitized under darkfield illumination on a Gould FD-5000 interfaced with a VAX 11/750 minicomputer; the Nissl sections and digitized images were overlayed and the architectonic borders on the former transferred onto the latter. The volume of each architectonic area and its mean total density of terminations were quantified. Qualitative assessment involved determination of the degree of columnar and laminar distribution of the terminal boutons.

The mean total density of axonal terminals in area 2 was inversely proportional to the degree of asymmetry of this area. Visual examination suggested that this was so because the brains with more symmetrical area 2's had fewer projection-weak regions. Further analysis revealed that the pattern of callosal projections in brains with asymmetrical sensorimotor/somatosensory areas was significantly more restricted to discrete regions than that in brains with symmetrical areas ($t = 12.65$, $df = 6$, $p < 0.001$). In the rodent at birth the pattern of callosal projections is relatively diffuse throughout the cortex, and subsequently establishes an appearance of discrete areas of projection by withdrawal of axons. This pruning process appears to occur to a lesser extent in brains with symmetrical areas than in those with established asymmetry. (Supported by NIH grant HD 19819 and a grant from the Hood Foundation).

18.4 MORPHOLOGICAL STUDY OF HEMISPHERIC ASYMMETRIES IN NORMAL AND ACALLOSAL MICE. S.L.Schmidt*,E.M.A.Caparelli-Daquer*,E.Volchan*(SPON:L.R.G.Brito). department of Neurobiology,Institute of Biophysics C.C.F9, U.F.R.J.; Inst. of Biology,U.E.R.J. , Rio de Janeiro , Brazil

It has been suggested that the normal development of the corpus callosum (C.C.) is responsible for the establishment of brain asymmetries.Cerebral asymmetries have been described in several species.In particular,right-left anatomical differences have been reported in male rodents. As the C.C. fails to develop in many mice of the BALB/cCF strain we decided to look for morphological asymmetries in the brain of these abnormal animals, reasoning that their occurence would disproof the determinant participation of the C.C. Forty - six adult males of the BALB/cCF strain were intracardially perfused under ether anesthesia with saline (0.9% NaCl) followed by a 4% solution of formaldehyde. After several weeks postfixation , the brains were removed from the skull and photographed from above. The dorsal area of each hemisphere was measured from the photographic prints, with the aid of a computer. The cerebral hemispheres were split apart by cutting the brain through the midsagittal plane and weighed on a digital balance. The left hemispheres were embedded in paraffin and cut into 10 μm parasagittal sections. These sections were stained with cresyl-violet and charted with a camera lucida.The midsagittal area of the C.C. was measured from these chart (n=46) in order to descriminate normal from abnormal animals. The C.C. was considered normal if its area exceeded 0.64mm² (n=24) and abnormal if its area was below 0.44mm² (n=13). In normal animals the mean difference between left and right dorsal areas demonstrated a significant absolute asymmetry the left hemisphere being consistently greater than the right hemisphere. The same was true for weight,the left hemisphere being heavier than the right hemisphere. In mice with callosal deffects, however there was no significant mean difference between dorsal areas as well as between weights of the hemispheres.Our results suggest that the C.C. does play a role in the development of morphological asymmetries. We do not know if these anatomical differences are related with functional asymmetries. The indication that the C.C. is implied in the establishment of laterality is supported by neuropsychological studies of human patients with callosal agenesis,but interpretation of these results is still controversial.Furthermore the use of anatomical measures for assessing asymmetry and establishing structure - function correlation, should be approached with caution, since recent studies have showed that they cannot predict reliably the occurrence of functional asymmetry in particular cortical areas.

18.5 RELATIVE CONTRIBUTION OF THE CORPUS CALLOSUM TO THE BILATERAL RECEPTIVE FIELD OF CELLS IN S-11. J.P. Guillemot, D. Petit* and F. Leporé. Dept of kinanthropologie and Psychologie, UQAM and Univ. de Montréal, C.P. 6128, Succ A, H3C 3J7.

The corpus callosum contributes to the interhemispheric transfer of somatosensory information. Since the somatosensory pathways are essentially crossed, many studies have postulated that the corpus callosum may be responsible for the presence of bilateral receptive fields (RFs) in cortical area S-11. On the other hand, subcortical structures as well as commissures other than the corpus callosum, may also contribute to the bilateral nature of these cells. In order to assess the relative contribution of the corpus callosum, this study compared corpus callosum-sectioned cats to normal cats. The location and RF properties of cells with bilateral RFs were compared. Single cell recordings in anesthetized and paralyzed cats were carried out with glass micropipettes and responses were amplified by conventional methods. Stimuli used to elicit responses within RF were: light touch, pressure, air puffs and passive movements of the limbs.

Results showed that the corpus callosum makes an important contribution to bilateral activation of cells in S-11, since less than half the number of cells with bilateral RF is found in corpus callosum-sectioned cats as compared to normal cats. The decrease in proportion of bilateral RF is found for all body regions with the exception of the face. However, the substantial proportion of bilateral RFs remaining in callosotomized cats indicate that this structure is not the only contributor to bilateral activation of cells in S-11. The relative importance of the callosal contribution to bilateral RFs to different body regions is also assessed and discussed with respect to the roles normally attributed to the corpus callosum.

18.6 **ASSYMETRIC HAND PREFERENCE IN FOUR SPECIES OF CAPTIVE APES** J. E. Heestand*. (SPON: J.S. Lockard). Univ. of Wash., Seattle, WA. 98195.

Interest in the evolution of neural lateralization in Man has led investigators to employ the comparative method to examine asymmetry in other primates, both on a neurologic and behavioral level. One theory maintains that the origin of language was gestural in nature and was preceded by hand preferences developed through the performance of bimanual tasks requiring asymmetrical use (Hewes, G., Cur. Anthro., 14:5-24, 1973). It has been suggested that the development of hand preferences was fostered by asymmetrical orienting during foraging (Sanford et al., Brain Beh. Evol., 25:217-224, 1985) when one limb performs a strength and the other a dexterous function. This leads to a prediction that the degree of preference should be related to the extent to which strength is an integral part of the foraging pattern.

Studies on monkeys have typically made extensive use of lesions or commissurotomy and few studies, using either monkeys or apes, have focused on observations of naturally occurring behavior in social groups. In the present study 70 apes representing four species [Gorilla gorilla, Pongo pygmaeus, Pan troglodytes and Symphalangus syndactylus] were observed in social groups in semi-natural captive environments at six zoos. Using the focal animal sampling technique the behavior in which the animal was engaged, the limb being used and the nature of the limb usage were recorded for a total of 12 hours. Direct-testing for eating involving fine manipulation was conducted with 11 animals.

Results revealed that there were significant species-typical hand/limb preferences (walk/run category) for orangutans ($p=.04$), siamangs ($p=.05$), chimpanzees ($p=.001$) and gorillas ($p=.01$). In addition, the bias for each species was in the same direction, namely a right preference that was significant ($p<.05$). Also, hand preferences were obtained for a significant number of the individuals under direct-testing although no preference was evident during free feeding.

Unlike human populations, non-human primate populations have not demonstrated the presence of a genetic component to handedness since, from population to population, consistent left-right proportions have not been found. This inconsistency has led many investigators to state that hand preferences in monkeys is a learned trait with no specific underlying neurologic correlate. Notably, in the present study, the four species of apes evinced the same hand/limb preference for a single behavioral category. These findings indicate that a limb preference is important in locomotion. As such, the findings of the present study suggest the presence of a neurologic correlate in apes.

18.7 THE HUMAN CORPUS CALLOSUM: AN MRI STUDY VARYING SEX, HANDEDNESS AND AGE. RM Harris, JW Sundsten* and RA Fischer-Wright*. University of Washington, Seattle, WA 98195.

Several recent studies have investigated variations in the cross-sectional area of the human corpus callosum or of its parts, especially the splenium, with respect to gender or handedness. Although initial studies using autopsied brains showed significantly larger values in females over males and left-handers over right-handers, later studies using magnetic resonance imaging (MRI) have failed to confirm these differences. In order to evaluate the effects of both gender and handedness simultaneously, as well as age, we have undertaken an MRI study of 24 normal human volunteers. These included equal numbers (12 each) of males and females, left and right-handers, and ages less than or greater than 30. Midsagittal spin-echo images 5 mm thick were obtained and the region of the corpus callosum magnified by a factor of 3. Cross-sectional areas were digitized for the entire corpus callosum (average value 6.34 $\pm$ .99 cm^2) and its posterior fifth, the splenium (1.83 $\pm$ .31 cm^2), as well as the maximal splenial width in the dorsal-ventral direction (1.62 $\pm$ .23 cm). An analysis of variance for the factors gender, handedness and age showed no statistically significant variations for any of the three callosal variables ($p > .16$ for all cases). For each variable, however, the average value for females was larger than that for males, for left-handers larger than for right handers, and for older subjects larger than for younger subjects. In an attempt to correct for differences in brain size, each variable was divided by the cross-sectional area of the cerebral cortex, obtained from the mid-sagittal scan. An analysis of variance for these ratios again showed no significant differences, with the exception of the splenial width divided by the cortical area, in which females were larger than males (0.020 $\pm$ .003 vs. 0.017 $\pm$ .002) at the $p = 0.02$ level.

The most obvious feature of these data was the large natural variations in callosal measurements within groups. For example, the total callosal area varied between 4.36 and 9.45 cm^2. Although the average values for the callosal measurements were somewhat larger for females and for left-handers, the large variability makes it unlikely that these are significant differences.

18.8 SIMPLE REACTION TIMES TO LATERALIZED LIGHT FLASHES IN NORMAL SUBJECTS, FOUR COMMISSUROTOMIZED PATIENTS, AND A CALLOSAL AGENESIS BOY: VARIETIES OF INTERHEMISPHERIC COMMUNICATION ROUTES. J. M. Clarke* and E. Zaidel* (SPON: D. Zaidel). Dept. of Psychology, Univ. California, Los Angeles, Los Angeles, CA 90024-1563.

In 1912, Poffenberger (Archives of Psychology, 23:1, 1912) first demonstrated that simple reaction times to lateralized light flashes are a few milliseconds longer when the responding hand and the visual hemifield in which the light flash appears are on opposite sides (crossed condition) than when they are on the same side (uncrossed condition). The crossed-uncrossed difference (CUD) appears to be a measure of interhemispheric transmission time across the corpus callosum in normal individuals.

Using a computerized tachistoscope, we had subjects respond to 10 blocks of 80 trials. To assess whether visual information was being transferred, two different light intensities were used. Intensity and visual field of presentation varied randomly within blocks of trials, while response hand alternated after each block. For 20 undergraduates the mean CUD was 2.1 ms (SD=3.7), which was invariant over light intensity despite a significant main effect of light intensity on overall reaction times. The absence of an effect of light intensity on the CUD suggests that motor rather than visual information was transferred.

Sergent and Myers (Perception & Psychophysics, 37:571, 1985) found notably larger mean CUDs (30 & 50 ms) in two commissurotomized subjects. However, the two light intensities they used were not sufficiently differentiated to affect overall reaction times. Therefore, it was not clear whether the CUDs represented visual transfer across the superior colliculus, subcallosal transfer of a motor code, or ipsilateral motor control.

In the present study, the overall CUDs from four completely commissurotomized subjects varied from 34 to 61 ms, and the intensity manipulation had significant effects on reaction times. The CUDs remained invariant over the two intensities for patients L.B. and A.A., suggesting a motor route. The CUD was inversely affected by light intensity for patient N.G., probably representing transfer of visual information across the superior colliculus. Surprisingly, the CUD for patient R.Y. was significantly larger for brighter flashes. However, R.Y. exhibited an unusual inability to respond to many of the light flashes that appeared in the right visual hemifield when he was using his left, but not right, hand. An idiosyncratic route of noncallosal transfer may be responsible for this latter result.

An eight-year old boy (M.M.) with callosal agenesis had an overall CUD of 20 ms, which is comparable to CUDs observed in similar patients (eg. Milner, A. D., et al., Neuropsychologia, 23:323, 1985). However, contrary to previous findings, the CUD for M.M. was unaffected by different light intensities, despite a main effect of intensity, suggesting a nonvisual route.

18.9 USE OF THE INTRACAROTID SODIUM AMYTAL PROCEDURE TO PREDICT SIDE AND SITE OF FOCAL LESION. Peter J. Snyder, Robert A. Novelly*, and Maria D. Lifrak*. Dept. of Psychology and Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824-1117

Although the intracarotid sodium amytal procedure (ISA) was developed to determine hemispheric dominance for speech in humans (Wada, 1949), the procedure was later expanded by Branch, Milner & Rasmussen (1964) in order to discern patency of memory in the hemisphere contralateral to the side of proposed surgery. Their extended procedure was designed to evaluate the risk of inadvertent production of an amnestic syndrome following unilateral temporal lobectomy.

We propose that standardized administration and quantification of ISA memory assessment in each hemisphere may lead to the further development of this procedure for independent diagnosis of side and site of focal epileptogenic lesions. Our data suggest that patients with epileptogenic lesions of the left (L) versus right (R) temporal lobes, and frontal versus temporal lobes can be differentiated with this procedure.

Of 70 epilepsy surgery patients seen at the Yale - West Haven, CT., VAMC Epilepsy Center, 67 met criteria for a single focal epileptogenic lesion (determined by a combination of scalp EEG, CT scan, and/or chronic depth EEG). Site and side of lesions were: Temporal - R=26 / L=25; Frontal - R=10 / L=6. A standardized and quantifiable ISA memory procedure, developed by the 2nd author, was administered to all subjects. No differences were found between the four groups with regard to amount of sodium amytal administered, age of onset of the discharging lesion, or Wechsler IQ indices.

A 2x4 factorial ANOVA, with hemisphere assessed and side/site of lesion as the 2 factors, was conducted on the memory data. A significant 2-way interaction effect was obtained, $F(3,126)=21.576$, $p<.001$. A posteriori analysis (Newman-Keuls, $p<.001$) showed significantly lower memory values for the lesioned hemisphere of both R and L temporal lobe patients compared to the non-lesioned hemispheres of these two groups. In contrast, no significant differences were observed between memory values for the lesioned vs. non-lesioned hemisphere of either R or L frontal lobe patients.

Despite individual exceptions, a pattern of memory preservation emerges that could extend the use of the ISA beyond its classical applications, mentioned above. Since it is often difficult to discriminate frontal vs. temporal seizure foci via standard diagnostic techniques, an adjunct and independent methodology, such as the ISA memory procedure, could be of considerable practical value.

18.10 ASYMMETRIES IN NEGLECT AND PATTERN OF RECOVERY FOLLOWING LEFT VS. RIGHT MEDIAL PRECENTRAL PREFRONTAL CORTEX LESIONS IN RATS. J.M. VARGO*, J.V. CORWIN*, V. KING* AND R.L. REEP (SPON: J. McLean) Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148 and Depts. of Physio. Sci. and Neurosci., Univ. of Florida, Gainesville, FL 32610.

Human brain organization is characterized by lateralization of function. An increasing number of studies are now documenting lateralization of nonhuman brain organization. Previous research has indicated that behavioral asymmetries are produced by unilateral destruction of medial precentral prefrontal cortex (MPP) in rats (J.V. Corwin et al., 1986, Soc. Neurosci. Abst., 12:1537). The present parametric study is a further examination of the patterns of behavioral asymmetries and recovery of function from multi-modal neglect produced by unilateral destruction of MPP.

The subjects were 42 male Long-Evans hooded rats: 12 received unilateral lesions of the left MPP (L-MPP), 18 received unilateral lesions of the right MPP (R-MPP), and 12 were controls. Nine of the controls had an equal amount of cortex removed unilaterally at a location just lateral to MPP, the remaining 3 subject's received skull removal only. Total neglect scores were calculated using "blind" ratings of the degree of orientation to the presentation of visual, somatosensory and auditory stimuli to each side of the body. Testing was conducted 3 times a week for a minimum of 3 weeks or until recovery (2 consecutive test days with the ratio of neglect side/non-neglect side ≥ 0.60).

There were no significant differences between the lesion extents of any of the surgical groups. Significant differences were found in each paired comparison between L-MPP operates, R-MPP operates and pooled controls for total neglect scores and for scores within each modality during weeks one (all p's<0.01) and two (all p's<0.05), with the exception of R-MPP and control somatosensory scores during week two. L-MPP operates demonstrated significantly more severe neglect than R-MPP operates during week one ($p<0.05$). The L-MPP group showed consistent contralateral neglect. In contrast, 12 of the 18 R-MPP operates showed consistent ipsilateral neglect, and the remaining six exhibited ipsilateral or contralateral neglect one day and opposite-sided neglect on subsequent test days. These animals "switched" neglect sides from one to three times before recovery. In contrast to our previous findings, no R-MPP animals exhibited exclusively contralateral neglect. There was no significant difference in the rate of recovery between the L-MPP and R-MPP operates.

These results are in accord with both anatomical (Van Eden et al. (1984) Dev. Brain Res., 12:146-153) and neurochemical (Slopsema et al. (1982) Brain Res., 250:197-200) findings indicating asymmetries in the MPP of rats.

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18.11 THE ISSUE OF BRAIN WEIGHT AND NEW MEASURES OF SEXUAL DIMORPHISM IN STUDIES OF THE HUMAN BRAIN. T. Adesanya*, D. J. Woodward & M-C de Lacoste (SPON: R. J. Kosinski). *Dept. Cell Biology and Anatomy., UTHSCD, Dallas, Texas 75235.*

The advent of **Nuclear Magnetic Resonance Imaging** techniques has made it possible for investigators to obtain gross brain measurements *in vivo* and attempt to correlate those with neuropsychological profiles [e.g., sex, handedness, lateralization of function] and/or psychiatric history [e.g., schizophrenia]. However, one of the determinants of gross measurements is overall brain size and, hence, unless one is assured that there is no variation in brain weight in a particular sample, it must considered as one of the variables in the data analysis. Since complete series for each brain of MRI images are usually difficult and expensive to obtain, investigators have had to depend on select linear measurements or single-section surface areas to estimate sample variation in brain size. This study was undertaken to isolate a set of gross brain measurements that could readily be identified on mid-sagittal MRI scans and used to accurately predict brain weight. A second aim was to determine if a subset of these measurements [exclusive of callosal area] could function to study sex differences in the brain in normal populations or hormonally aberrant groups.

Whole brain [N=80] perfused in situ with embalming fluid and stored in 10% buffered formalin were acquired from the UTHSCD Willed Body Program. Brain weights were recorded at different time periods but those utilized in this study were obtained immediately prior to the compilation of an extensive battery of gross measurements. Mid-sagittal sections were photographed and these were used to obtain surface areas of the entire section and of select regions [e.g., cuneus] using the laboratory CARP software. Our sample size was statistically age- and sex-matched and evinced the expected sex difference in brain weight[$p = .003$]. As anticipated a number of measures were statistically correlated with brain weight although none had a Pearson r of greater than .70. Multiple regression techniques further isolated six of these variables [including total surface area, inferior-superior frontal dimensions, etc. but not anterior to posterior distance] as the most useful for predicting brain weight [Multiple R=.9238]. However, not one of the selected variables reflected the observed sex difference in brain weight. In other words, measures that could be used to reliably estimate brain weight were nearly identical between the sexes. Yet, ANOVA techniques demonstrate a consistent sex and brain effect on the size/area of a number of brain measurements. In practical terms, this means that in examining the relationship between sex and the size of individual brain structures, one must obtain an accurate measurement of brain weight to control for the influence of that variable. Perhaps, better estimates of brain weight can be acquired from a number of serial MRI sections or from skull measurements.

We were able to isolate a subset of variables that appear to be consistently statistically sexually dimorphic These include distances between the callosal sulcus and the superior aspect of the brain and between the rostrum and splenium and, respectively, the frontal and occipital poles. Additionally, we observed that the calloso- marginal sulcus is more likely to bifurcate or radiate in the female right hemisphere. Finally, although we did confirm Papez observation of sex differences in the direction and degree of asymmetry in the cuneus, these differences were not statistically significant in this sample.

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18.12 SEX DIFFERENCES IN THE HUMAN MASSA INTERMEDIA. Laura S. Allen* and Roger A. Gorski (SPON: A. Adinolfi). Dept. of Anatomy and Lab. of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

Several neuroanatomical sex differences have been identified in birds and mammals which could underlie functional sex differences in reproductive behavior and physiology. However, relatively little is known about sexual dimorphism in the human brain. Sexually dimorphic cell groups in the preoptic-anterior hypothalamus (Swaab, D.F. and Fliers, E., Science, 228:1112, 1985; Allen L.S. et al., Endocrinology Suppl., 118:633, 1986) are larger in men and, at the midsagittal plane of the brain, several structures may be larger in women. The massa intermedia is more often present in females than in males (Morel, F., Acta Anatomica, 4:203, 1948), and the cross-sectional area of the anterior commissure (Allen, L.S. and Gorski, R.A., Anat. Rec., 214:3A, 1986) and the corpus callosum (DeLaCoste-Utamsing, C. and Holloway, R.L., Science, 216:1431, 1982) are greater in women than in men. We examined the possibility that the mid-sagittal cross-sectional area of the massa intermedia (MI) in post-mortem human tissue is also sexually dimorphic. The region of the brain containing the thalamus was sectioned midsagitally. Therefore, the MI, if present, protruded from the thalamic wall of the third ventricle. The midsagittal surface of each MI was placed against a glass, with an adjacent ruler taped at the same plane, and photographed. The image was projected onto white paper at a magnification of 12X, and the border of the MI was traced. The area of each tracing was determined using a bioquant hipad digitizer. Our results are based on analysis of the brains of 48 females which ranged in age from 1 to 87 years (X=63±21), and 36 males from 1 to 81 years (X=54±22). The massa intermedia was present in the brains of 40 (83%) of the females and in 26 (72%) of the males. The area of the MI in females, which ranged from 0 to 1.279 cm^2 (X=.354±.341) averaged 68% larger than the area of the MI in males, which ranged from 0 to .814 cm^2 (X=.211±.183) (p=.026). The more frequent presence of the MI in females does not account for this sex difference since in these 40 individuals the MI is 45% larger (X=.42±.330) than in the 26 males with an MI (X=.292±.150). These differences are present despite the fact that the male brains were an average of 14% larger in weight than the female brains. Since the role of gonadal hormones on the sexually dimorphic structures in the human brain cannot be determined by experimental manipulation of humans, as has been demonstrated in other animals, it is possible that radiological techniques such as magnetic resonance imaging in humans with abnormal hormone exposure, may further elucidate the role of sex hormones in influencing the structure of the human brain as well.

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18.13 ANALYSIS OF THE MAGNETIC ALPHA RHYTHM IN SIGNAL SPACE. R.J. Ilmoniemi and S.J. Williamson. Neuromagnetism Laboratory, Depts. of Physics and Psychology, New York University, 4 Washington Place, New York, NY 10003.

We have investigated the magnetic field produced by spontaneous alpha activity using two seven-channel SQUID gradiometers positioned bilaterally over the occipital head of normal subjects. This provides 14 simultaneous, independent measures, which are sufficient to determine a wide variety of possible source configurations. Nevertheless, we have analyzed the data in a more general sense by exploiting the advantages of a 14-dimensional signal space. The analysis is therefore independent of any specific models of the source configuration.

Each of the several trials in a session consisted of eighteen 16-second epochs of data collection, with a two-second separation between epochs. Subjects were instructed to keep eyes closed except for three 18-second periods in each trial, during which the characteristic suppression of alpha activity could be observed when the subject looked at a visual pattern. In some trials, hemifield visual presentation was applied. To facilitate the handling of the large amounts of data collected in this study, an automatic detection scheme for alpha spindles was developed. Each detected spindle was characterized by its duration, frequency, time of occurrence, and by the rms-amplitudes in the 14 signal channels; these characteristics formed the basis for subsequent analysis. Since there may be multiple simultaneous sources distributed over large areas of cortex, we look at the behavior of the field pattern from spindle to spindle or within spindles, thus avoiding uncertain assumptions about the sources.

The measured field values are conveniently represented as coordinates of a vector in the 14-dimensional signal space. Using methods of cluster analysis, we observed that there are intervals of time when the signal vector is confined to a small region of signal space, implying that these spindles are produced by nearly identical source configurations. At other times, possibly contingent on the experimental situation, locations of spindles in signal space appear to bear little relation to their predecessors. We will show how representing each spindle on a two-dimensional surface allows us to observe dependencies of various characteristics of the spindles on source configuration. Similarly, this representation reveals dependencies of the source configuration on experimental conditions.

In order to infer regions of activity that give rise to the observed spindles, we represent the source of each spindle by an equivalent current dipole and show relationships between source position in the occipital-parietal regions and characteristics of the spindle.

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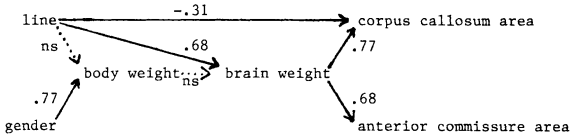
18.14 COMPARISON OF RIGHT- VS. LEFT-HAND SOMATOSENSORY ACTIVATION: A POSITRON EMISSION TOMOGRAPHIC STUDY. F. Yoshii*, M.D. Ginsberg, R.E. Kelley, J.Y. Chang, W. Barker, G. Ingenito, A.M. Apicella, R. Duara, and T.E. Boothe. Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami School of Medicine, Miami, FL; and Section of Positron Emission Tomography, Department of Radiology, Mount Sinai Medical Center, Miami Beach, FL.

The left brain and right brain are differently organized. Speech is a left-hemisphere function and visuospatial function is related to the right hemisphere. Anatomical hemispheric asymmetries, such as a larger left than right planum temporale and a longer and lower left than right sylvian fissure are well known. However, functional differences of the somatosensory cortices of the two hemispheres have not been studied. We compared the activation capacity of the right and left somatosensory cortices in normal right-handed volunteers using positron emission tomography (PET) and the double-injection kinetic model of 18-F)fluoro-deoxyglucose (Chang et al, J Nucl Med, 1987). This model allowed us to use each subject as his own control; thus, cerebral metabolic rate for glucose (CMRglc) could be assessed in two behavioral states -- rest and activation.

Ten subjects were males and 6 were females. Somatosensory activation was performed by palpation and sorting of mah-jongg tiles with one hand (Ginsberg et al, Neurology, in press). In 8 subjects (mean age ± SD: 50.8 ± 21.0 y/o) who used the right hand for activation, CMRglc in the left (contralateral) somatosensory cortex (SSC) increased 16.2 ± 6.1% during the activation task, while the CMRglc increase in the right (ipsilateral) SSC was 3.8 ± 4.8%. In 8 subjects (40.0 ± 20.1 y/o) who used the left hand for activation, the CMRglc increase in the right (contralateral) SSC was 12.1 ± 2.9% and that in the left (ipsilateral) SSC was 5.4 ± 2.3%. In both groups, activation of the contralateral SSC was significantly higher than that of the ipsilateral SSC (for the left hand, $P < 0.05$; for the right hand, $P < 0.01$). Thus, contralateral SSC stimulation did not differ significantly between the left- and right-hand groups. However, when an index of interhemispheral activation asymmetry was computed by dividing the CMRglc increase in the ipsilateral by that of the contralateral SSC, the index in subjects using the right hand was 0.23 ± 0.22, while in subjects using the left hand it was 0.45 ± 0.20. Our findings suggest that, in right-handed individuals, right- and left-hand somatosensory stimulation gives rise to equal degrees of contralateral SSC activation, while the tendency toward ipsilateral/contralateral symmetry of activation is greater when the left hand is stimulated. (Supported by USPHS Grants NS21720, NS05820 and NS 22603.)

18.15 BRAIN WEIGHT AND CORPUS CALLOSUM SIZE IN MICE SELECTED FOR HIGH AND LOW DEGREES OF PAW PREFERENCE. B. Cassells, R.L. Collins, and D. Wahlsten. Dept. of Psychology, Univ. of Waterloo, Waterloo, ON Canada, and Jackson Laboratory, Bar Harbor, ME 04609.

Mice (n=109) from the 29th generation of lines selected for high and low degrees of paw preference were tested for direction and degree of paw preference at 6 to 10 weeks. At 10 to 14 weeks, they were perfused intracardially with 0.9% saline followed by 10% buffered formalin and their brains removed. The myelin staining intensity of the genu of the corpus callosum and morphometric indices of all major myelinated forebrain commissures were assessed from 25μ frozen midsagittal sections stained with Sudan Black B. All measures were adjusted to a perfusion age of 90 days. A stepwise discriminant analysis followed by a path analysis based on multiple regression revealed that the data were consistent with the following causal model:



Positive coefficients indicated high > low and male > female or positive covariation of measures. The nonsignificant (ns, $p > .01$) effects remaining in the diagram were included for theoretical reasons. Gender significantly influenced body weight only. The brains of the high line mice were relatively larger (by 45 mg or 11%). No significant effects on anterior commissure area independent of brain weight were evident. However, the callosal areas of the low line mice were larger (by .092 mm^2 or 12%) relative to their brain weights. Because the lines did not differ on myelin staining intensity of the genu, selection for high degree of paw preference may have produced an increase in relative brain size that surpassed the increase in the number of callosal axons linking the two cerebral cortices and, perhaps, greater hemispheric independence and functional specialization. However, in the total sample, relative brain weight (but not relative callosal area) was significantly related (positively) to degree of paw preference. This suggests that relative brain weight (but not degree of interhemispheric connectivity) is an important influence on degree of functional lateralization. No differences between lines or genders in percentage of area in or shape of the posterior fifth of the corpus callosum or in the absolute or relative areas of the hippocampal or dorsal fornical commissures were evident.

18.16 EEG COHERENCE AND INTELLIGENCE IN CHILDREN. R.S. Hernandez, A.T. Arenander and R.W. Boyer*. Dept. of Physiol., and Dept. of Psych., Maharishi Int'l Univ., Fairfield, IA 52556.

EEG coherence (COH) is considered to be an estimate of the stability of the phase relationship between any two scalp recorded signals within a given frequency band. COH is thought to reflect the amount and strength of cortical connectivity, and thus, high COH is considered to represent a state of low functional neural differentiation. During periods of high COH neurons may be functionally more coupled corresponding to periods of lower, perhaps less independent, information processing. Current research has indicated apparent paradoxical conclusions regarding the relationship between standardized IQ scores and measures of COH during eyes-closed (EC) conditions: a robust inverse correlation in children, and a positive correlation in adults. The purpose of the present study was to examine the correlation across a range of cerebral function; from low to high levels of excitation. A sequence of different task conditions were employed to modify brain state in order to create a dynamic range of activation.

EEG was recorded from 48 right handed normal children aged 10 to 16 years during the following sequence of conditions: rest/EC, rest/eyes-open (EO), mental arithmetic/EC, word generation/EC, Transcendental Meditation (TM) practice/EC, rest/EO, rest/EC. TM was included because this mental procedure is reported to produce substantially de-excited brain states, and high COH. Raw EEG from 16 scalp locations was digitized at 120 Hz, and 25 artifact-free seconds of data were analyzed using complex demodulation, giving phase and power spectrum information across standard EEG frequency bands. COH, the ratio of the square of the cross spectrum to the product of the autospectrum, was computed for all combinations of lead pairs. The Weschler Intelligence Scale for Children-Revised (WISC-R) test was also administered.

Preliminary polynomial regression analyses, using full scale IQ as the dependent variable, indicate an inverse correlation between IQ and COH in frontal derivations in alpha and theta frequencies across all conditions, with rest/EC, rest/EO and mental arithmetic sigificant at the $p > .005$ level.

18.17 VISUAL SEARCH IN HYPOTHETICALLY PSYCHOSIS-PRONE COLLEGE STUDENTS. J. W. Jutai* (SPON: R.E. Walley). Dept. of Psychology, Univ. of Alberta, Edmonton, Canada T6G 2E9.

This research examined the possibility that a lateralized cerebral hemispheric abnormality is present in individuals at risk for the development of psychotic disorder, and that this abnormality is the same as that hypothesized to underlie cognitive dysfunction in schizophrenia. It has been suggested that the primary, initial disturbance in schizophrenia occurs in the right cerebral hemisphere and results in damage to a cortical network involved in attention. A key aspect of attention particularly sensitive to right-hemisphere damage is its distribution within extrapersonal space. Eight hundred college students were screened using psychometric measures of psychiatric vulnerability. Experimental subjects (n=51) were those with extreme high scores on at least one of four psychosis-proneness scales developed by Chapman and colleagues at the University of Wisconsin. Other research has shown that many of these individuals show cognitive deficits similar to those of adult diagnosed schizophrenics. Control subjects (n=21) scored with 0.5 S.D. of the mean on all scales. Subjects were tested using letter/shape cancellation tasks designed by behavioral neurologists to detect abnormalities in the spatial distribution of attention. If the prepsychotic period in the development of psychotic disorder is associated with dysfunction of right hemisphere mechanisms of attentional control, it was expected that experimental subjects would show evidence of deviant visual search on the cancellation tasks. This hypothesis was supported by the data. More than 50% of experimental subjects employed an erratic search strategy typically observed in right-brain damaged patients, compared with less than 20% of controls. Also presented will be the results of further analyses which examined search strategy as a function of type of prepsychotic indicator. Discussion will place the findings in the context of the existing body of research which has demonstrated 'schizophrenic-like' information-processing abnormalities in hypothetically psychosis-prone young adults.

18.18 ANALYSIS OF SPECIALIZED COGNITIVE FUNCTIONS IN DEAF AND HEARING SIGNERS. D. MCKEE* AND H.W. GORDON. Department of Special Education, California State University, Northridge, CA 91330.

This study analyzed the performance of deaf adults on specialized cognitive functions which are associated with the left and right hemispheres, and explored the relationship between the cognitive profile and a number of variables: etiology, age of onset and degree of hearing loss, and age of sign language acquisition. Two sequential tests in the Cognitive Laterality Battery (CLB), developed by Gordon (1986), were modified so that deaf subjects could process the sequential stimuli by the visual modality instead of the auditory. There was significant correlation betwen the original auditory and modified visual tests ($r=.62$, $p<.001$), although the scores on the modified tests were higher than the scores on the original tests. Using the norms for the hearing tests would overestimate a subject's performance. The other tests on the CLB were adapted directly using videotaped instructions in Sign and 35mm slide projector for presentation. Nevertheless, deaf subjects performed below the norms on these modified sequential tests. For the deaf sample as a whole, cognitive function was below average for the verbal and sequential skills associated with the left hemisphere ($p<.001$) and above average on the visuospatial skills associated with the right hemisphere ($p<.05$). In other words, the cognitive profile of the deaf sample significantly favored processing associated with the right hemisphere by one-half standard deviation.

When the variables related to deafness, i.e., age of sign language acquisition, were examined in terms of cognitive functioning among deaf subjects, there were no significant differences among groups categorized by language acquisition. The results were inconclusive as to whether sign language acquisition affects cognitive functioning, partly due to insufficient information on fluency skills and codes of sign language. Hence, further investigations were recommended in this area. Hearing subjects whose parents were deaf and who used American Sign Language exclusively at home, performed nearly normally in comparison to the norms but performed significantly better than deaf subjects of deaf parents on left hemisphere function ($t=2.77$), $p<.05$). Use of Sign as a first language was ruled out as a factor in cognitive functioning. It is concluded that lack of auditory experience may have an influence on the cerebral development and organization of certain higher cognitive functions such as temporal processing and visuospatial skills.

18.PO **HAND PREFERENCE AND SEX DIFFERENCES IN THE ISTHMUS OF THE CORPUS CALLOSUM.** S. F. Witelson. Department of Psychiatry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

The size and morphology of the midsagittal section of the corpus callosum in individuals varying in hand preference and sex were further evaluated by subdividing the callosum into seven divisions, roughly representing functionally different cortical regions. Geometric divisions along the maximal anteroposterior axis of the callosum were used to divide the callosum into the genu, rostrum, splenium and four trunk regions. Area measures of these subdivisions were made on brains obtained from 56 consecutive autopsies from adults who underwent neuropsychological testing prior to death. The total sample analyzed was 51, with five cases excluded due to incomplete data. This sample includes the 42 cases reported previously (Witelson, Science, 229:665,1985; Neurology & Neurobiology, 17:117, 1986) to show hand differences in total area but no hand or sex differences in the splenium.

Hand preference was now found to vary with size of the callosum, particularly in the region of the isthmus, the posterior part of the trunk, which houses the callosal fibers connecting the right and left parietotemporal cortical regions. Hand preference was dichotomously classified into consistent-right-handers (n = 33) and non consistent-right-handers (including all mixed and left handers, n = 18). The non consistent-right-handers had a larger overall callosal area, mainly due to larger anterior and mid regions, with no difference in the splenium. The most marked difference occurred in the isthmus ($\bar{X}$ scores = 72.4 vs 61.5 mm^2, $F = 6.9$, $p = .01$). Moreover, there was a significant interaction between hand preference and sex ($F = 10.1$, $p = .003$), which indicated that the difference between the hand groups was more marked in males than females.

Sex differences were also found in callosal size and morphology. Absolute area of the total callosum was statistically larger in males than females. The splenial area was almost significantly larger in men ($\bar{X}$ scores = 186.8 vs 173.6 mm^2, $F = 3.0$, $p = .09$), comparable to the results of most previous reports (reviewed in Witelson & Kigar, Wenner-Gren Int. Symp. Series, 47: 466, 1987). In contrast, in the isthmus, females had a significantly larger absolute area than males, among right handers ($\bar{X}$ scores = 64.4 vs 54.0 mm^2, $t = 2.29$, $p = .03$). Analyses controlling for sex difference in brain size and total callosal size highlighted the sex differences in callosal anatomy. The variation in size of the callosum, in particular the region serving the parietotemporal cortex, and its correlation with hand preference and sex will be discussed in terms of differences in patterns of hemisphere specialization and cognition between right and left handers, and males and females.

(Supported by NIH contract NS 62344 and grant NS 18954)

BIOLOGICAL RHYTHMS I

19.1 **THE EFFECTS OF HYPERGRAVITY ON THE CIRCADIAN RHYTHM OF CONIDIATION IN *NEUROSPORA CRASSA*.** H.N. Krum*[1], C.A. Fuller[2], F.M. Sulzman*[3] and J.S. Ferraro[1] (SPON: E. Barca). [1]Dept. of Biol. Sci., SUNY-Binghamton, Binghamton, NY, 13901; [2]Dept. of Animal Physiol., UC-Davis, Davis, CA 95616; [3]Div. of Life Sci., NASA, Washington, DC 20546.

To determine whether the circadian rhythm of conidiation in *Neurospora crassa* is endogenously derived or is driven by some geophysical time cue, an experiment was conducted on space shuttle flight STS-9, where inoculated race tubes were exposed to the microgravity environment of space. The results demonstrated that the rhythm can persist in space. However, there were several minor alterations noted; an increase in the period of the oscillation and the variability of the growth rate and a diminished rhythm amplitude, which eventually damped out in 25% of the flight tubes. On day seven of the flight, the tubes were exposed to light while their growth fronts were marked. It appears that some aspect of this marking process reinstated a robust rhythm in all the tubes which continued throughout the remainder of the flight.

We have hypothesized that the damping found prior to the marking procedure on STS-9 may have been a result of the hypergravity pulse of launch and not due to the microgravity of the orbital lab; furthermore, that the marking procedure, by exposing the samples to light, had reinstated rhythmicity. To test this hypothesis, we conducted an investigations into the effects of acute and chronic exposure to hypergravity. The acute studies consisted of a 10 minute 3G exposure (similar to launch). A chronic (7 day) 3G exposure was used in order to determine if there were any long term effects of hypergravity. Both BND and CSP strains of *Neurospora* were used. The onset of both the acute and chronic exposures to the 3G force was evenly distributed through-out the circadian cycle; i.e. packages filled with inoculated race tubes were placed on the centrifuge at circadian time (CT) 3, 9, 15, & 21. Two groups of the acute exposure group were exposed to light shortly after being removed from the centrifuge to determine whether the effects of the 3G force could be reversed through the use of a light pulse as had been suggested.

While chronic exposure of *Neurospora* to a 3G force had no damping effect, an acute exposure to hypergravity causes significant damping of the circadian rhythm of conidiation. Furthermore, a brief light pulse given 36 hours after the acute exposure eliminates any effect of the acute 3G exposure on damping. BND was more susceptible to the hypergravity perturbation than CSP. The average free-running period increases with chronic hypergravity similar to what was seen during spaceflight. Moreover, the period of each individual cycle continues to increase throughout the exposure period. It is not known whether this increase in cycle length possesses a threshold, since the period continued to increase for the entire length of the experiment. These results suggest that most, if not all, of the aberrant effects observed on STS-9 spaceflight can be explained by the hypergravity encountered during launch and not due to the lack of geophysical zeitgebers in space. Supported by NASA Grant NAG236 (JSF).

19.2 DEVELOPMENT OF CIRCADIAN RHYTHMS IN THE *LIMULUS* VISUAL SYSTEM. R. B. Barlow, Jr. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Circadian rhythms characterize the *Limulus* visual system. They are generated by a circadian clock which transmits efferent optic nerve activity from the brain at night to all major visual organs. The clock's input to the lateral eye produces pronounced circadian changes in retinal anatomy, physiology and metabolism that adapt the eye for diurnal changes in ambient illumination. (Barlow, R. B., Jr., J. Neurosci., 3:856, 1983).

How do the circadian rhythms develop? Is the clock programmed genetically or environmentally? To answer these questions, we fertilized eggs and raised them in the laboratory under three conditions: cyclic lighting (LD), constant light (LL), and constant dark (DD). Most eggs hatched after one month. After about 6 months, the animals reached the third juvenile stage (4 to 5 mm across the carapace). At this stage, the lateral eyes were large enough to produce stable electroretinographic responses (ERGs) when the animals were placed in darkness. Changes in the ERG amplitude were used as indicators of circadian rhythms in retinal sensitivity.

All LD 12:12 animals exhibited circadian rhythms in retinal sensitivity, but LL animals did not. DD animals had degenerate eyes and did not yield recordable ERGs. Animals raised on non-24 hr light cycles of LD 15.5:15.5 and LD 8.5:8.5 exhibited either a damped circadian rhythm in retinal sensitivity or no rhythm at all.

Circadian rhythms could be generated in most LL animals by exposure to multiple LD 12:12 cycles. In some animals, sustained circadian rhythms could be triggered by just a single light pulse of 6 hr or less in duration.

In sum, both genetic and environmental factors influence the development of circadian rhythms in the *Limulus* visual system. The genes set the length of the period to approximately 24 hr. Environmental lighting triggers the rhythm, perhaps by synchronizing the activity of multiple oscillators.

Supported by NSF grant BNS-8320315 and NIH grant EY-00667.

19.3 A CIRCADIAN RHYTHM RECORDED IN VITRO FROM THE OPTIC LOBE OF THE COCKROACH. Terry L. Page, Department of General Biology, Vanderbilt University, Nashville, TN 37235.

The timing signal that regulates the circadian rhythm of locomotor activity in the cockroach Leucophaea maderae originates in the optic lobes of the protocerebrum. Results from previous studies have shown that the pacemaking system is composed of two mutually coupled oscillators, one located in each optic lobe. One remaining issue is whether or not the optic lobe can independently generate a circadian oscillation in the absence of neural or hormonal communication with the rest of the animal.

To answer this question optic lobes were removed from male cockroaches and maintained in either Mark's M-20 medium (J. Insect Physiol. 18:847, 1972) or the "5+4" medium of Chen and Levi-Montalchini (PNAS 66:32, 1970). Results were similar for both media. Spontaneous multiunit activity was recorded from the cut end of the optic tract with a suction electrode which was also held the optic lobe at the surface of the medium. Action potentials were electronically counted and printed to paper tape every hour. The tissue was maintained in constant dim light at constant temperature (23 $\pm$ 0.2°C).

In these conditions optic lobes exhibited spontaneous neural activity for up to 10 days, with usual survival times of 3-5 days. Multiunit activity was recorded from a total of 26 lobes for 3 or more days. Activity was judged to exhibit a circadian rhythm if plots of activity vs. time subjectively appeared periodic and if times of peak activity Days 1 and 2 and Days 2 and 3 were approximately 24 h apart (dissection on Day 0). Based on these criteria, activity in 6 of 11 optic lobes maintained in 5+4 medium and 7 of 15 optic lobes in M-20 medium was rhythmic. Analysis of freerunning period and phase with respect to the prior light cycle was also based on the time of the maximum hourly activity in each cycle. The average value of the period of the rhythms was 23.8 $\pm$ 1.90 h (N=13). Peak activity generally occurred during the early subjective day. For optic lobes of animals that had been maintained in a light cycle (LD 12:12) with lights-on at 08:00, average peak time was 12.0 $\pm$ 2.19 h on Day 2 and 11.8 $\pm$ 3.56 h on Day 3 (N=6). For optic lobes from animals from a light cycle with lights-on at 22:00 time of peak activity was 21.8 $\pm$ 3.77 on Day 2 and 23.2 $\pm$ 3.89 h for Day 3 (N=5).

These data indicate that the isolated optic lobe is able to generate a circadian oscillation in the frequency of action potentials in the optic tract, and that the phase of the rhythm is dependent on the prior lighting conditions. Supported by NIH Grant No. GM30030.

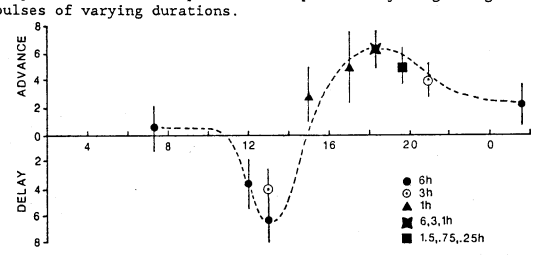
19.4 CIRCADIAN RHYTHMICITY OF BEHAVIORAL RESPONSES TO LIGHT IN THE CRAYFISH. F. Fernández de Miguel* and H. Aréchiga, Depto. de Fisiología, Biofísica y Neurociencias, CINVESTAV, México, D. F.

Light is known to affect crustacean behavior in a variety of ways, ranging from attraction induced by low levels of illumination, to avoidance reactions under strong light pulses. Little is known however, about the physiological mechanisms involved in these behaviors. In the present work, we analyse the effects of light intensity on locomotor behavior, as a function of the 24-hour cycle, and provide some information on possible neurophysiological mechanisms involved.

The experiments were conducted in adult crayfishes Procambarus clarki, of either sex and in intermolt. Locomotor activity was recorded from freely moving animals, placed in actographic two-compartment chambers, similar to those used by Aréchiga and Atkinson (Mar. Biol. 32: 63, 1975). Light intensity was externally controlled in the chambers and the position and rate of movement of individual animals was detected by means of a multichannel interface. Recordings of electrical activity from retinal elements and visual neurons were conventionally made. Two types of locomotor responses were detected: a) light attraction, as defined by displacement of the animals from a dark compartment to the illuminated one and b) light avoidance, when the movement was in the opposite direction. In either case, the time alloted as latency of the response was shorter than the average interval between spontaneous changes of compartment in darkness. Light attraction was induced in dark-adapted animals at day-time at 0.8 + 0.1 lux, whereas at night, the threshold value was 0.3 $\pm$ 0.2 lux. Above that level of intensity, the average latency of the response remained constant. The threshold for avoidance reaction was 15 lux at day-time and 9 lux at night. In animals kept during several days under recording, the changes in responsiveness to light closely parallelled the circadian rhythm of locomotor activity, which displayed a periodicity of τ=22.3 + 0.8 hs. in D:D and 24.52 + 0.8 hs in L:L, with two peaks of activity at subjective night. Light responsiveness showed the shortest latency at that time of day. The light intensity values for the switch from one behavior to the other also vary along the 24-hour cycle. The threshold for the avoidance reaction corresponds to the necessary intensity to promote the dispersion of proximal shielding pigments within retinal photoreceptors. The whole range of both the attraction and avoidance reactions falls within the dynamic range of the V-log I curve of electroretinogram and the F-log I curve of the light responses in sustaining fibers in the optic peduncle, suggesting that this neural pathway may subserve the light-input of both reactions.

19.5 PHASE-RESPONSE CURVE OF THE ISOLATED PINEAL GLAND OF *ANOLIS* M.Max*, S.Wisner*M.Menaker, Dept Biol, U of Va, Ch'lle, Va 22901

The isolated pineal of the iguanid lizard, *Anolis carolinensis* has been shown to display the three main properties of a circadian clock/system: It is rhythmic in constant conditions (freeruns), it synchronizes to a light:dark cycle (entrainment), and its period of oscillation is only slightly dependent on ambient temperature (temperature compensation)(Menaker and Wisner 83). Here we report that it possess another classic property of circadian systems, phasic response to single light pulses. Isolated glands superfused for four days in the dark are exposed to single light pulses delivered at specific times during the second day of culture. Alloquiots of superfusate from individual glands are collected every 90 minutes throughout the four days of culture, and assayed by RIA for melatonin. Phase shifts are determined by comparing the time of the fourth peak of light pulsed glands with controls that have received no light pulse. We find that glands shift their peak of melatonin secretion to an earlier time (advance) in response to a light pulse given late in the subjective night (CT 15-22), to a later time (delay) in response to light pulses early in their subjective night (CT 12-14), and do not shift in response to light pulses given during their subjective day. Single light pulses of durations from 15min. to 6hr given during the late subjective night (CT 18-19) produce equivalent 6hr. phase advances. A light pulse of 6hr. given early in the subjective night (CT 13) produces a 6hr. delay while a 3hr. light pulse given at the same circadian time results in a delay of only 3hr. While it is clear that the duration of the light pulse is not important in determining the magnitude of the advancing phase shift, we are less sure that this is true for delaying light pulses. Further experiments employing short duration light pulses around CT 13 will answer this question. The phase-response curve shown below is a composite of the mean phase shifts produced by single light pulses of varying durations.



19.6 OSCILLATION OF CYCLIC AMP AND MELATONIN RELEASE IN CHICK PINEAL CULTURES. S. S. Nikaido and J. S. Takahashi. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

Dissociated chick pineal cells express a circadian oscillation of serotonin N-acetyltransferase (NAT) activity and of melatonin release in vitro. The cell cultures are photosensitive and the melatonin oscillation can be entrained by light cycles in culture. Previous studies have suggested that cAMP may be involved in the regulation of NAT in the chick pineal.

To determine whether cAMP levels oscillate, dispersed chick pineals were grown on Cytodex 3 beads for 2 days and then were transferred to a flow-through culture system (about 2 pineal equivalents per culture). Both melatonin and cAMP were measured by radioimmunoassay from the same samples collected every two hours over a period of 6 days. Chick pineal cultures expressed an oscillation of cAMP efflux on a LD 12:12 light cycle. The cAMP oscillation was in phase with the rhythm of melatonin release. The level of cAMP efflux oscillated with trough values around 0.3 pmol/hr during the day and peak values of 0.6-0.8 pmol/hr during the night. Corresponding melatonin release levels were 20 pmol/hr during the day and 200-400 pmol/hr during the night. In the presence of 0.25 mM isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, the levels of cAMP efflux increased about 10-fold with trough values of 1-2 pmol/hr and peak values of 2-8 pmol/hr while melatonin release oscillated between 50 pmol/hr and 400-800 pmol/hr.

We have also found a day-night oscillation in cAMP content in chick pineal cell cultures. Dispersed chick pineal cells were plated at 1.25 x 10^5 cells per well and cultured for 36 hrs before the first sample was taken. Under these conditions, the day value for cAMP content was 6.89 $\pm$ 1.32 fmol/well (mean $\pm$ S.E.; n=8) and the night value for cAMP content was 22.5 $\pm$ 2.44 fmol/well. The cAMP content was correlated with cAMP efflux during the previous 6-hr interval (day: 7.69 $\pm$ 1.20 fmol/well; night: 34.8 $\pm$ 2.6 fmol/well). With a 15-min pretreatment with IBMX, day content value increased to 11.3 $\pm$ 2.34 fmol/well and night content value increased to 45.7 $\pm$ 1.35 fmol/well.

In order to investigate the mechanisms controlling the oscillation of cAMP, we have initiated a series of experiments studying the role of G-proteins. Pertussis toxin treatment of static cultures during the light phase of a LD 12:12 cycle resulted in a dose-dependent elevation of melatonin release during the day. Whereas, pertussis toxin treatment during the dark phase did not elevate melatonin release. These results indicate a differential effect of pertussis toxin with time of day and suggest a possible role for pertussis-toxin sensitive processes in the regulation of the melatonin rhythm. (Supported by NIMH grant MH39592, Searle Scholars Award 85-H-107 and NSF PYI award DCB-8451642)

19.7 EFFECTS OF PERTUSSIS TOXIN ON LIGHT-INDUCED REDUCTION IN MELATONIN RELEASE AND PHASE-SHIFTING RESPONSE OF CIRCADIAN OSCILLATOR IN CHICK PINEAL CELLS. L.M. Robertson and J.S. Takahashi. Neurobiology & Physiology, Northwestern University, Evanston, IL 60201.

Chick pineal cells express a circadian oscillation of melatonin release. Light regulates the melatonin rhythm by the process of entrainment (which involves control of phase and period) and acute exposure to light causes a reduction in melatonin release. The mechanisms underlying phototransduction in the avian pineal gland are poorly understood. In retinal photoreceptors, the G-protein, transducin, is neccessary for phototransduction: ADP-ribosylation of transducin by pertussis toxin (PT) blocks the effect of light. We explored the effects of PT on both the acute and phase-shifting effects of light in cultured chick pineal cells. Cells were plated on 48-well dishes in medium-199 with serum and were maintained on LD 12:12. Beginning at circadian time (CT) 14 or 17 (lights off at CT12) of the 5th night in culture, cells were exposed to 0, 100, or 300ng/ml of PT. Medium was changed at CT20 with the same doses of PT. One-half of each group was exposed to 3 hr of light (125 lux) beginning at CT20. Control cultures were maintained in darkness. Medium was sampled at the end of the 3-hr and assayed for melatonin by RIA. Exposure to light caused a reduction in melatonin release relative to dark controls. Treatment with 100 or 300ng/ml PT from CT14-23 completely blocked the light-induced reduction in melatonin. A partial block was also observed with 100 or 300ng/ml PT from CT17-23. We used a flow-through culture system to determine whether PT treatment also blocked the phase-shifting effects of light. Chick pineal cells were cultured and light-induced phase shifts measured as previously described (Robertson and Takahashi, Soc. Neurosci. Abst. 12:221, 1986). Previous experiments showed that a 3-hr 125 lux light pulse caused a submaximal phase shift of the melatonin rhythm relative to dark controls. Cultures exposed to a 3-hr light pulse (125 lux) beginning at CT20 were advanced 4.95 $\pm$ 0.37 hr ($\bar{X} \pm$ SEM) relative to dark controls. PT (100ng/ml) treatment for 9 hr (CT14-23) had no effect on the phase of the rhythm in constant darkness. Cultures treated with PT (100ng/ml) for 9 hr (CT14-23) and exposed to a 3-hr light pulse (CT20-23) were phase advanced 5.05 $\pm$ 0.39 hr relative to dark controls. Therefore, PT did not block the light-induced phase shift. However, analysis of melatonin release during the light pulse revealed that PT blocked the light-induced acute reduction in melatonin release in the same experiment. In summary, treatment with pertussis toxin can block the acute reduction of melatonin without blocking the phase-shifting effect of light. These results suggest a differential effect of pertussis toxin on the acute and entraining effects of light. (Supported by NIMH grant MH39592, Searle Scholars award 85-H-107 and NSF PYI award DCB-8451642 to JST and NIMH award F31 MH09465 to LMR).

19.8 FURTHER CHARACTERIZATION AND REGIONAL DISTRIBUTION OF 2-[^{125}I]-IODOMELATONIN BINDING SITES IN HAMSTER BRAIN MEMBRANES. M.J. Duncan, J.S.Takahashi and M.L. Dubocovich, Dept. Pharmacol., Northwestern Univ. Med. School, Chicago, IL 60611 and Dept. Neurobiol. and Physiol., Northwestern Univ., Evanston, IL 60201.

We have previously characterized a high affinity 2-[^{125}I]-iodomelatonin binding site in membrane preparations from Syrian hamster whole brains (Duncan, M.J. et al., Eur. J. Pharmacol. 132:333, 1986). The present studies determined the subcellular and regional localization of 2-[^{125}I]-iodomelatonin binding sites and the potencies of various compounds that act at monoaminergic sites to compete for 2-[^{125}I]-iodomelatonin binding in hamster brain membranes. Membranes from hamster whole brains were prepared in ice-cold assay buffer (50 mM Tris-HCl, pH=7.7 at 25°C). Assays containing membranes (150-250 ug protein), 2-[^{125}I]-iodomelatonin (85-125 pM) and various drugs were incubated at 0°C for 1 h. Binding was terminated by filtration. Specific binding, defined as the difference between binding in the absence and presence of 10 uM 6-Cl-melatonin, was 65-75% of total binding. When specific binding (fmol/mg prot) of 2-[^{125}I]-iodomelatonin (85 pM) was measured in three membrane fractions (N=3), the highest amount of binding was found in the P_2 fraction (1.29 $\pm$ 0.23) as compared to the P_1 fraction (0.37 $\pm$ 0.03) or the total washed membrane fraction (P_1 + P_2). 2-[^{125}I]-iodomelatonin binding sites (fmol/mg prot) were found to be unevenly distributed in eight brain regions (N=3) examined: hypothalamus (6.20 $\pm$ 0.24), brainstem (5.87 $\pm$ 0.36), retina (5.24 $\pm$ 1.43), striatum (4.25 $\pm$ 0.89), olfactory bulbs (3.44 $\pm$ 0.08), cerebral cortex (2.96 $\pm$ 0.38), cerebellum (2.74 $\pm$ 0.41) and pituitary (1.88 $\pm$ 0.11). The affinity (K_i) of 6-Cl-melatonin for the 2-[^{125}I]-iodomelatonin binding site was 15.8 $\pm$ 2.3 nM (N=6). Compounds known to act at serotonin, dopamine and alpha receptor sites were markedly less potent than 6-Cl-melatonin in competing for 2-[^{125}I]-iodomelatonin binding [(IC_{50}, nM) ketanserine (600), bufotenine (1,590), fluphenazine (1,800), spiperone (2,000), serotonin (4,000), propranolol (5,000), yohimbine (>70,000); methysergide, cinanserine, cyproheptidine, phentolamine, dopamine, norepinephrine and epinephrine were inactive at concentrations up to 100,000 nM]. These results suggest that 2-[^{125}I]-iodomelatonin does not bind to monoaminergic receptor sites and in conjunction with our previous report indicate that 2-[^{125}I]-iodomelatonin can be used to selectively label melatonin binding sites in the brain. Furthermore, the presence of a high level of 2-[^{125}I]-iodomelatonin binding in the hypothalamus is consistent with previous studies suggesting that this region is a target site for the photoperiodic effects of melatonin.

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19.9 **TEMPORAL ORGANIZATION OF CRITICAL PROTEIN SYNTHESIS IN THE BIOLOGICAL CLOCK IN THE APLYSIA EYE.** S. J. Yeung* and A. Eskin (SPON: G. F. Gwilliam). Biol. Dept., University of Houston, Houston, Tx 77004.

Protein synthesis seems to be a general requirement for circadian timing. We have attempted to define when proteins important for timing are synthesized in the Aplysia eye.

Rothman and Strumwasser (1976), Jacklet (1977) and Lotshaw and Jacklet (1986) generated phase-response curves (PRC) for relatively long pulses (6hr) of anisomycin and puromycin. Although recovery of protein synthesis after a pulse of anisomycin was reported to be rapid in cultured cells, Lotshaw and Jacklet and we found that recovery was slow (2.5hr to recover to 40% of control and more than 8hr to fully recover) in Aplysia eyes. Therefore, it is difficult to infer accurately when protein synthesis is important from the anisomycin PRC's. Thus, a protein synthesis inhibitor whose effect reverses quickly needs to be identified.

We studied recovery from inhibition of protein synthesis after 2hr pulses of emetine ($2X10^{-5}$M) and O-methylthreonine (4mM). Both emetine and O-methylthreonine seemed to reverse no faster than anisomycin. Cycloheximide (CHX) reversed faster than the others. CHX 10mM (1hr) produced 89$\pm$4% (N = 4) inhibition of ^{3}H-leucine incorporation and within 3 hr after the pulse, the recovery was 85$\pm$22% (N = 4).

We obtained a PRC using 1hr pulses of CHX (10mM). The magnitudes of the phase shifts were small (largest phase shift: -1.2$\pm$0.6hr, N = 7). CHX produces no phase shift from CT11 to CT21. There may be a small advance phase shift at about CT09. Delay phase shifts occur from CT18 to CT09. Can the CHX PRC be explained by different kinetics of recovery at different phases? Slow recovery (longer duration of inhibition) at certain phases may produce phase shifts whereas fast recovery at other phases may not produce phase shifts. No difference in the recovery time was observed when recovery from inhibition by CHX was measured at two phases (a phase when CHX produces no phase shift and a phase when CHX delays the circadian rhythm). Synthesis of proteins important for the timing mechanism seems to occur during late subjective night and most of the subjective day (i.e. from CT18 to CT09). We are searching for proteins synthesized only during that time period and comparing them with proteins whose synthesis is modified by phase-shifting treatments of light and serotonin.

Supported by NSF BNS8216756 and NIMH MH41979.

19.10 PHASIC DISCHARGE OF NEURONS IN THE RAT SUPRACHIASMATIC NUCLEUS. J. D. Miller and C. A. Fuller. Department of Animal Physiology, University of California, Davis, CA 95616.

This study reports data from a subpopulation of single suprachiasmatic nucleus (SCN) units recorded in ongoing electrophysiological investigations of SCN neurons and their afferent inputs. Male albino rats (Wistar, $\bar{x}$ = 263 $\pm$ 23 g) were anesthetized with urethane and catheterized in the femoral vein. Standard electrophysiological techniques were used to record the activity of single units in the SCN and adjacent areas (e.g., medial preoptic and retrochiasmatic areas). A fiber optics light source was used to apply photic stimuli directly to the eye (85 Lux). In approximately 19% of fast green labelled placements in the SCN and immediately adjacent optic chiasm (OC), but not in adjoining peri-SCN regions, we observed phasic discharge (bursting) with a period of approximately 3 min (n = 11, $\bar{x}$ = 199 $\pm$ 28 sec). The action potential waveforms were either biphasic (n = 5) or triphasic (n = 6), with an average duration of 3.0 $\pm$ 0.3 msec. These parameters differ considerably from the characteristics of chiasmatic fibers (monophasic waveforms with durations typically $\leq$1 msec). Interspike interval (ISI) histograms were generated for these cells for sequential periods of rapid (active state) and slow (inactive state) discharge. These cells periodically decreased their firing rates from a relatively active state ($\overline{ISI}$ = 87.3 $\pm$ 31 msec) to an inactive state ($\overline{ISI}$ = 233 $\pm$ 68 msec) by 147% (t for related measures = 4.4, df = 10, p <0.01). This population was also examined for burst activity in the msec range. Burst length averaged 3 to 4 spikes/burst in the active or inactive states. Decremental bursting was not observed. The mean percent change in bursts/total spikes increased by 18.6 $\pm$ 7.1% (t = 2.6, n = 11, p <0.05) from the active state (mean % bursting = 15.7 $\pm$ 6.1%) to the relatively inactive state (mean % bursting = 34.3 $\pm$ 6%). No parametric differences were observed between placements in the SCN proper and placements in the OC. Only VIP and GRP immunoreactive neurons have been observed in the dorsal OC. Through the period augmenting effects of strong coupling mechanisms as proposed in some mathematical models, a relatively small number of SCN neurons could be sufficient to generate circadian periodicity. It is possible that bursting neurons such as the ones described, with ultradian periodicity approximating that of many well-known biochemical reactions, may be the ultimate substrate of the circadian clock of the SCN.

This research was supported by NIMH Grant 7RO1-MH41477-01.

19.11 ELECTRICAL AND METABOLIC ACTIVITY OF THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE GOLDEN HAMSTER IN HYPOTHALAMIC SLICES. S. Shibata* and R.Y. Moore (SPON: A.Rosen). Depts. of Neurology and Neurobiology, SUNY-Stony Brook, Stony Brook, NY 11794 and Dept. of Pharmacology, Kyushu University, Fukuoka 812, Japan

The evidence for the SCN as a circadian pacemaker in the mammalian brain includes observations of circadian rhythms in glucose metabolism in vivo (Schwartz et al, 1980) and in vitro (Newman and Hospod, 1986) and electrical activity in vivo (Inouye and Kawamura, 1979) and in vitro (Shibata et al, 1982). These observations have been obtained primarily from studies on the rat but, in contrast, the hamster is the species most often chosen for behavioral investigation of the functional properties of the circadian system. In this context, the present study was undertaken to determine whether a circadian rhythm in SCN metabolism and single unit activity can be shown in hamster hypothalamic slices in vitro.

Adult male golden hamsters weighing 120-150 g maintained in a normal or reversed light-dark cycle were used. After decapitation, the brain was quickly removed and coronal hypothalamic slices (400 μm), including the SCN and the anterior hypothalamus (AHA), were prepared using a tissue chopper. Preparations were pre-incubated for one hour and transferred either to a recording chamber for electrophysiology or an incubation chamber for analysis of glucose metabolism using the 14C-2-deoxyglucose technique. Neuronal activity was recorded extracellularly through glass electrodes. Incubations with isotope were carried out for 20 min and then removed from the chamber and rinsed for 30 min. These slices were cryostat-sectioned at 20 μm, and the sections exposed against X-ray film for 2 weeks. The autoradiographs are then analyzed by densitometry and a simple estimate of local glucose utilization (LGU, μ mole glucose/100g tissue/min) was obtained.

Single unit activity is highest at circadian time (CT) 4-6 (CT00.00 is the time of lights on) with a mean firing rate of 8.6±1.0 imp./sec (Mean ± S.E.). It is lowest at CT16-18, 2.3±0.4. Although we compared the neuron activity between ventrolateral and dorsomedial SCN, no difference was observed in the firing rates between these areas. Autoradiographs of SCN and AHA were obtained from slices during subjective day (CT0600) and subjective night (CT1800). The SCN appears as a pair of densely labeled areas above the optic chiasm during subjective day but are lightly labeled or not evident during subjective night. The LGU of SCN is approximately three times higher during subjective day than subjective night whereas the LGU of AHA is nearly identical at these time points.

Since apparent circadian rhythms are demonstrated in SCN single unit activity as well as in SCN 2-DG utilization, we conclude that the brain slice method is useful for analysis of circadian function in the hamster SCN. Supported by NS-16304.

19.12 **EFFECTS OF IONIC MANIPULATION ON THE CIRCADIAN RHYTHM OF NEURONAL FIRING RATE IN THE SUPRACHIASMATIC BRAIN SLICE.** Martha U. Gillette. Department of Physiology & Biophysics and Neural & Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

Understanding of the circadian pacemaking properties of the mammalian suprachiasmatic nuclei (SCN) has been enhanced by study of their physiology in vitro. Circadian rhythms of neuronal firing rate and vasopressin secretion can be measured in rat SCN maintained in culture demonstrating that a circadian oscillator is endogenous to this tissue. As a prelude to examining cellular mechanisms which underlie nighttime entrainment, we have examined the effect of in vitro depolarizing pulses of K^+ on the subsequent cycle of firing rate in SCN neurons.

Hypothalamic slices (500 μ thick) containing the SCN were prepared from Long-Evans rats at a phase-neutral time (CT 10-11), and maintained in a brain slice chamber perfused with HEPES-buffered Earle's Balanced Salt Solution. In order to determine the sensitivity of the oscillator to depolarization, three 10 min pulses of 50 mM KCl, separated by 10 min periods with normal media (KCl= 5.4 mM) were applied to the tissue. This stimulation regime has been shown to relieve general depressive effects on neuronal excitability of long-term K^+ exposure (Hartter & Ramirez, Endocr. 107: 375, 1980). High K^+ medium was maintained isosmotic by adjusting the NaCl content and was preheated and oxygenated for >5 min before use.

50 mM K^+ pulses applied during CT 17-19 had no effect upon the viability of the slices, nor upon the ability of SCN neurons to generate their circadian rhythm in firing rate (n=6). The peak of this oscillation occurred at CT 1.53±0.82 in K^+ treated slices. This represents a mean phase advance of 6.7 hr from the unperturbed time of peak in vitro at CT 7.87±0.36.

These investigations demonstrate that this regime of depolarizing K+ pulses is a potent phase-shifting treatment to the isolated oscillator. We are now in a position to test the ability of other medium replacements to permit or antagonize depolarization-induced phase changes. Further, these data support the notion that dissection-induced K+ release may have contributed to the phase-shifting effects of slice preparation during the animal's night (Gillette, Br. Res. 379:176, 1986). Supported by PHS Grant NS22155.

19.13 PROGRESSIVE AND DIFFERENTIAL DEGENERATION OF CIRCADIAN RHYTHMS IN AGING FEMALE RATS. B. Tate-Ostroff. Department of Anatomy and Cellular Biology, Harvard Medical School, and Department of Psychiatry, McLean Hospital, Belmont, MA 02178.

The virgin, aging female rat experiences a loss of normal estrous cyclicity and the onset of persistent vaginal estrus (PVE) at approximately 8 months of age. PVE appears similar to that induced by disruptions of normal circadian rhythmicity, for instance by lesions of the suprachiasmatic nuclei or by exposure to constant bright light. It has been suggested that PVE in aging females is the result of age-related alterations in the circadian system. However, we have examined the circadian rhythms of gross motor activity and body temperature in rats undergoing the transition to PVE and found no alterations in circadian rhythms in animals exposed to normal lighting schedules (Tate-Ostroff and Bridges, 1986). Our initial studies were performed in animals exposed to a regular lighting schedule (LD14:10) and it is possible that rhythmicity was induced by this alternating environmental cue instead of being endogenously generated. We therefore extended our studies to PVE females free running in costant conditions. Middle-aged female rats (N=5, 7-9 months of age) that had entered PVE within the month preceding data collection and young females (N=3, 3 months of age) showing normal estrous cycles, were transferred from LD14:10 to dim, constant light of less than 4 lux (LL). Body temperature and gross motor activity data were simultaneously collected from each animal via implanted telemeters (Mini-mitters). Daily vaginal smears were also taken from each animal. Both PVE, middle-aged females and normally cycling, young females showed free running circadian rhythms. We conclude that PVE is probably the result of age-related alterations in the reproductive system and not a secondary effect of age-related alterations in the circadian system.

Since other laboratories have reported abnormal circadian rhythms in PVE rats, some PVE animals were monitored for months after the onset of PVE. These animals did eventually showed abnormal rhythms, with abnormalities in activity rhythms being displayed before abnormalities in body temperature. The sequence of loss of normal activity rhythms before the loss of normal body temperature rhythms may be a consequence of the strenght of coupling between these rhythms and the primary oscillator.

19.14 INFLUENCE OF AGE AND ACTIVITY RESTRICTION ON SLEEP-WAKE AND DRINKING RHYTHMS IN THE MOUSE. D. M. Edgar, T. S. Kilduff and W. C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94305.

Previous studies in this laboratory have implicated activity as an important determinant of sleep and wakefulness consolidation in the mouse. Activity restriction, imposed by locking an otherwise freely rotating running wheel, decreases consolidation of wakefulness during the subjective day and dampens the amplitude of the circadian sleep-wake cycle. These altered sleep-wake patterns are remarkably similar to that observed in aged animals. In the present study, we investigated the generality of activity restriction effects by simultaneously monitoring drinking patterns and arousal states. We have also compared the effects of activity restriction in young (< 6 mos.) and older (18-22 mos.) mice.

Young and old male Mus musculus (C57/BL/6Nnia) were surgically prepared with chronic implants which permitted EEG and EMG recording. Animals were individually housed in filter-top cages, each equipped with a swivel commutator and running wheel. Ambient temperature was maintained at 24-25°C, lighting was LD 12:12, and both food and water were available ad libitum. Drinking and sleep-wake stages (awake, slow wave, and paradoxical sleep) were monitored in 10 sec epochs using a recently developed microcomputer based sleep scoring system. Parity between human and computer based sleep-wake determinations was validated in excess of 97%. Mice were monitored 20-30 d with freely rotating running wheels, for 16-24 d with wheels locked, and then for an 14 d of free wheel running opportunity.

With running wheels unlocked, young mice exhibited robust circadian rhythms in sleep-wakefulness and drinking which were entrained to the light-dark cycle. Older mice exhibited circadian rhythms lower in amplitude and less stable in waveform. When running wheels were locked, all animals exhibited an initial diminution of circadian amplitude of the sleep-wakefulness and drinking rhythms. Partial restoration of sleep-wake rhythm amplitudes and total restoration of the drinking rhythm was observed 6-10 days after wheel locking was initiated. After wheel availability was reestablished, sleep-wake rhythm amplitudes were completely restored. Total sleep time per day did not differ appreciably between old and young animals while wheels were locked (about 750 min/24 hrs). However, the patterns of wakefulness observed in the older animals during the wheel lock period showed noticeably greater wake fragmentation. These observations support the notion that activity plays an important role in sleep consolidation, but may have less influence on the drinking rhythm. In addition, activity may be of particular importance for the consolidation of sleep-wake rhythms in older animals (Supported by NRSA AG05397 to DME, AG06490, and MH05804 to WCD).

19.15 CIRCADIAN EFFECTS AND AVOIDANCE LEARNING IN GENETICALLY DEFINED MICE. D. F. Peeler, Department of Neurosurgery, University of Mississippi Medical Center, Jackson, MS 39216

Circadian rhythms characterize a variety of behaviors and have been reported to be at least in part a function of genetic influences. The genetically defined mouse strains C57BL/6 and BALB/c have been shown to exhibit variation in several activity measures as a function of both strain and time of day. Genetic but not circadian influences have been reported for these strains on another behavioral measure - shuttle box avoidance. It consistently has been reported that C57BL/6 mice are inferior to BALB/c in rate and degree of acquisition. Recent attempts to replicate earlier findings from this and other laboratories of superior performance in a shuttle box task by BALB/c have given mixed results. Examination of the data revealed that C57BL/6 and BALB/c adult male mice which were trained to avoid shock by crossing a hurdle in a shuttle box at times primarily late (after about 15:00 hours) or earlier (before about 11:00 hours) in the working day produced the typical pattern of avoidance response acquisition (i.e., BALB/c acquire the response more readily and to a greater proficiency than C57BL/6). (Animals are maintained on a 12 hr. light/dark cycle, with light on from 6:00 to 18:00.) However, if training is conducted at an intermediate time (between about 13:00 and 15:00), this relationship between strains is changed: the C57BL/6 exhibit a considerable, statistically significant improvement in both rate and final level of acquisition. They achieve essentially the level of the BALB/c tested at earlier or later times. BALB/c at the 13:00 to 15:00 period show a non-significant trend toward performance decrement. The seven RI strains derived from these progenitor strains give mixed results: those RI strains having poorest performance at the earlier or later times tend to improve, while those having good acquisition tend to remain in this category. Thus, the distribution of scores for avoidance performance is in part dependent upon the time of day (or position in the light/dark cycle) at which testing is conducted.

C57BL/6 mice have been reported to have a trough of tyrosine hydroxylase activity at about this (intermediate) point in the light/dark cycle, while they and the BALB/c show out of phase levels of tryptophan hydroxylase, serotonin and its metabolites in selected brain sites at this time. Also, this change in performance occurs at approximately the same time of day that BALB/c and some of the RI strains (but not C57BL/6 and the other RI strains) exhibit an increase in emotionality (measured as defecation during initial exposure to activity testing), and show a reduction of investigatory but not locomotor activity.

Thus, several behavioral and neurophysiological variables exhibit comparable, in phase circadian cyclicity. The implication is that some of these characteristics may be manifestations of genetic determinants at the same or linked loci.

19.16 SENSORIMOTOR BIASING OF SYNCHRONIZATION TIMING. H. Kim, D. Hary, and G.P. Moore*. School of Engineering, University of Southern California, Los Angeles, CA 90089-1451.

In studies of temporal tracking behavior, subjects were asked to synchronize the sound produced by tapping a Morse key with the sound from a loudspeaker driven by a periodic metronome source (interval 700 ms). The sound produced by the Morse key was a click delivered through a second loudspeaker. The Morse key itself was silent in operation. The tap-triggered sound was subject to selected delays, ranging from 0 to 60 ms. When the delay was set to 0 ms, all subjects led the metronome (mean 21 ms, 6 ms s.d.), confirming numerous earlier studies (Hary and Moore, Human Neurobiology, 4:73, 1985). As the delay time between tap and the tap-evoked sound increased, subjects would have to tap earlier in the metronome cycle in order to maintain the same relative synchrony. 6 of 14 subjects did this. The remaining 8 subjects, although also tapping earlier in the cycle, all achieved near-synchrony (an average of 0 ms, 4 ms s.d.) when the delay between tap and sound was made large enough, some achieving synchrony when the delay was as small as 30 ms. We hypothesize that these latter subjects, by dissociating the proprioceptive/motor components of their response from the auditory product of the response were able to attend to the audio error and correct it to a value not significantly different from zero. The inability of the other subjects to achieve synchrony is a matter of continuing investigation.

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19.17 CORTICAL COMPONENTS OF THE EEG REVEALED BY REAL-TIME OPTICAL IMAGING OF NEURONAL ACTIVITY IN CAT CORTEX. A. Arieli*, R.D. Frostig, E.E. Lieke, R. Hildesheim*, and A. Grinvald. The Weizmann Institute of Science, IBM Thomas J. Watson Res. Ctr., and Laboratory of Neurobiology, The Rockefeller University.

Optical imaging of fast cortical activity *in vivo* provides information about electrical activity of neuronal assemblies with millisecond time resolution, and a few hundred microns spatial resolution. Since the optical signals are restricted to their site of origin within the upper cortical layers, comparisons between electrically recorded EEG waves and spatio-temporal optical maps provides a new approach for analysing the origin of individual EEG components, event related potentials, and interactions among distant neuronal assemblies.

The exposed cat visual cortex was stained for 90-120 min prior to optical imaging. We used a 12×12 diode array and found that the styryl dye RH-795 provides relatively large signals in the cat cortex. To investigate the cortical components of the EEG, two types of experiments were performed. In the first one, spontaneous epileptiform activity was induced in the cortex by topical application of bicucculine. In the amplitude domain the activity was relatively uniform. However the foci of activity and propagation of the epileptic waves was determined by analysis of the millisecond temporal domain (similar to rat experiments by Orbach et al. 1985, London et al. 1986). The spatio-temporal patterns of repetitive epileptic bursts were mapped for 30 seconds continuously, and such measurements repeated up to 10 times (the fractional change in fluorescence was 0.3%). A comparison with surface recording of the EEG indicated that only negative waves with a distinct time course originated from the upper layers of the stained cortex. Therefore, optical imaging can determine the cortical origin of components of the EEG waves.

In the second experiment, normal spontaneous activity of stained cortex was optically monitored. Although the optical signals were much smaller in this experiment, they were resolved without averaging when large sleep spindles occurred. The spatial distribution of the optical signals which correlated in time with the largest sleep spindles was nonuniform: large optical signals were observed at different sites. In addition the particular nonuniform patterns varied from one spindle to the next. These observations suggest that optical imaging can reveal the spatial and temporal organization of interacting neuronal assemblies.

An alternative way to analyse the data would be to calculate the cross correlation between all possible pairs from 128 sites. The results of such an analysis, which is likely to increase the sensitivity and spatial resolution, will be discussed.

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19.18 ENDOGENOUS POSTICTAL ANTICONVULSANT SYSTEMS THAT CONTROL SEIZURES IN GERBILS AND HAMSTERS. Paul L. Prather* and W. B. Iturrian, Pharmacology, Univ. of Georgia, Athens, GA. 30602.

In epileptics, a postictal phase prevents seizure recurrence for several days. In rodents, paired electroshock shows a two phased postictal period consisting of a brief period of hyperexcitability in which a second tonic extensor seizure can be elicited, followed by a prolonged period of depression and anticonvulsant effects. The spontaneous seizures of the gerbil model of epilepsy do not appear to occur randomly but rather show a periodicity across days. Each individual possesses its own rhythm suggesting that seizure diathesis involves an oscillator of brain excitability modified by postictal mechanisms. The neurological basis for a long term oscillator or postictal protection is not known but endogenous anticonvulsant chemical(s) are attractive possibilities since they can be modified by genetic, behavioral, and pharmacological methods. We suggest that several different endogenous systems are activated by seizure and that they act in cascade-like sequence. Furthermore, deficits in different parts of the cascade contribute to the hereditary spontaneous seizure of gerbils and hamsters.

Our inbred, genetically epileptic, black gerbils have brief explosive tonic-clonic seizures elicited by handling but become refractory with optimal anticonvulsant effects occurring biphasically at 15 min and at 2 to 3 days. In contrast, our genetically epileptic sz hamster (derived from Bio 86.93) have BDZ deficits. Therefore, their seizures are bouts of increasing severity becoming tonic by 30 minutes and persist for 3 hours without an apparent postictal protective phase. Although sound does not precipitate seizure in either species, it can either increase or decrease brain excitability depending upon the temporal relationship between sound and seizure testing. Brief (sec) sound (100dbA) blocks gerbils' postictal effect occurring at 15 min but then protects both gerbils and hamsters from seizure for many days. Indomethacin (10 mg/kg) also blocks the 15 min postictal event. Although the sz hamster outgrows spontaneous seizures, deficits in postictal inhibition persist since paired electroshock elicits a 2nd tonic extensor seizure without any depression phase. Morphine sulfate (10 mg/kg) reduces the incidence of 2nd seizure by 39% without significantly altering the initial seizure. This protective effect was reversed by the opiate antagonist naloxone. In hamsters without the sz trait, naloxone (4 mg/kg) blocks the postictal anticonvulsant effect of paired electroshock and incidence of 2nd tonic extensor seizure increased to 88%. Indomethacin is anticonvulsant for sz hamsters but blocks postictal effects in controls. Numerous endogenous chemicals are released following a seizure (or auditory stimulation) that regulate brain excitability but short term and long term effects may differ dramatically.

20.1 REPRODUCTIVE OUTPUT OF PAIRED MALE RATS AS A FUNCTION OF SOCIAL DOMINANCE. J.A. Witcher*, W.R. Bachman*, and N.T. Adler, Dept. Psychology, Univ. Pennsylvania, Philadelphia PA, 19104.

Dominant rats sire more offspring in a competitive mating situation than submissive rats. To investigate the mechanisms of these effects a number of reproductively relevant variables were investigated. These variables included mating behavior during the first ejaculatory series, number of sperm ejaculated and transported, and copulatory plug within female reproductive tract after the first ejaculatory series.

Virgin Long-Evans and Sprague-Dawley male rats (90 days) were initially housed, alone in hanging metal cages (43X25X18cm; 14L:10D lights on 0600). An estrous female was introduced into each male's cage and remained until the male ejaculated. Twenty min after ejaculation sperm within the female reproductive tract were counted and copulatory plug assessed. This was repeated four times with each male with no less than 3 days between mating sessions. Each male was given 4 mating sessions.

Pairs of males were established by introducing a Sprague-Dawley male from one cage into the home cage of a Long-Evans male of matched weight or vice versa. Following observation of agonistic behavior, dominant and submissive status was assigned to each male. After one week of the male's co-habitation estrous females were introduced one at a time to each pair of males until each member of the pair had attained an ejaculation. Sperm counts were performed 20 min after ejaculation. This was repeated for each pair until each male had mated with five females, with no pair being tested within less than 3 days of a previous mating session.

Therefore, data from 4 mating sessions in a non-competitive mating situation and 5 mating sessions in a competitive mating situation were obtained. In the non-competitive situation no differences existed between rats that were to be dominant, as opposed to submissive, with respect to mating efficiency, sperm output, or copulatory plugs. However, after the first competitive mating, sperm recovery in females mated with dominant males was slightly elevated when compared to the four non-competitive matings; while sperm recovery from females mated with submissive males dropped by more than 50%. Over the next 4 competitive matings the submissive animals' sperm output gradually rose to non-competitive mating levels while, the dominant males' sperm output was maintained at non-competitive mating levels. In addition, submissive males' copulatory efficiency and copulatory plug weight was reduced when compared to that of dominant males. Copulatory efficiency and plug weight did not display recovery over the mating sessions, unlike sperm output which did recover.

Pairing of adult male rats results in a dominance relationship. Dominance appears to confer a reproductive advantage via both acute and chronic mechanisms. The acute mechanism is reflected in a transient lowering of sperm output by a submissive male while a more chronic mechanism is manifest in behavior and copulatory plug characteristics.

20.2 RETENTION OF SEXUAL BEHAVIOR AFTER CASTRATION : ENVIRONMENTAL INFLUENCES. B. E. F. Wee, K. L. Sinchak*, and L. G. Clemens. Department of Zoology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

The extent to which sexual behaviors are retained after castration varies among different strains of mice, as well as among individuals of the same genotype (Wee et al., Neurosci. Abstr. 1985). Two groups of castrated B6D2F1 mice were distinguished on the basis of their behavioral response to castration. The males designated as the "continuers" (C) retained the ejaculatory reflex for 25 weeks after surgery; the "noncontinuers" (NC) did not retain this reflex. These two groups of castrates did not differ in their levels of plasma testosterone or nuclear estrogen receptors in the medial preoptic area (Clemens et al., submitted). These results ruled out the possibility that C had higher levels of non-gonadal androgens or estrogens than NC.

An alternative hypothesis is that the behavioral differences between C and NC are due to environmental factors. The present study examined the role of the amount of behavioral testing after castration and the testing environment in the retention of masculine sexual behaviors after castration by B6D2F1 (F1) mice. EXPERIMENT 1 Male F1 mice received six weekly sex behavior tests in 10 gallon aquaria converted to testing arenas, followed by either sham operation or castration. Weekly testing resumed one week later for half of the animals (the continuously tested (CT) group); the other animals (the delay tested (DT) group) received six additional weekly tests only after a 12 week delay.

Approximately 40% of all the castrates continued to display the ejaculatory reflex 18 weeks after surgery. No significant differences were observed between the CT and DT castrates for any of the behavioral latencies or for the percent of tests with ejaculation. Further, the percent of males designated as C varied between the two groups of castrates: 9 out of 48 (19%) CT versus 17 out of 45 (38%) DT. Sham operated CT and DT males continued to show high levels of copulatory behavior throughout the experiment. These results indicate that continuous behavioral testing after castration is not necessary for the retention of sexual behaviors. EXPERIMENT 2 The animals from Expt. 1 received an additional four behavior tests in either the test arenas or the animal's home cage. The percent of tests with behaviors was not significantly different between home and arena tests for the sham operated males or the continuers for any of the behaviors. In contrast, among NC (castrates that stopped showing sex behavior when tested in the arena), those tested in the home cage had significantly more tests with sex behavior (MWU, $p < .01$) than the arena-tested mice. These results suggest that among arena-tested castrates, NC are more sensitive than C to possible inhibitory influences from the test arenas.

Thus, the retention of copulatory behaviors by some, but not all, F1 castrates appears to be related, at least in part, to environmental factors.

20.3 **BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF DIHYDROTESTOSTERONE AND TESTOSTERONE IN CASTRATE RATS MATCHED FOR CIRCULATING LEVELS OF ANDROGENS.** K. J. Streator*, P. K. Westfahl* and J. A. Czaja. Miami University, Oxford, Ohio 45056

It has been suggested that, compared to its parent compound testosterone (T), the relative ineffectiviness of dihydrotestosterone (DHT) on male rat sexual behavior may relate to differences in bioavailability of these androgens. If this is so, then administration of larger doses of DHT should be sufficient in themselves to increase levels of male rat sexual behavior. A dose-response study was run to assess this possibility. Male Long-Evans rats were castrated and then injected for 34 days with either oil vehicle or dihydrotestosterone propionate (DHTP, either 316 µg, 1000 µg, or 3160 µg). For comparison, additional groups of rats were treated with testosterone propionate (TP, either 3.16 µg, 10 µg, 31.6 µg, 100 µg, or 316 µg). At the end of this treatment schedule, animals were tested for mating behavior and then killed for autopsy.

Compared to injections of oil alone, the DHTP treatments maintained significantly higher levels of sexual behavior (Log[1/latency to ejaculation], $F[3,45]=9.870$, $p<.001$), accessory tissue weights (Log[mg seminal vesicle weight], $F[3,45]=847.637$, $p<.001$), and circulating androgens (Log[pg/ml DHT], $F[3,45]=74.832$, $p<.001$), and did so in a dose-dependent manner. At the same dose level (316 µg), TP injections were more effective than DHTP injections in maintaining seminal vesicle weights ($t[23]=2.497$, $p<.05$) and sexual behavior ($t[23]=3.894$, $p<.001$). However, radioimmunoassay of serum for T and DHT revealed that the TP injections induced significantly higher circulating levels of androgens than did the DHTP injections (Log[pg/ml], $t[23]=2.921$, $p<.01$). Data were therefore reanalyzed for 19 pairs of animals matched for levels of circulating androgens. Matched this way, given levels of circulating DHT were found to be significantly more effective than the same levels of circulating T in maintaining peripheral organ weights ($t[18]=6.860$, $p<.01$), although DHT was still less effective than T in maintaining mating behavior (Log [1/latency to ejaculation], $t[18]=3.518$, $p<.01$).

The same dose of DHTP and TP did not result in comparable circulating levels of these hormones, a difference in bioavailability which was reflected in the effects of these treatments on peripheral target tissues. It is possible that similar factors may produce even greater differences in bioavailability of these hormones to the brain, differences which would at least partially account for the reduced effectiveness of peripheral DHTP treatments on sexual behavior of male rats.

20.4 TESTOSTERONE REINSTATES THE LATERALIZED HYPERACTIVITY SECONDARY TO CORTICAL LESIONS IN CASTRATED MALE RATS. S.E. Starkstein*, T.H. Moran, J.A. Bowersox*, L. Shnayder*, R.G. Robinson (SPON: P.R. McHugh). Dept. of Psychiatry, Johns Hopkins Univ., Balt., MD 21205.

In adult male rats, focal suction lesion of the right frontal cortex (FC) produce hyperactivity, while similar lesions of the left hemisphere fail to produce this behavioral change. This lateralized phenomenon is gender-related, as female rats do not develop hyperactivity after a right FC suction lesion, and age-related, as prepuberal male rats do not develop hyperactivity after similar lesions (Soc Neurosci Abst, 12:841, 1986). Sexual maturation also plays an important role as prepuberally-castrated adult male rats do not develop hyperactivity after receiving a right suction FC lesion. In the present experiment, we examined the role of steroid hormones in the production of this lateralized behavior. Male rats were castrated prior to puberty and placed in running wheel cages. Following when puberty would normally occur, rats were divided into the following groups: FC suction lesion with testosterone implant, FS suction lesion with sham implant, sham lesion with testosterone implant and sham lesion with sham implant. Lesion or sham procedures were performed on the right or left hemispheres. Testosterone implants were made to mimic normal circulating testosterone levels. Post-operative activity was monitored for 30 days. Overall, testosterone increased activity levels. Rats with right FC suction lesions and testosterone implants showed a significantly higher degree of hyperactivity ($F(1,23)= 5.23$, $p<.05$). For the left hemisphere group there were no differences in activity between rats with a FC suction lesion and testosterone implants and rats with sham lesions and testosterone implants. These results demonstrated that: 1) testosterone reinstates the hyperactivity phenomena in prepuberally castrated adult male rats after a FC suction lesion. 2) this is a lateralized phenomena, as only rats with right hemisphere lesions showed this response, and 3) this is also a gender-related phenomena, as female ovariectomized rats with a right FC suction lesion and testosterone implants do not develop hyperactivity (Soc Neurosci Abst 12: 841, 1986). This study suggests that lateralized hyperactivity is the consequence of a specific interaction between steroid hormones and brain structures.

20.5 LOCAL SITE OF ACTION FOR TESTOSTERONE'S ANABOLIC EFFECT ON RAT BULBOCAVERNOSUS. M.N. Rand and S.M. Breedlove, Psychology Department, U.C. Berkeley, Berkeley, CA 94720.

Testosterone has an anabolic effect on muscle, and lack of testosterone decreases the size of many muscles, including the bulbocavernosus (BC) and levator ani (LA) of rats. It is not known whether testosterone exerts this influence directly upon muscle cells or whether it acts elsewhere, e.g. via motoneurons. We attempted to determine the general site of testosterone action in adult male rat BC muscles and their motoneurons comprising the spinal nucleus of the bulbocavernosus (SNB).

Adult male rats 60-90 days of age were castrated and implanted with two capsules, one containing testosterone (Sigma) and the other anti-androgen (SCH16423, Schering Corp.). These capsules had a single small diffusible Silastic surface (approx. 2 mm^2) and were sutured one on each side of the BC so that the diffusible surface faced the muscle. After 30 days the animals were sacrificed and the BC, spinal cord, and seminal vesicles were dissected out. The muscles were trimmed, cut in half medially and then weighed without knowledge of their steroid treatment. Muscle half weights were compared using a repeated measures t-test.

Muscle halves receiving local testosterone treatment were found to be significantly heavier than those receiving local anti-androgen treatment ($n = 11$, $p < 0.01$).

The difference in BC half weights cannot be due to central effects of testosterone and anti-androgen since the laterality of steroid treatment could not have been retained at the distance of the SNB cell bodies which innervate the BC. Testosterone must therefore act at or near the muscle for its anabolic effect.

Rat BC muscles and SNB motoneurons are sexually dimorphic and mediate sexual function in males. Rat SNB dendritic length is also increased by administration of testosterone in adulthood (Kurz et al., Science 232:395, 1986). We are currently measuring SNB motoneuron dendrites filled with cholera-toxin HRP conjugate to see if there is a unilateral effect on dendritic length in the adult male rat SNB with the peripheral, lateralized administration of testosterone to the BC. In any case, the direct anabolic effect of androgen on the BC muscles themselves may play an important role in male copulatory behavior.

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20.6 SPINAL BLOCK REVEALS ROLES FOR BRAIN AND SPINAL CORD IN MEDIATION OF MALE RAT SEXUAL REFLEXES. B. D. Sachs and D. Bitran*. Dept. of Psychology, U-20, Univ. of Connecticut, Storrs, CT 06268.

The potential for penile erection varies as a function of antecedent copulation. As measured by erection latency in ex copula tests, the erectile potential of rats increases after 1-3 ejaculations and is depressed after 6 or more ejaculations. The contributions of spinal and brain sites in mediating these changes are unknown. Surgical transection of the thoracic spinal cord reduces erection latency and increases the number of penile reflexes during ex copula tests. We explored these phenomena further with a reversible "functional" spinal transection.

Tetracaine (TET; 1.4 mg/5 ul saline) or the vehicle alone (SAL) was infused into the intrathecal space of adult male rats via catheters terminating at the T9-T10 spinal segments. In penile reflex tests immediately after infusion, TET males had much shorter latencies than SAL males to the first penile body erection ($\bar{X}$ = 1.5 min vs 11.1 min, $p < .0001$) and to first glans erection ($\bar{X}$ = 3.0 min vs 11.0 min, $p < .0005$). However, the number of responses displayed by the two groups of males was similar. Thus, TET treatment mimicked the accelerative but not the "productive" effects of surgical transection, partially supporting previous conclusions of supraspinal inhibitory influences on penile erection.

The anatomical specificity of the effect of TET in the spinal cord was explored in another study by delivery of TET or SAL to the L5-L6 spinal segments known to regulate penile reflexes ex copula. The proportion of TET males responding in this condition (2/9) was lower than when TET was delivered to the thoracic cord (10/13; $p < .025$). Hence, the effect of TET in the spinal cord is site-specific.

In a third experiment, males copulated to sexual exhaustion (6-12 ejaculations) prior to infusion with TET or SAL directed at T9-T10. None of the 5 males in each group displayed penile reflexes in subsequent tests. From these data we infer (1) that the presumed release from supraspinal inhibition effected by intrathecal TET could not override the depressive effects of repeated ejaculations on penile reflex potential, and (2) that these depressive effects are largely endogenous to the lumbosacral cord.

These experiments establish for the first time that the spinal cord mediates some changes in reflex potential consequent to ejaculation, and support the view that the brain and the spinal cord interact in modulating the effects of copulation on penile reflex potential.

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20.7 EFFECTS OF INTRATHECAL TESTOSTERONE ADMINISTRATION ON PENILE REFLEXES IN RATS. R. P. Horodnic*, B. D. Sachs and R. E. Leipheimer* (SPON: R. L. Gilliland). Dept. of Biol. Sci., Youngstown State Univ., Youngstown, OH 44555.

Actions of the perineal muscles are known to regulate specific components of penile reflexes in rats. Studies have demonstrated that these reflexes are dependent on androgens. However, the specific effects of testosterone (T) on the spinal motor nuclei in regulating penile responses remain unclear. Hart (Physiol. Behav. 3:735-738, 1968) reported an increase in penile reflexes following spinal implantation of (T) into the cauda equina of the spinal cord in castrated, spinally transected rats, suggesting a direct action of (T) on spinal neurons mediating penile responses. In order to test this hypothesis in spinally intact animals, male rats known to respond in ex copula tests were implanted with cannulae in the subarachnoid space terminating in the L5-L6 spinal segments. Four weeks after castration penile responses declined and the rats were injected with 250 ug testosterone benzoate (TB) sc to increase their baseline penile reflex activity and tests were done 3 and 6 days later. Latency to first glans response decreased 3 days after sc injection ($P < .001$). The mean number of glans responses increased during this same interval ($P < .01$). By 6 days after sc injection penile responses began to decline: Latency increased ($P < .05$); and mean responses decreased ($P < .05$). Because penile responses had declined, rats received intrathecal (IT) injections of 10 ng TB on day 7, and were tested on day 10. Latency decreased on day 10 ($P < .02$), and mean responses increased to levels comparable to those seen after sc injection of 250 ug TB. By day 13 (6 days after IT injection), penile responses again declined: Latency increased ($P < .05$); and mean responses declined ($P < .05$). To test whether the effects of IT injection of TB were due to systemic absorption, the rats were injected sc with 10 ng TB. Penile reflex tests conducted 3 days after sc injection of 10 ng showed that mean responses were less than those seen 3 days after IT injection ($P < .05$).

Therefore, these results suggest that the facilitatory effects of IT injection of TB are due to actions on the spinal neurons which regulate penile reflexes and not to systemic effects.

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20.8 GABAERGIC MEDIATION OF SEXUAL REFLEXES IN LUMBOSACRAL SPINAL CORD IN MALE RATS. D. Bitran*, S. A. Miller*, D. B. McQuade*, R. E. Leipheimer*#, and B. D. Sachs (SPON: M. Wilson). Dept of Psychology, University of Connecticut, Storrs, CT 06268 and #Dept of Biology, Youngstown State University, Youngstown, OH 44555

Systemic administration of baclofen (BAC), a $GABA_B$ receptor agonist, was previously reported (Leipheimer & Sachs, Soc Neurosci Abst, 1986) to inhibit ex copula penile reflexes but not to affect copulatory behavior. In contrast, treatment with $GABA_A$ agonists did not affect penile responses either in or ex copula. The spinal cord is a likely site of action for the observed effects of BAC on penile reflexes, since GABAergic terminals and receptors have been located in the cord, including the L5-L6 spinal segments that contain the motoneurons innervating penile muscles.

In the first experiment, intrathecal injection of 0.8 ug BAC aimed at the L5-L6 spinal cord decreased the proportion of animals displaying penile reflexes (0/8 BAC vs 5/8 saline, $p < .02$). A smaller dose of BAC (0.2 ug) decreased the number of erections ($\bar{X}$ = 5.8 vs 22.0, $p < .03$) and number of reflex clusters ($\bar{X}$ = 3.0 vs 6.6, $p < .02$) relative to saline controls. An intermediate dose of 0.4 ug BAC had intermediate inhibitory effects. None of these doses prevented rats from copulating to ejaculation.

A potentiation of penile reflexes occurs after 1 to 4 ejaculations as reflected by a shorter latency to the first reflex. Apparently copulation acts to reduce a source of tonic inhibition. In a second study, we found that antecedent ejaculation eliminated the inhibitory effects of low doses of intrathecal BAC (0.2 and 0.4 ug) but not a high dose (0.8 ug). Thus, copulation prior to ex copula tests for penile reflexes shifted to the right the dose-response curve of BAC. The anatomical specificity of this inhibition was then tested by intrathecal injection with 0.8 ug BAC directed at the T9-T10 spinal segments. In contrast to the effects of lumbosacral injection, BAC onto the thoracic cord did not affect any measure of penile reflexes following an ejaculation.

The role of spinal $GABA_A$ receptors on penile reflexes was assessed by intrathecal injection of THIP (0.5, 1, 2 ug) onto the lumbosacral spinal cord. Hints of facilitative effects at 1 ug proved to be unreliable, and a weak inhibitory effect was observed at 2 ug. Specifically, a decrease in the number of erections ($\bar{X}$ = 5.8 vs 14.3, $p < .05$) and erections per cluster ($\bar{X}$ = 1.4 vs 2.8, $p < .05$) were observed.

In summary, the data indicate that stimulation of $GABA_B$ receptors in the lumbosacral spinal cord inhibits penile reflexes in a context-sensitive and dose-related fashion. We can not rule out a potential role for $GABA_A$ receptors, but it appears that spinal inhibition of penile reflexes by GABA is mediated to a greater extent by $GABA_B$ receptors.

20.9 EVIDENCE FOR OXYTOCIN INNERVATION OF PERINEAL MOTOR NEURONS IN RATS. E. P. Monaghan and S. M. Breedlove. Psychology Dept., U. C. Berkeley, Berkeley, CA 94720.

Swanson and McKellar (J. Comp. Neurol., 188: 87-106, 1979) found oxytocin (OT) innervation throughout the spinal cord of rats. OT injections i.c.v. elicit penile erections in male rats (Melis, et al., Soc. Neurosci. Abstr. 12:223, '86) and plasma concentrations of OT are elevated after ejaculation (Stoneham, et al., J. Endocrinol. 107:97 '85). In rats, the bulbocavernosus (BC) muscles are involved in producing erections and in copulatory behavior. We decided to take a detailed look at OT innervation at the level of the spinal cord containing motor neurons which innervate the BC muscles, namely the spinal nucleus of the bulbocavernosus (SNB).

Adult male rats received six hourly intra-gastric infusions of tap water prior to being sacrificed and perfused with 0.9% saline and 4% paraformaldehyde. The hydration procedure enhances the OT immunoreactivity in the spinal cord (Swanson & McKeller, 1979). The area of the spinal cord between L5 and L6 was cut into 50 µm sections. Labelled fibers were localized using rabbit anti-OT (Immuno Nuclear Group, Stillwater Minn.) as the primary antibody in ABC immunohistochemistry using diaminobenzidine as a chromogen.

Immunoreactive fibers were found between lumbar levels 5 and 6, including the region of the SNB. The paraventricular nucleus (PVN) has been implicated as a possible afferent to the SNB (Monaghan & Breedlove, Soc. Neurosci. Abstr. 12:1219, '86); these findings that OT-like fibers are present in the area of the SNB support the possibility that the PVN provides input to the perineal motoneurons. Such OT innervation of perineal motoneurons may play a significant role in male reproductive behavior.

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20.10 APOMORPHINE AND HALOPERIDOL, BUT NOT DOMPERIDONE, AFFECT PENILE REFLEXES IN RATS. E. A. Pehek*, R. C. Eaton*, T. J. Bazzett* and E. M. Hull (SPON: C. J. Smith). State University of NY at Buffalo, NY 14260.

Other investigators have shown that dopamine (DA) agonists have a biphasic effect on penile erection in rats, with low to moderate doses facilitating, and high doses inhibiting, erection. These studies did not distinguish between erection and seminal emission. One aim of the present studies was to examine the effects of apomorphine (APO), a DA agonist, and haloperidol (HAL), a DA antagonist, on penile reflexes and seminal emission in the supine rat. A second aim was to determine whether the effects on penile responses were mediated by actions on the central nervous system (CNS). This question was investigated by comparing the actions of HAL and domperidone (DOM), a DA antagonist that does not cross the blood-brain barrier. Penile reflexes were evoked via retraction of the penile sheath, and usually occurred within ten minutes following retraction.

A moderate dose of APO (100 ug/kg) decreased reflex latency whereas a higher dose (500 ug/kg) decreased the total number of reflexes. Both doses increased the incidence of seminal emission. HAL (300 ug/kg) reduced the number of reflexes and increased reflex latency. APO administration antagonized the effects of HAL on reflex latency and reflex number. HAL antagonized both the facilitative effect of 100 ug/kg APO on seminal emission and the inhibitory effect of 500 ug/kg APO on reflex number. DOM produced no effects on its own, and did not block any of the actions of APO.

These results demonstrate that APO has a biphasic effect on penile reflexes, with a moderate dose facilitating, and a higher dose impairing, penile reflex potential. APO facilitated seminal emission at both doses, suggesting that separate populations of dopamine receptors may regulate penile reflexes and seminal emission. These effects appear to be pharmacologically specific to the DA receptor, since HAL and APO have antagonistic actions. Furthermore, since DOM administration did not affect penile responses, the present results suggest that the effects of dopaminergic agents on penile responses are mediated by actions on the CNS.

20.11 MICROINJECTION OF CIS-FLUPENTHIXOL, A DOPAMINE ANTAGONIST, INTO THE MEDIAL PREOPTIC AREA IMPAIRS SEXUAL BEHAVIOR OF MALE RATS. E. M. Hull, E. A. Pehek*, R. K. Warner*, T. J. Bazzett*, D. Bitran*, L. C. Band*, and R. C. Eaton*. Dept. of Psychology, SUNY at Buffalo, Amherst, NY 14260.

We have previously shown that microinjection of the dopamine (DA) agonist apomorphine (APO) into the medial preoptic area (MPOA) of male rats facilitated several measures of sexual behavior. If DA receptors in the MPOA are important for the control of male sex behavior, then blocking DA receptors with cis-flupenthixol (FLU) should impair copulation. Furthermore, concurrently administered FLU should block the facilitative effects of APO.

In Experiment 1 male rats received 0, .2, 2, or 10 µg FLU, microinjected into the MPOA 5 min before each of four weekly tests with a receptive female. These doses of FLU did not significantly affect copulation.

In Experiment 2 doses of 0, 20, or 40 µg FLU were injected into the MPOA. Both 20 and 40 µg dramatically reduced ejaculation frequency. Fewer males mounted, intromitted, and ejaculated after FLU. Furthermore, among those animals that did copulate following FLU, ejaculation frequency was reduced, and copulation was slowed. Thus, higher doses of FLU dramatically impaired copulation.

Experiment 3 tested the ability of FLU to block the facilitative effects of APO. FLU (10 µg, ineffective alone in Experiment 1), APO (.5 µg, previously shown to facilitate copulation), the combination of the two, or vehicle were microinjected in counterbalanced order. APO injected alone increased ejaculation frequency, as before. FLU completely blocked this facilitation, but had no effect of its own on this measure. However, FLU injected alone slowed the rate of copulation. Neither drug produced major changes in activity or nonsexual motivation in home cage tests in the absence of a female.

These experiments support our hypothesis that DA receptors in the MPOA are important for the facilitation of sexual behavior of male rats.

20.12 **EFFECTS OF CASTRATION, STEROID REPLACEMENT AND COPULATORY EXPERIENCE ON SEXUAL AROUSAL AND MESOLIMBIC DOPAMINE** J. B. Mitchell and J. Stewart, Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Quebec, Canada, H3G 1M8.

A number of studies have implicated dopamine (DA) in the control of male sexual behavior (e.g. Malmnas, J. Endocrin 73:187, 1977; Baum and Starr, Pharm, Biochem & Behav, 13:57, 1980; McIntosh and Barfield, Behav Brain Res, 12:267, 1984). Alderson and Baum (Brain Res, 218:189, 1981) have reported that concentrations of DA and its metabolites are decreased 30 days post-castration. However, they have also reported a failure to replicate this finding (Baum, Melamed, and Globus, Brain Res Bull, 16:145, 1986). The present studies were undertaken to investigate the effect of castration on the concentrations of DA and the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the mesolimbic and nigrostriatal DA systems using the brain microdissection technique of Palkovits (Palkovits and Brownstein, 1983) and HPLC with electrochemical detection. Behavioral tests for sexual arousal and copulation were undertaken to determine if changes in DA content could be related to deficits in sexual behavior. Measures of sexual arousal included precopulatory behavior as well as the latency to initiate mounting (Grant and McIntosh, Behav, 21:246, 1963; Heimer and Larsson, Brain Res, 3:248, 1966; Stewart and Kaczender-Henrik, Hormones and Behav, 2:255, 1971).

DA and DOPAC concentrations in the nucleus accumbens, but not the caudate putamen, decreased by as much as 30 % after castration, falling to their lowest levels 4 weeks after gonadectomy. Castrated animals also displayed significantly less precopulatory behavior. Both the decrease in mesolimbic DA and the deficits in sexual behavior were prevented by post-castration androgen administration. Neither castration nor steroid administration affected the concentrations of norepinephrine, serotonin, or the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in any brain area assayed. In males allowed post-castration copulatory experiences the castration-induced deficit in sexual arousal was attenuated; once copulation had been initiated the two groups of castrates did not differ significantly.

These findings indicate that androgens are important for the maintenance of sexual arousal and the initiation of copulation, but that sexual experience can partially compensate for the absence of androgens. Furthermore, the biochemical data suggest that the mesolimbic DA system is affected by androgens and that it may be involved in mediating sexual arousal.

20.13 EFFECTS OF AN OPIATE ANTAGONIST MICROINJECTED INTO THE MPOA ON MALE COPULATORY BEHAVIOR. Linda C. Band* and Elaine M. Hull (SPON: R. McIsaac). Psychology Department, SUNY at Buffalo, NY 14260.

We have previously reported that a hydrophilic form of the opiate antagonist naloxone microinjected into the medial preoptic area (MPOA) of male rats facilitated copulatory rate in poor copulators. Morphine microinjected into the same site produced a delayed cessation of copulation.

In subsequent studies, naloxone microinjected into the MPOA of average copulators produced a dose-related impairment of copulatory rate: .1 ug produced no effects; .5 ug reduced intromission rate and intromission ratio; 1 ug decreased ejaculation frequency, slowed intromission rate, and tended to lengthen ejaculation latency and the postejaculatory interval; 10 ug reduced intromission rate and frequency. Maximal effects on copulatory rate were seen approximately 15 minutes after injection.

Apparently contradictory data both within and between laboratories, concerning the effects of opiate receptor blockade on copulation, may be resolved by noting baseline performance, hormonal state, drug dosage, and site of injection.

20.14 COPULATORY AVOIDANCE IN MALE RATS: ALTERATIONS IN SERUM LEVELS OF TESTOSTERONE AND LUTEINIZING HORMONE. G. J. Lawrence, S. W. Kiefer, & C. W. Metzler*, Dept. of Psychology, Kansas State University, Manhattan, KS 66506.

Male rats demonstrate conditioned avoidance to mating behavior when illness is paired with copulatory encounters with estrous females that have been sprayed with a novel almond odor; only a weak avoidance is noted when male rats are conditioned without the almond odor (Lawrence & Kiefer, Behav. Neuro., 1987, 101). Levels of serum testosterone and luteinizing hormone (LH) were measured in two experiments in the present study to determine what changes occur in the hormone levels (a) in untrained male rats following ejaculation and (b) in conditioned males that display copulatory avoidance behavior.

Following each encounter with an estrous female, experimental male rats were made ill with lithium chloride (LiCl) by intubation immediately following ejaculation or at the termination of a 20-min session. One-half of the experimental males (Odor-Sex/Ill group) were trained with estrous females that had been sprayed with a 2% almond solution; the remaining experimental males (Sex/Ill group) were trained with estrous females without the almond odor. Controls were included for the effects of almond odor, illness, test environment, and ether anesthesia. Blood samples were collected by cardiac puncture within 90 s after the induction of light ether anesthesia, immediately following the copulatory encounters. In Experiment 1, control males were yoked to experimental males, and blood was collected at baseline, after first ejaculation, and after the second consecutive trial in which experimental males failed to ejaculate. In Experiment 2, blood was collected at baseline, and after Trials 4 and 8.

Serum levels of both hormones showed significant surges following ejaculation. However, in the males that exhibited avoidance to copulatory behavior, serum levels of both hormones were significantly decreased from baseline values. Results indicated that male rats conditioned to avoid copulatory behavior exhibited significant decreases in serum levels of testosterone and LH, reductions which were correlated with the behavioral changes.

20.15 **TEMPORAL ASPECTS OF THE NUCLEAR UPTAKE OF ^{3}H-TESTOSTERONE AND ITS METABOLITES IN THE BRAIN OF THE MALE RAT.** **R.W. Bonsall*, H.D. Rees and R.P. Michael. Department of Psychiatry, Emory University School of Medicine, Georgia Mental Health Institute, 1256 Briarcliff Rd, Atlanta, Georgia 30306.**

In male mammals, testosterone (T) is secreted by the gonads in an episodic manner in response to stimulation by gonadotropins. To investigate the significance of episodic secretion for the nuclear uptake of T and its metabolites in target organs, including the brain, 24 adult male rats (250-330 g) were adrenalectomized and castrated. Three days later each rat was injected with ^{3}H-T (1 μCi/g body weight) as a bolus via the jugular vein. Four rats were killed at each of the following intervals after injection: 30, 60, 90, 120, 180 and 240 min. Brains were rapidly removed and dissected to obtain the following blocks: hypothalamus and preoptic area (HYP/POA), amygdala (AMG), parietal cortex (PCX), and cerebellar cortex (CBL). Samples from each animal were analyzed separately (168 samples). Brain blocks, as well as pituitary gland, seminal vesicles (SV) and prostate (PRO) were homogenized and centrifuged to obtain purified nuclear fractions. Ether extracts of the nuclei were chromatographed on reverse-phase, high performance columns and fractions representing estradiol (E), T and dihydrotestosterone (DHT) were analyzed for radioactivity. In the brain, ^{3}H-E was detected in HYP/POA and AMG, but not in PCX or CBL, and levels declined from a maximum at 60 min (HYP/POA: 5,780 ± 630 dpm/mg DNA, mean ± SEM; AMG: 8,997 ± 1,088 dpm/mg DNA) to a minimum at 240 min (HYP/POA: 691 ± 691 dpm/mg DNA; AMG: 3,618 ± 474 dpm/mg DNA). Unchanged ^{3}H-T was present in the brain cell nuclei of HYP/POA and AMG, but not of PCX or CBL and declined from a maximum at 30 min (HYP/POA: 2,962 ± 304 dpm/mg DNA; AMG: 2,722 ± 465 dpm/mg DNA) to undetectable levels at 90 min. ^{3}H-DHT was present only as a minor metabolite of ^{3}H-T in nuclei from HYP/POA and AMG of some males. In pituitary gland, unchanged ^{3}H-T was the major form of radioactivity; maximum levels occurred at 30 min (13,689 ± 2,385 dpm/mg DNA) and concentrations declined rapidly thereafter. In genital tract structures, ^{3}H-DHT was the major form of radioactivity and was highest at 90 min after the injection (SV: 918,560 ± 122,618 dpm/mg DNA; PRO: 944,698 ± 257,446 dpm/mg DNA) while ^{3}H-T concentrations, in contrast, were highest at 30 min (SV: 64,046 ± 3,244 dpm/mg DNA; PRO: 52,824 ± 3,862 dpm/mg DNA) and declined with a half-life of about 30 min. Thus, in brain, pituitary gland and genital tract, the nuclear uptake of ^{3}H-T was much shorter-lived than that of its metabolites. However, T is more effective in restoring the sexual activity of castrates than either its estrogenic or androgenic metabolites, and the present results suggest that the unique temporal aspects of the nuclear uptake of unchanged T may be significant for the behavioral actions of this potent hormone.

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20.16 MEASUREMENT OF CYTOSOLIC AND CELL NUCLEAR ANDROGEN RECEPTORS IN MICRODISSECTED RAT BRAIN NUCLEI. C.E. Roselli, R.J. Handa*, and J.A. Resko*. Department of Physiology, Oregon Health Sciences University, Portland, OR 97201 and Oregon Regional Primate Center, Beaverton, OR 97006

Androgens are important regulators of neuroendocrine function and reproductive behavior in male rats. The binding of androgens to specific high affinity receptor sites in brain tissue is postulated as an initial event in the mechanism of central androgenic action. In vivo autoradiography has established that androgen receptors (ARs) are distributed heterogeneously throughout the hypothalamus and amygdala. However, it is important to establish not only the location, but also the concentration of ARs in the central nervous system so that an accurate representation of the functional capacity of this system can be obtained under various physiological conditions. As an initial part of this effort, we have measured the concentration of both cytosolic (ARc) and nuclear androgen receptors (ARn) in microdissected brain samples from intact male Sprague-Dawley rats. Twelve individual nuclei were bilaterally removed from 300-μm frozen sections and pooled from 6 rats for each determination. The content of AR was measured with single point in vitro assays that used saturating concentrations of ^{3}H-dihydrotestosterone (S.A. 141 Ci/mmol) and either the 105,000 x g cytosol or a nuclear extract. Nuclei were purified by centrifugation through 2.15 M sucrose at 50,000 x g. Androgen receptor was then extracted from the purified nuclei with 0.8 M KCl. We found that ARc and ARn were distributed similarly throughout the hypothalamus and amygdala. The highest levels of both were measured in the ventromedial nucleus of the hypothalamus (ARc = 16.8 ± 4.2 [SEM] fmol/mg protein; ARn = 235.6 ± 23 fmol/mg DNA). High levels of receptor were also detected in the medial amygdala (ARc = 8.6 ± 1.5 fmol/mg protein; ARn = 153 ± 21 fmol/mg DNA); periventricular preoptic area (ARc = 7.2 fmol/mg protein; ARn = 145 ± 6.9 fmol/ mg DNA); and arcuate nucleus - median eminence (ARc = 6.6 ± 1.2 fmol/mg protein; ARn = 138 ± 18 fmol/mg DNA). Low but detectable ARc levels were measured in the lateral preoptic area (1.7 ± .2 fmol/mg protein) and cortical amygdala (2.0 ± 0.3 fmol/mg protein), however, we did not consistently measure ARn in either of these nuclei. Our results provide a quantitative profile of AR concentrations in specific hypothalamic and limbic nuclei of the rat brain. These data show that in intact males the distributions of ARc and ARn are similar.

(Support by HD 18196).

20.17 **NUCLEAR UPTAKE OF ^{3}H-TESTOSTERONE AND ITS METABOLITES IN THE BRAIN AND PITUITARY GLAND OF MALE CYNOMOLGUS MONKEYS: A COMBINED AUTORADIOGRAPHIC AND CHROMATOGRAPHIC STUDY.** **H.D. Rees, R.W. Bonsall* and R.P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Briarcliff Road, Atlanta, GA 30306.**

Administration of testosterone to castrated male macaques restores sexual behavior and retards the frequency of luteinizing hormone pulses. To investigate how these effects may be mediated, autoradiography was used to map target cells and high performance liquid chromatography (HPLC) was used to measure radioactive metabolites in the brain after the administration of ^{3}H-testosterone to male cynomolgus monkeys. Two adult males (5.2 and 5.3 kg) castrated 3 days earlier were injected i.v. with 3 mCi [1,2,6,7-^{3}H]testosterone (92 Ci/mmol) and killed after 30 min. The left halves of the brains were frozen, and 4-micron sections were thaw-mounted on emulsion-coated slides and processed for autoradiography. A cell was considered labeled if its nucleus had a silver grain density at least twice that of adjacent tissue. In the brain, all labeled cells appeared to be neurons. The highest percentages of labeled neurons were found in the ventromedial nucleus (n.) (mean 34%), anterior hypothalamic area (27%), accessory basal amygdaloid n. (26%), medial preoptic n. (23%), bed n. of stria terminalis (21%), and cortical amygdaloid n. (21%). Fewer neurons were labeled in the arcuate n. (18%), lateral septal n. (16%), medial amygdaloid n. (14%), and paraventricular n. (9%). In the pituitary gland, labeling was found in only about 0.5% of pars distalis cells. Samples of 14 brain regions were dissected from the right halves of these same brains and homogenized. Purified cell nuclei were prepared and ether extracts were analyzed by reverse-phase HPLC on Spherisorb 5-ODS columns using 50% aqueous acetonitrile. Unchanged ^{3}H-testosterone was present in nuclear fractions from all brain areas. ^{3}H-Estradiol was a major metabolite in nuclei from three areas: hypothalamus (38%), preoptic area (47%), and amygdala (55%). The proportion of the total extracted radioactivity represented by ^{3}H-dihydrotestosterone was highest in the mammillary body area (27%) and thalamus (9%) and did not exceed 6% in any other brain region. In the pituitary gland, 96% of the extracted nuclear radioactivity was in the form of ^{3}H-testosterone and 2.6% was in the form of ^{3}H-estradiol. These results in male cynomolgus monkeys are in agreement with those in male rhesus monkeys. The autoradiographic data indicate that target neurons for testosterone are located primarily in circumscribed regions of the hypothalamus, preoptic area and amygdala. The biochemical data suggest that in these brain areas the actions of testosterone depend on unchanged testosterone or on estradiol formed locally by aromatization, rather than on dihydrotestosterone. These results may help explain behavioral data showing that testosterone is much more effective than dihydrotestosterone in restoring the sexual behavior of castrated males (Michael, R.P. et al., 1986, Physiol. Behav., 36: 349-355).

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20.18 A QUANTITATIVE GOLGI ANALYSIS OF THE NEURONS IN THE MEDIAL NUCLEUS OF THE AMYGDALA IN MALE GOLDEN HAMSTERS: CASTRATION ALTERS DENDRITIC LENGTH. D.Gomez*, S.Newman. Dept.of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI. 48109.

The medial nucleus of the amygdala (M) is an important area for controlling mating behavior in the male Golden Hamster (Mesocricetus auratus). M receives chemosensory input from both olfactory and vomeronasal systems which are essential for this behavior and it projects directly to other mating control centers of the brain. Neurons in M also actively accumulate androgens. We are investigating the detailed structural organization of these neurons to determine how these inputs are integrated to guide mating behavior. We hypothesize that an important role of androgens in this system is to maintain the dendritic length and arborization of these neurons.

The normal morphology of the neurons in M have been studied in adult male hamsters using a Ramón-Moliner modification of the Golgi technique. The neurons throughout M are primarily of two types. Type I cells are few in number, have either a pyramidal or multipolar shaped perikarya (long axis = 20-24 µm), and have at least three primary dendrites which radiate and branch sparingly. Type II cells are more numerous and generally have smaller cell bodies (long axis = 9-21 µm) which are spherical, ovoid or fusiform in shape. One to three primary dendrites extend out from the long axis of the cell body and branch sparingly.

The caudodorsal region of M is an area in which neurons actively accumulate androgens and are primarily Type II cells. Measurements on the length of all the dendritic processes per neuron in this specific region of M were made using a Tektonics digitizing pad on tissue from 4 long-term castrated animals (4-6 months) and 5 intact animals. Thirty-six neurons from each group were analyzed and the sum of the lengths of all dendritic processes per neuron (DL) was calculated. The average DL was 495 µm for the neurons from the intact group and 355 µm for the castrated group. This indicates a 28% decrease in DL after castration in this brain area ($P < .05$). The level of dendritic branching per neuron was also determined and the percentage of neurons having each level of branches are listed in the table below.

ORDER	1st	2nd	3rd	4th	5th
GROUP					
Intact (neurons=36)	100%	89%	58%	22%	6%
Castrated (neurons=36)	100%	83%	31%	6%	0%

Fewer neurons from the castrated group have third order branches and beyond than those in the intact group.($P < .05$). This suggests that androgens may be involved in maintaining the dendritic length and the distal dendritic processes of the neurons in this area. (Supported by NIH,NS #20629 to SWN).

20.19 STEROID MODULATION OF MONOAMINE LEVELS AND TURNOVER IN THE VOCAL CONTROL SYSTEM AND HYPOTHALAMUS OF THE MALE ZEBRA FINCH. S.R.Barclay, A.Gardner and C.F.Harding. Biopsychology Program, Hunter College, C.U.N.Y., New York, N.Y. 10021.

In many vertebrate species, testosterone plays a critical role in the activation of male sexual behavior. Singing behavior in passerine birds serves multiple reproductive functions including attracting females (courtship behaviors) and helping to repel other males from the vicinity (territorial behaviors). Courtship behaviors, including singing are abolished by castration and can be restored by testosterone administration. Behavioral and biochemical studies indicate that most of male courtship behavior requires the combined action of estrogens and androgens. These steroids are concentrated in specific vocal control nuclei and induce changes in neural functioning which activate behavior. A growing body of evidence suggests that one mechanism by which steroids exert their effects is through modulation of neural transmission. Considerable evidence from mammalian species indicates that steroid effects in modulating male sexual behavior are mediated by steroid-induced changes in catecholaminergic (CA) neurotransmission.

The purpose of this study was to measure the effects of behaviorally effective steroids on CA levels and turnover in steroid-sensitive brain areas (vocal control nuclei: RA, HVc, ICo, MAN, Area X and hypothalamic nuclei: POA, PVM) in zebra finches. Three weeks after castration, the males used in this experiment were implanted with Silastic capsules containing either androstenedione (AE) or cholesterol and placed in an individual cage with a female for one week. To allow for calculation of CA turnover rates the birds were divided into two subgroups: one received an injection of α-methyl-para-tyrosine (αMPT), the other a control injection of saline. The appropriate dosage and time course of MPT treatment was empirically determined in a preliminary study. Brain nuclei were microdissected from 180µ frozen sections using the Palkovits punch technique and expelled into sodium acetate buffer containing an internal standard. The concentrations of CA and serotonin were determined by electrochemical detection in an HPLC system.

Steroid treatment significantly affected norepinephrine turnover rates in two hypothalamic nuclei and three vocal control nuclei (Area X, HVc and RA). Less systematic effects were seen in other neurotransmitters examined. Dopamine levels and turnover were affected in POA, MAN and RA. Serotonergic function was affected in HVc and POA. Research is currently in progress to determine if the effects of AE treatment are due to one class of metabolites (androgenic or estrogenic) or if these effects on monoaminergic turnover result from the interaction of androgens and estrogens. (Supported by grant HD-15191 and MH-00591 to CFH)

20.20 ANDROGEN ELEVATED BUT CORTICOSTERONE UNAFFECTED BY ACUTE AND CHRONIC SOCIAL STRESS IN LIZARDS

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Previous work has demonstrated that socially subordinate male lizards, Anolis carolinensis, have elevated circulating levels of corticosterone if intact but not if castrated (Greenberg et al. 1983). In a subsequent study, evidence was provided that chronically elevated corticosterone is a key factor in an anomolous adrenal catecholamine (epinephrine) response to sexually receptive females. The present work was undertaken to further characterize the relationship between aggressive and reproductive behavior, social dominance, plasma androgen, and corticosterone.

After agonistic encounters between territorially competitive male lizards, winners show a striking elevation in total plasma androgen while androgen levels in losers are unaffected. Subsequent cohabitation with the loser leads to a more modest but nevertheless significant elevation in androgen at one week, post-encounter. Androgen levels in losers, however, are unaffected. Winners recover pre-encounter levels of courtship behavior by one-week post-encounter but losers remain relatively depressed in reproductive activity. Plasma corticosterone levels are not affected either immediately or at one week post-encounter. This finding, inconsistent with the earlier study, may reflect the differences between the time scale (in the earlier study, animals cohabited for several weeks before blood sampling) or activity levels (in the earlier study subjects were not exposed to receptive females).

20.21 ACTIVITY, APPETITE, AND WEIGHT GAIN IN THE ADRENALECTOMIZED MALE ZUCKER RAT. Thomas W. Castonguay, Lori Ann Roth*, and Judith S. Stern*. Food Intake Laboratory and Nutrition Department, University of California - Davis Davis CA 95616

Many of the anomalies associated with genetic obesity are attenuated (if not reversed) subsequent to adrenalectomy (ADX). ADX promotes decreased levels of activity (revolutions in running wheels) in Sprague-Dawley rats. Conversely, restricted access to food can promote increases in activity. The present study was conducted to observe the effects of ADX on running wheel behavior of obese and lean Zucker rats. Obese and lean males were housed in Wahmann running wheels or standard, rack-mounted stainless steel cages. At surgery, rats were assigned to active or sedentary groups and monitored for 4 weeks. Body weight and food and water intake were determined daily. All ADX rats had ad libitum access to .9% NaCl. In addition to these groups, obese sham operated active animals were pair fed to obese ADX active rats. On Day 1, obese rats weighed more than did their lean littermates (343.9 ± 11.2 g vs 266.6 ± 6.3 g). No other differences were noted. By Day 28, the body weights of active obese and lean groups did not differ from sedentary controls. ADX failed to promote differences between active and sedentary groups of either genotype. On Day 1, obese sham operated sedentary and active rats ate more per day than any of the other rats. ADX rats of both genotypes ate less/day than did their sham operated controls. Obese ADX active and sedentary groups ate less than did the lean sham operated sedentary group. By Day 28, differences between surgical conditions, activity conditions, and genotypes were not observed. On Day 1, lean ADX active and sedentary groups drank more than did sham operated controls. By Day 28, all ADX groups were drinking more fluid/day than did their sham operated controls. Activity failed to influence daily fluid intake. Finally, on Day 1, lean sham operated rats ran more than either ADX or sham operated obese groups, but not more than the lean ADX group. By Day 28, there were no differences between lean ADX and sham operated groups. Obese sham operated rats that were pair fed to ADX obese rats ran more than the obese sham operated controls as well as more than the obese ADX ad libitum fed group. The decrease in intake following ADX in the obese fails to promote the increase in activity that follows caloric restriction. Unlike many of the other anomalies associated with genetic obesity, the hypoactivity of the obese rat is not easily or quickly reversed following ADX. (Supported in part by NIH grants DK18899 and AM35747)

20.22 CHRONIC CORTICOSTERONE ADMINISTRATION INCREASES ESCAPE BEHAVIOR IN RATS. M. Egawa*, E.A. Stone and B.M. McEwen, (SPON: D. J. Micco) Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016 and Lab. Neuroendocrinology, The Rockefeller Univ., New York, NY 10021.

In the course of previous biochemical studies of the effects of chronic corticosterone treatment in rats we made the unexpected behavioral observation that the treated animals showed a pronounced tendency to escape while be held. Behavioral changes that occur with chronic corticosterone treatment are of potential clinical interest because of their possible relevance to the affective and psychotic changes produced by this hormone in humans. Since this tendency to escape had not to our knowlegde been reported previously we undertook the following systematic study of it.

Rats were given corticosterone dissolved in ethanol in their drinking water (200 µg/ml, final ethanol conc. 1.2%). Controls received water with ethanol only. Daily tests were conducted for escape behavior (pulling away or backing-up while being held by the tail on a horizontal cage top for 30 sec.) and spontaneous motor activity (rearing and ambulation during 3 min in a behavioral test chamber). Dose response studies were carried out using 25-200 µg corticosterone administered for 3 days. For studies of habituation to stress rats were subjected to restraint (2 hrs/day for 4 days). Plasma corticosterone (trunk blood) levels were measured by RIA.

It was found that corticosterone markedly stimulated tail-pulling behavior during the escape test. The effect was evident beginning on day 2 and peaked on days 3 and 4 of treatment (percent of controls: day 3, 406 ± 78, $p < .01$ and day 4, 562 ± 90, $p < .005$). The hormone produced the effect in a dose dependent manner. Some spontaneous tail-pulling behavior was also evident in nontreated rats. Repeated stress for 4 days produced a reduction in the spontaneous behavior (56.3 ± 8%, $p < .05$) due to habituation. This reduction was markedly enhanced by prior adrenalectomy (83 ± 7%, $p < .005$). In contrast to its effect on escape behavior, corticosterone reduced motor activity. The latter effect however was observed only in repeated behavioral tests and not in the single test used in the dose response study.

From the above results, it is evident that elevated corticosterone level caused by exogenous hormone administration produces a dramatic rise in escape behavior in rats. Furthermore, the effect of adrenalectomy on spontaneous escape behavior in stressed rats suggests that increased release of endogenous corticosterone may serve to maintain levels of this behavior during adaptation to stress. Since exaggerated escape behavior can be a manifestation of psychotic disturbance this behavioral effect in rats may be related to the abnormal changes in CNS function produced in humans by the latter hormone. (Supported in part by grant MH22768).

20.23 SALIVARY CORTISOL ANALYSIS - FURTHER EVIDENCE AS TO ITS CLINICAL UTILITY IN THE DST, D. M. Martin, C. E. Turner*, D. H. Sherman*, M. A. Grimes*, Psychiatric Diagnostic Laboratories of America, Inc., South Plainfield, NJ 07080

The Dexamethasone Suppression Test (DST) is the most widely applied laboratory determination used as a state marker for endogenous depression and objective predictor of treatment response. The test is used both in outpatient and inpatient settings and requires at least two post dexamethasone blood draws for cortisol analysis. These draws require a skilled phlebotomist to collect the sample and a centrifuge to prepare the sample for refrigerated storage until analysis.

Salivary cortisol parallels the circadian rhythms observed in serum and responds to stimulation by ACTH or suppression to dexamethasone. Our group investigated its use as an alternative to the standard blood collection technique.

Seventy (70) inpatients with a preliminary diagnosis of major affective disorders were asked to participate in the study. If the DST was determined to be clinically indicated, the patients were asked to provide approximately 2-3 mls of saliva in a plastic centrifuge tube prior to venipuncture for cortisol analysis. Serum cortisol was determined by a standard radioimmunoassay technique and salivary concentrations were determined by a modification of the same technique.

Using the criteria of nonsuppression in serum of 5 ug/dl and 90 ng/dl in saliva, 61 of the 70 patients had similar findings in saliva and in serum. These data suggest that salivary cortisol determinations can be used in the DST without the loss of sensitivity or specificity. Compared to venipuncture, the collection of saliva is more convenient, painless and suitable for both inpatient and outpatient applications. This noninvasive approach requires no skilled personnel, equipment or preparation, and the sample is stable at room temperature for several days.

21.1 TOPOGRAPHIC ORGANIZATION IN THE SPINAL PROJECTION TO THE LATERAL RETICULAR NUCLEUS IN THE RAT.
N. Rajakumar*, A.W. Hrycyshyn* and B.A. Flumerfelt. (SPON: K. Elisevich). Department of Anatomy, The University of Western Ontario, London, Canada. N6A 5C1.

The organization of the afferent projections from the spinal cord to the lateral reticular nucleus (LRN) in the rat was studied using anterogradely transported lectin conjugated horseradish peroxidase (WGA-HRP). The tracer was placed at various levels of the spinal cord. A hemicordectomy was done just caudal to the placement of the WGA-HRP to ensure that no tracer was taken up by fibres of passage originating at lower levels. Sections through the LRN were examined microscopically under bright-field and polarized light after treatment with tetramethyl benzidine (Mesulam, 1978).

The results revealed that the cervical spinal cord projects mainly to the magnocellular portion of the caudal two-thirds of the contralateral LRN with only a sparse projection to the ipsilateral nucleus. The lumbar spinal cord projects mainly to the ventrolateral portion of the middle third of the LRN, with a sparse projection to the subtrigeminal portion and to the rostral third of the magnocellular LRN. Following placement of the tracer in the thoracic spinal cord, moderate labelling was seen in the intermediate zone between the cervical and lumbar projection areas within the LRN.

The above data indicate that the spinal projection to the LRN is topographically organized in the rat. The topographic pattern within the LRN shifts from caudo-medial to rosto-lateral with descending levels of the spinal cord. These results are consistent with the view that the neck and forelimb spinal projection terminates within the forelimb area of the LRN, while the hindlimb spinal input terminates within the hindlimb area of the LRN. Thus the pattern of connectivity within the LRN and that of its connectivity to the cerebellum is consistent with the hypothesis that the LRN plays an important role in coordinating motor activity.

Supported by the Medical Research Council of Canada

21.2 IMMUNOHISTOCHEMICAL LABELING OF MONOAMINERGIC TERMINALS IN PHRENIC NUCLEUS OF THE RAT. W.-Z. Zhan*, H.H. Ellenberger and J.L. Feldman. Systems Neurobiology Lab., Kinesiology Department, UCLA, Los Angeles, CA 90024-1568.

The termination patterns of monoaminergic axons in the phrenic nucleus were studied in Wistar rats. The phrenic nerve was dipped in biotinylated horseradish peroxidase (Bi-HRP) to retrogradely label phrenic motoneurons. After a 48 hr. survival time the animals were sacrificed and perfused; the cervical spinal cord was then sliced into 20-30 μm sections. The transported Bi-HRP was visualized using an avidin-biotin reaction (ABC kit, Vector Labs) with a heavy metal enhanced, black diaminobenzidine (DAB) reaction product. Then the tissue was incubated with antisera against dopamine β-hydroxylase (DBH), phenylethanolamine-N-methyltransferase (PNMT) and serotonin to identify the terminations of monoaminergic neurons. From the C3 to C5 spinal cord, DBH-positive terminals with varicosities formed a dense network, with presumptive synaptic contacts on dendrites and somas of phrenic motoneurons. There were fewer PNMT-positive terminal arborizations in the cervical spinal cord compared to thoracic spinal cord; PNMT terminals were not seen in the vicinity of phrenic motoneurons. Numerous serotonin containing axons and terminals contacting phrenic motoneurons were also labeled. These monoaminergic inputs may not be respiratory specific, however, since we also observed similar termination patterns in other adjacent (non-respiratory muscle) motoneuron pools. The supraspinal origins of the serotonin and norepinephrine projections to the phrenic nucleus are not from the dorsal or ventral respiratory groups (Ellenberger et al., this volume), but were not otherwise determined. The results suggest that phrenic motoneuronal activity can be influenced by monosynaptic supraspinal inputs from norepinephrine and serotonin containing neurons. This input appears to be shared by other spinal motoneurons rather than a respiratory specific influence limited to phrenic motoneurons. Supported by NIH Grants NS 24742, HL 37941 and HL 07363.

21.3 ASCENDING AND DESCENDING PROJECTIONS TO MEDULLARY RETICULAR SITES WHICH ACTIVATE EPAXIAL MUSCLES IN THE RAT. A. Robbins, S. Schwartz-Giblin and D.W. Pfaff. Lab of Neurobiology and Behavior, Rockefeller University, New York, N.Y. 10021.

The epaxial muscles, lateral and medial longissimus (LL and ML), are active during lordosis, the sexual posture of the female rat characterized by dorsiflexion of the back and elevation of the tailbase. Stimulation of the medullary reticular formation (MRF) can activate these muscles (Femano et al., Am. J. Physiol., R389:246, 1984). Since electrical stimulation of other lordosis-relevant brain sites and peripheral nerves can facilitate MRF-induced muscle activity, this experiment aimed to precisely determine which brain and spinal cord neurons project to effective sites in the MRF using electrophysiological and neuroanatomical techniques.

In anesthetized female rats, the MRF was explored with electrical stimulation using currents less than 50uA, and EMG activity was recorded from ipsilateral LL and ML muscles. When an effective site was found, the retrograde fluorescent tracer, fluoro-gold, was applied to the site in a cannula prepared as follows: a 15% polyacrylamide gel was drawn into a 32g cannula and the cannula tip was soaked in a 20% fluoro-gold solution. The gel allows for slower release and a more confined deposition site (Fahrbach et al., J. Comp. Neurol., 225:605, 1984). The cannula was inserted into a guide cannula attached to the stimulating electrode, to a depth equal to the tip of the stimulating electrode. The cannula containing fluoro-gold remained in the brain 10-13 hrs and the animals survived for 4 days.

Effective stimulation sites and corresponding fluoro-gold deposition sites were located in the dorsal n. gigantocellularis. Labeled cells with descending projections were seen in contralateral n. gigantocellularis, parvocellular reticular n., medial and lateral vestibular n., midbrain central gray, n. lateral lemniscus, tegmentum, lateral hypothalamus, amygdala, paraventricular n., anterior hypothalamic area, and the bed n. stria terminalis. Of interest was the paucity of labeled cells in the ventromedial n., a crucial hypothalamic area for lordosis behavior. Labeled cells with ascending projections were observed in the cervical and lumbar spinal cord, primarily in lamina V, VIII and X. The number of cells observed in the lumbar cord was much smaller than in the cervical cord.

Experiments are being conducted to determine if the afferent projections to ineffective MRF sites differ from the pattern observed to effective sites. Preliminary evidence indicates that, in paired comparisons, there is a tendency toward a larger number of projections from contralateral MRF, midbrain central gray and cervical spinal cord to effective MRF sites than to ineffective MRF sites.

21.4 ORIGINS OF BRAINSTEM AFFERENTS AND BULBOSPINAI PATHWAYS IN THE PARROT. D.M.S. Webster and J.D. Steeves. Dept. of Zoology, University of British Columbia (UBC), Vancouver, B.C., V6T 2A9.

The purpose of this study was to correlate, in dexterous prehensile and non-prehensile birds, the origins of descending brainstem-spinal pathways, as well as the afferent projections from rostral brain to brainstem.

True blue (TB) dye (5%), or wheat germ agglutinin-horseradish peroxidase (WGA-HRP) (2%), was injected (5 uL) bilaterally into the high lumbar spinal cord in 4 Sulphur-crested cockatoos, and 1 Eastern rosella parrot. Examination showed that the distribution of retrogradely labelled neurons within the medullary and pontine reticlar formation, raphe nuclei, red nucleus, descending and lateral vestibular nuclei, lateral and periventricular hypothalamic nuclei, was essentially the same as previously described for the duck and goose (Webster and Steeves, 1986. Soc. Neurosci. Abs. **12**:882).

In parrot, duck and goose, TB was also used to determine the source of afferent projections to the medullary reticular formation and other brainstem areas known to play a role in the initiation of locomotion (Steeves et al., 1987. Brain Res. **401**:205). Following unilateral injection of TB (0.1 uL) into the nucleus reticularis gigantocellularis (Rgc), labelled cells were found in the vestibular nuclei, cerebellar nuclei, inferior olive, contralateral reticular formation, nucleus intercollicularis, interstitial and hypothalamic nuclei. A few labelled cells were also found in the wulst and the paleostriatum primitivum, but only following larger injections of TB (1.0 uL) into the Rgc.

Similar results were found in all avian species (Sulphur-crested cockatoo, Eastern rosella, Pekin duck, and Canada goose). Therefore, we conclude that there were little or no differences in 1) the afferent projections onto brainstem reticular formation neurons, or 2) descending bulbospinal projections in either prehensile and non-prehensile birds that might underlie the observed pedal dexterity of parrots .

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21.5 PROJECTIONS OF THE LOCUS COERULEUS TO BRAINSTEM NUCLEI: AN ANTEROGRADE TRANSPORT STUDY IN THE RAT. R. Grzanna and J.-M. Fritschy* (SPON: A. Georgopoulos). Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

It is a widely held notion that locus coeruleus (LC) efferents have a widespread and diffuse distribution throughout the central nervous system. We have recently reported that in the spinal cord LC axons are distributed preferentially in the dorsal horn with only a sparse input to the ventral horn. In the present set of experiments, we studied the distribution of LC axons in the brainstem. The anterograde tracer phaseolus vulgaris leukoagglutinin (PHA-L) was iontophoresed unilaterally into the LC. After a two week survival period rats were perfused and brainstem sections were processed for PHA-L immunocytochemistry using the avidin-biotin peroxidase complex method. To determine definitively whether PHA-L labeled fibers were noradrenergic (NA) axons, we processed a separate series of sections for double immunohistochemical staining using antibodies to PHA-L and antibodies to dopamine-beta-hydroxylase.

Numerous PHA-L labeled LC axons were observed in the spinal cord, the cerebellum and the inferior colliculus. An unexpected finding of our experiments was the low density of PHA-L labeled axons in the hindbrain compared to that seen in either the spinal cord or the cerebellum. Extensive regions of the brainstem were almost devoid of PHA-L labeled LC axons including regions known for their dense NA innervation such as the nucleus of the solitary tract, dorsal motor nucleus of the vagus and the raphe nuclei. The highest density of PHA-labeled LC axons was observed in the cochlear nuclei and throughout the rostro-caudal extent of the sensory trigeminal nucleus bilaterally. Very few PHA-L labeled LC axons were observed in the motor nucleus of V and in somatic motor nuclei of cranial nerves. In contrast to the very low density of PHA-L labeled fibers in these nuclei, the motor nucleus of the facial nerve contained a moderate number of LC axons. Analysis of PHA-L LC axons revealed a broad spectrum of morphologically different types of fibers varying in their diameter and the shape and spacing of their varicosities. The entire range of fiber types was represented in the trigeminal complex.

The results of the present study reveal a distinct regional distribution of LC axons in the brainstem and provide further evidence against the hypothesis that the LC exerts a global influence on brainstem functions. The absence of LC axons in extensive regions including motor nuclei of cranial nerves suggests a highly restricted influence of the LC in the brainstem. Based upon the observation of numerous LC axons in the trigeminal nuclear complex, we propose that the LC influences primarily the processing of sensory inputs. (Support: MH 41977 and NS 16654).

21.6 CORTICAL AND CEREBELLAR PROJECTIONS IN THE NUCLEUS OF DARKSCHEWITSCH (ND) IN THE CAT. J.G. Rutherford, D.G. Gwyn and A. Zuk-Harper*. Dept. of Anatomy, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

The ND is a small midbrain nucleus traditionally classified as an accessory oculomotor nucleus. However, this cell group is known to be the source of a substantial projection to the inferior olive (IO) in the cat (J. Comp. Neurol., 212:278, 1982). In this study, anterograde terminal and retrograde neuronal labeling in the ND following horseradish peroxidase (HRP) injections into the cerebral cortex, and anterograde labeling in the ND following HRP injections into the deep cerebellar nuclei (dCN), is described and discussed.

Injections of from 0.36 to 2.00 µl of wheat germ agglutinin-HRP (WGA-HRP) were placed unilaterally in the cerebral cortex anterior and posterior to the cruciate sulcus in each of 9 male and female cats. In a further 4 animals, from 0.12 to 0.38 µl of WGA-HRP was injected unilaterally into the dCN, using stereotaxic coordinates for guidance. Following survival times ranging from 1 to 4 days, cats were sacrificed, and 40 µm frozen sections were cut in the transverse plane through the injection sites, and in the transverse or horizontal planes through the midbrain and caudal diencephalon. Sections were reacted for HRP activity using tetramethyl benzidine as the chromogen.

Cortical injections of WGA-HRP produced anterograde labeling of varying density throughout the ND, from its caudal pole rostrally to the level at which the fasciculus retroflexus passes laterally adjacent to the periaqueductal gray (PAG). The labeling sharply defined the boundaries of the ND, and was predominantly, but not exclusively ipsilateral to the injection sites. Retrogradely labeled somata were found in the PAG adjacent to the ND, and in the ND itself, where the distribution of anterograde labeling overlapped the position of the cortically projecting neurons. Retrogradely labeled cells increased in number at more rostral levels of the ND. Following cerebellar injections, anterograde labeling was noted in the ND contralateral to the injection sites, and again extended throughout the rostro-caudal length of the ND. Thus the cerebellar input to this region overlapped the anterograde and retrograde labeling resulting from cortical injections of WGA-HRP.

Convergence, and potentially integration, of cortical and cerebellar input in the ND, a nucleus projecting substantially to the IO, suggests that this cell group is a functional adjunct to the red nucleus, rather than an accessory oculomotor nucleus. Additionally, the ND may be involved in relaying combined cerebellar and cortically derived information to the cerebral cortex. Supported by the MRC of Canada.

21.7 DESCENDING PROJECTIONS FROM THE PERIAQUEDUCTAL GREY (PAG) AND SURROUNDING TEGMENTUM, Petra Smulders (1) and Gert Holstege (2) Dept. Anat. Erasmus University Rotterdam The Netherlands (1) and Dept. Anat. Univ. California San Fransisco CA 94143 (2) (SPON:Daunton)

The PAG is one of the largest structures in the central nervous system, but its function is not well understood. It is known that the PAG maintains reciprocal connections with many limbic areas in the di- and telencephalon and that it sends fibers to the nucleus raphe magnus and adjoining tegmental field. However, the descending pathways have never been described in great detail. Therefore, in 32 cases injections of 0,5 µL containing 50 µCi ^{3}H-leucine were made in various parts of the PAG and surrounding areas. In almost all cases a basic descending projection system was observed, in which a large stream of labeled fibers passed from the injection site ventrolaterally into the lateral mesencephalic tegmentum. In this area the fibers turned caudally to descend through the lateral tegmentum of caudal mesencephalon and pons. At the level of the caudal pons the fiber stream shifted ventromedially and descended further through the ventral part of the medial tegmentum of caudal pons and medulla. Many labeled fibers terminated in the areas through which they descended and in the area of the nucleus raphe magnus. However, this study also revealed some other projections, such as:

1: The basic pattern of descending fibers was also found in cases with injections in areas lateral and ventral to the PAG.
2: When the lateral and ventrolateral PAG was injected, a very specific distribution was observed to the nucleus retro-ambiguus.
3: In the cases with injections in the ventrolateral PAG labeled fibers were also distributed to the nucleus raphe pallidus.
4: In many of the cases some labeled fibers were observed in the solitary and dorsal vagal nuclei, but none to the motor trigeminal, facial and hypoglossal nuclei or to the dorsal group of the nucleus ambiguus.
5: In the cases with injections in the lateral PAG and laterally adjoining tegmentum, labeled fibers were found descending in all funiculi of the spinal cord untill mid-thoracic levels. From the ventral funiculi labeled fibers were distributed to laminae VIII and adjoining VII and to lamina X. From the dorsolateral funiculus a few labeled fibers were distributed to the upper thoracic intermediolateral (sympathetic) cell column. HRP injections in the spinal cord corroborated these autoradiographic findings.
6: In many cases some labeled fibers were distributed to the lateral tegmental field of caudal pons and medulla. These projections were best seen at caudal medullary levels. If the injection sites extended into the dorsal raphe nuclei, the projections to the caudal pontine and medullary lateral tegmental field were much stronger and labeled fibers were also found in the lateral facial subnuclei.
Supported by a grant of NASA/Ames Research Center to G.H.

21.8 SIZE DIFFERENCES IN CELLS OF ORIGIN OF FTG DESCENDING PATHWAYS IN THE CAT. A. Mitani, K. Ito, Y. Mitani and R.W. McCarley. Lab. Neuroscience, Dept. Psychiatry, Harvard Medical School, Brockton/West Roxbury VAMC, Brockton, MA 02401

Numerous physiological studies in the cat implicate neurons of the pontobulbar gigantocellular tegmental field (FTG) in control of events related to the sleep-waking cycle and motor activity. However it is not known if there is a morphological differentiation of FTG neurons corresponding to projections with different functional roles. This study sought to determine the relationship between morphological features of FTG neurons and their descending fiber trajectories. We first injected WGA-HRP and anterogradely labeled descending fibers (N=14 animals). We then injected HRP into the ventral (N=3) or lateral funiculus (N=7) of cervical spinal cord or into bulbar reticular formation (N=4) where the anterogradely labeled fibers had been found and determined the distribution and sizes of the retrogradely HRP-labeled neuronal somata.

Predominantly large to giant-sized neurons gave rise to reticulospinal projections that descended in the medial longitudinal fasiculus (MLF) and in ventral funiculus (VF): (1) Neurons in the pontine FTG (average soma diameter = 43.4 um) and rostral bulbar FTG (41.3 um) gave rise to reticulospinal fibers descending in the ipsilateral MLF and VF and distributed in laminae V-X with an ipsilateral predominance. These were primarily large diameter fibers; (2) Neurons (46.9 um) in the bulbar FTG gave rise to reticulospinal fibers descending in the contralateral MLF and VF. These were mainly large diameter fibers.

The projection patterns of smaller neurons were different in that: (3) Predominantly medium-sized neurons (29.5 um) in the pontine FTG gave rise to reticuloreticular fibers that descended directly to and distributed in the bilateral bulbar reticular formation. These were small diameter fibers; (4) Predominantly medium-sized neurons (28.9 um) in the bulbar FTG gave rise to reticulospinal fibers descending in the ipsilateral reticular formation and lateral funiculus. These were small diameter fibers. The differences in size distribution of cells of origin between these latter two groups and the MLF-VF fibers were highly statistically significant ($p's < .001$, Kolmogoroff-Smirnoff test). These findings confirm and enrich the data of our intracellular HPR studies of pontine FTG neurons and suggest neuronal size is an important organizing feature for reticular formation.

21.9 OPERANT CONDITIONING OF PRIMATE SPINAL REFLEXES: BEHAVIOR OF THE UNCONDITIONED LEG. J.G. Calaitges* and J.R. Wolpaw (SPON: E.W. Wolpaw). Wadsworth Labs, NYS Dept Hlth, Albany, NY 12201; and Depts Neurol & Anat, Albany Med Coll, Albany, NY 12208.

Operant conditioning of the wholly spinal and largely monosynaptic H-reflex (J Neurophysiol 57:443-459, 1987) may prove to be a powerful experimental model for the study of primate memory substrates. Evidence suggests that conditioning causes a persistent alteration in the lumbosacral spinal cord, a potentially accessible memory substrate. In the present study, we conditioned the H-reflex in one leg and simultaneously monitored the H-reflex in the other, unconditioned, leg.

Five monkeys (Macaca nemestrina) with triceps surae EMG electrodes and a posterior tibial nerve cuff in each leg learned to maintain stable background EMG bilaterally. At random times, bilateral nerve cuff stimuli just above M response threshold elicited the H-reflexes. In control mode, reward always followed. In HR↑ or HR↓ mode, it followed only if the H-reflex in one leg (the conditioned leg) was above (HR↑, 3 animals) or below (HR↓, 2 animals) criterion. Monkeys worked 3000-6000 trials/day over 4-6 months. Background EMG and M response were stable throughout. Under the control mode, H reflexes in both legs were stable.

Under the HR↑ or HR↓ mode, the conditioned leg's H-reflex changed in the expected direction over weeks and months in all 5 monkeys. In contrast, under the HR↑ mode, the unconditioned leg's H-reflex fell slightly in one monkey, rose slightly in one, and rose as much as the conditioned leg's in the third. Under the HR↓ mode, the unconditioned leg's H-reflex rose slightly in both animals.

The results indicate that operantly conditioned change in the H-reflex is relatively specific to the conditioned leg. The unconditioned leg usually shows little or no change in the conditioned direction. This finding suggests that conditioned H-reflex change is a result of asymmetrical activity in descending pathways. Furthermore, and most important, it supports current efforts to delineate the persistent spinal alterations by comparing the conditioned and unconditioned sides of the cord (Wolpaw & Lee, this vol).

(Supported by NIH 22189 and by United Cerebral Palsy)

21.10 **NEURAL RESPONSES TO ACOUSTIC STARTLE-ELICITING STIMULI IN THE PONTINE RETICULAR FORMATION OF THE CAT: RELATIONSHIP WITH NECK EMG STARTLE RESPONSE.** S. S. Suzuki, M.-F. Wu* and J. M. Siegel. Neurobiology Research, V. A. Medical Center, Sepulveda, CA 91343, and Department of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

The pontine reticular formation (PRF) has been implicated in the instigation of the acoustic startle reflex (ASR), by lesion, electrical stimulation, and anatomical tracing studies. However, except for a previous report from this lab, unit recording from this area in relation to ASR has not been reported. In the present study, single unit responses in the PRF to startle-eliciting stimuli (S), along with neck EMG responses, were studied in unrestrained, behaving cats under a variety of stimulus conditions.

A total of 105 cells, which showed a response to eliciting stimuli between 90 and 120 dB, were examined. Most of these cells had thresholds over 90 dB, and were found in nucleus reticularis pontis caudalis, and rostral nucleus reticularis gigantocellularis, between P3 and P7. The majority (71%) of the auditory-responsive cells had latencies less than 20 msec: 39% 3-7 msec, 32% 8-19 msec. The spinal projections of 41 responsive and 38 non-responsive cells were antidromically determined by electrical stimulation of the cervical (C2) and lumbar (L1) spinal cord. Reticulospinal (RS) cells were significantly more likely than non-RS cells to respond to auditory stimuli (65% vs 35%). 59% (24 of 41) of the responsive cells had spinal projections, with .5-1.5 msec latency to C2 stimulation. Adding an average of 7 msec response latency to eliciting stimulus, the total time required for these cells to execute a response is within the range of neck EMG response (6-10 msec latency, 5-15 msec duration), consistent with a direct role of these neurons in startle elicitation. Behaviorally, most of these cells were related to head and facial movements, and received convergent inputs from deep superior colliculus, midbrain tegmentum, motor cortex, vestibular nucleus, neck muscles, and spinal cord.

The magnitude of the mammalian ASR can be suppressed by a prestimulus (P), a phenomena which has been termed "reflex inhibition". We found that discharges to S of the eleven PRF cells tested were also inhibited by the prestimulus (30%-90% inhibition, with 80 dB P, 110-120 dB S, and 80-100 msec ISI). The amount of inhibition on the units examined was comparable to that of EMG responses measured simultaneously. Manipulation of the intensity of the eliciting stimulus showed that the responses of PRF reticulospinal neurons paralleled the EMG startle response.

These results support the hypothesis that PRF is directly involved in startle elicitation, and that the modulatory process exerted by the prestimulus is occurring at or before PRF RS neurons.

(Supported by the V. A. and USPHS grant NS14610.)

21.11 **MODULATION BY PRESTIMULUS AND BACKGROUND NOISE OF MULTIPLE-UNIT ACTIVITIES IN THE INFERIOR COLLICULUS OF THE RAT: RELATIONSHIP WITH THE ACOUSTIC PINNA REFLEX.** M.-F. Wu* (SPON: D. McGinty). Department of Psychology, University of Rochester, Rochester, NY 14627.

The inferior colliculus (IC) has been implicated in prestimulus inhibition of the acoustic startle reflex in the rat. The present study correlated multiple-unit activities of the IC neurons with the simultaneously measured acoustic pinna EMG response, which has been suggested to be a component of the startle response, in spinally transected rats.

The pinna reflex followed the whole-body startle in all aspects of the effects examined: prestimulus frequency, prestimulus intensity, inter-stimulus interval, and the frequency of noise and noise-offset. High trial by trial correlations between the amplitude of the EMG response and that of the whole-body startle, measured with an accelerometer, were found, suggesting that the acoustic pinna reflex is part of the startle response and that similar modulatory mechanisms must be involved for both measures.

Significant correlations, either negative or positive, between neural discharges to the prestimulus and EMG startle amplitude were found in about 50% of the unit-clusters examined, supporting the notion that the IC is involved in prestimulus modulation. These units were found in all divisions of the IC. The sign of correlation, however, depended on the best frequency (BF) of the unit: mid- (5-15 KHz) and high-frequency (>15 KHz) units showed negative while low-frequency (<5 KHz) units showed positive correlations.

About 50% of the IC unit-clusters showed discharge suppression by prestimuli. Most of these inhibited-units were found in the dorsal cortex and the dorsal and rostral portion of the external cortex, and tended to have an on or pauser type response pattern. However, only those with a BF in the midrange showed a pattern of prestimulus effect parallel to that of the pinna EMG. Most units were affected by the background noise, either inhibition or facilitation, depending on the frequency of the noise and the BF of the unit, with no clear response type or anatomical specificity. Only the effects of the midrange units paralleled that of the EMG. Together with correlational analyses between spike and EMG startle responses, the results suggest that the startle mechanism must be mediated by neurons in the lower auditory system having midrange BF's and a phasic response pattern, and that there is a modulatory process occurring in the afferent limb of the reflex circuit which may be responsible for part of the effects produced by prestimuli and background noise. A partial functional and anatomical segregation within the IC regarding those neurons mediating reflex modification and those related to reflex elicitation was suggested.

21.12 2-DEOXYGLUCOSE STUDY OF THE PLANTAR CUSHION REFLEX IN THE CAT: THREE-DIMENSIONAL RECONSTRUCTIONS. D.P. Crockett*, W.K. Smith, E. Proshansky*, J.S. Kauer, W.B. Stewart, D.J. Woodward, D.S. Schlusselberg and M.D. Egger. Dept. of Anatomy, UMDNJ-R.W. Johnson Med. Sch., Piscataway, NJ 08854 and Dept. of Anatomy & Cell Biol., Univ. of Texas Hth. Sci. Ctr., Dallas, TX 75235.

As part of our interest in functional neuroanatomy, we report on three-dimensional reconstructions of spinal cord activity associated with a simple, highly stereotyped behavior as revealed by ^{14}C-2-Deoxyglucose (2-DG) serial autoradiographs. Our model behavior is the plantar cushion (PC) reflex, which is a downward flexion of the toes in response to moderate stimulation of the PC in cats.

Four chronically (4-11 days) spinalized cats were injected (i.v.) with 2-DG (100 μCi/Kg). The PC on one side was stimulated transdermally through needle electrodes (0.5 ms/pulse; 3 Hz; 45 min). At the termination of stimulation, the animals were deeply anesthetized and the spinal cords were quickly removed. Transverse, 32 μm-thick sections were cut on a cryostat. In general, six neighboring sections were saved for autoradiography and the next six discarded. Approximately 40 autoradiographs from each cat were chosen (≈16 mm A-P) for computer analysis and 3-D reconstruction using CARP (Computer Assisted Reconstruction Package) on a Data General MV-8000 II computer equipped with an Ikonas (Adage 3000) color raster graphics system. The images were digitized directly from the X-ray films. Minor artifacts were eliminated by a "bubble removal" routine. The images were "stretched" to correct for the geometrical anisometry of the digitization process. The data were normalized using the dorsal column white matter as a standard for background or minimal 2-DG activity.

Several strategies of analysis were employed: Three-dimensional volume images were color-coded to represent different levels of functional activity. On the reconstructed volumes, virtual sections were made in the horizontal, sagittal and transverse planes in order to view regions of 2-DG activity. Activity in various regions within the grey and white matter was quantified to reveal differences between ipsi- and contralateral regions within a section, as well as possible variations between sections.

The three-dimensional reconstructions confirmed the existence of stimulation-elicited heightened 2-DG activity dorsomedially in the lumbar dorsal horn of unanesthetized spinal cords.

21.13 THREE DIMENSIONAL AND MORPHOMETRIC STUDIES USING A COMPUTERIZED SPINAL INJURY DEVICE. B.T. STOKES, M.S. BEATTIE, J.C. BRESNAHAN, S.K. SOMERSON, P.A. WALTERS and M.S. ROSS, Depts. Physiol., Surgery and Anatomy, Ohio State University, Columbus, OH 43210

Previous reports have emphasized the behavioral and physiological sequelae of injuries produced with a feedback controlled injury device (Somerson and Stokes, 1987, Exp. Neurol. in press). Here a detailed reconstruction of lesions was performed on the spinal cords of three previously defined groups of spinal cord injured rats. Displacement (time and magnitude) of the spinal cord was controlled using biomechanical feedback during the injury process; the displacement amplitude was kept constant (1.54 +/- .02 mm) while the time of compression was varied between the groups. The three levels of injury were designated as light, intermediate, and heavy; impact durations were 14, 19, and 24 msec., respectively. Laminectomies were performed at midthoracic cord (T-9 or T-10) and the vertebral column was rigidly fixed during injury by using vertebral clamps rostral and caudal to the exposed cord. Such paradigms have been shown in previous reports to result in predictable courses of partial behavioral recovery using several indices of locomotor mobility, postural compensation, and fine motor control.

Morphological results reinforced the behavioral and biomechanical data. Morphometric analysis using a Zeiss Videoplan system revealed that lesion length and to a lesser extent lesion volume (based on biconical frustum analysis) were correlated with biomechanical measures of the impact. An automated MINC-11/23 system was also used to produce three dimensional reconstructions of the lesions to test the assumption of the biconical nature of the lesion volume and see if the behaviorally identical heavy and intermediate groups had geometrically different lesions. As previously reported, the light injury group showed anatomical sparing at the lesion epicenter (66.2%); this evolved into an ovoid shaped lesion in the rostral and caudal neuropil. Intermediate and heavy impacts, however, produced lesions over much greater distances with a displacement of lesion sites toward the dorsal columns in adjacent rostral and caudal sections. Geometric analysis therefore suggests that although after a certain point, biomechanical parameters may lose their sensitivity as behavioral predictors (i.e. once the neuropil and fiber tracts are destroyed at the lesion epicenter), they continue to be useful as predictors of lesion geometry. Such analysis may be of some import to those therapeutic attempts (drugs or spinal transplants) which could potentially alter secondary necrosis after spinal injury. (Supported by USPHS-10165)

21.14 FAILURE TO DEMONSTRATE AXONAL SPROUTING AFTER SUBTOTAL SPINAL CORD LESIONS IN RATS. J.W. Little, R.M. Harris, R.C. Sohlberg*. Seattle V.A.M.C. and Univ. of Washington, Seattle, WA, 98108

Significant recovery of motor function is common following subtotal spinal cord lesions in experimental animals and human patients. Axonal sprouting or growth of new terminals by spared descending pathways is one postulated mechanism to explain this motor recovery. This study attempts to demonstrate sprouting by spared descending pathways in rats undergoing subtotal spinal cord section, utilizing anterograde labeling with horseradish peroxidase (HRP).

Adult female rats underwent partial section of the spinal cord in the mid-thoracic region, sparing either the left lateral and/or ventral funiculi. After four weeks of motor recovery in which considerable return of hindleg postural and locomotor abilities was observed, an injection of 30% HRP, using a glass micropipette and iontophoresis, was made into the left side of the lumbosacral enlargement. Anterograde labeling of commissural fibers and terminals in these rats was compared with similar labeling in non-lesioned controls. In all cases the injections themselves were confined to the left half of the cord, but anterogradely labeled fibers were seen crossing to the right side of the cord.

At high magnification, numerous labeled terminals could be seen along axons, both as en passant boutons and at the ends of short collateral branches. No significant differences could be found in the location or extent of crossing fibers or boutons between the lesioned rats and the control rats. No abnormal terminal morphology was observed in the lesioned rats which might indicate sprouting. The average number of terminals per unit length of individual axons was not increased in lesioned rats over controls. We have thus found no evidence for sprouting of commissural axons four weeks after a subtotal spinal cord lesion. This may be due to technical problems in demonstrating sprouting, or may indicate that axonal sprouting does not play a major role in motor recovery.

21.15 EFFECT OF 4-AMINOPYRIDINE ON ACTION POTENTIAL CONDUCTION IN MYELINATED AXONS OF CHRONICALLY INJURED SPINAL CORD A. R. Blight, Departments of Neurosurgery and Physiology & Biophysics, NYU Medical Center, New York, NY 10016.

Severe mechanical trauma to the spinal cord results in rapid local destruction of most axons, followed by a slower demyelination of many of the surviving axons. Partial remyelination occurs in succeeding weeks, but leaves many axons thinly and irregularly myelinated at the lesion site. This is reflected in deficits of action potential conduction, including conduction block at physiological temperature (Blight, *Neuroscience*, 10: 1471, 1983).

The K^+ channel blocker 4-aminopyridine (4-AP) may be useful in treatment of demyelinating conditions (reviewed by Stefoski et al., *Ann. Neurol.*. 21: 71, 1987). The drug was therefore tested in an *in vitro* preparation of injured spinal cord, prior to *in vivo* tests on conduction in descending pathways (see Gruner et al., this meeting). The recording techniques and injury model have been described in detail (Blight, 1983; Blight & DeCrescito, Neuroscience 19: 321, 1986). In this study, the spinal cords of 12 adult female cats were injured, under pentobarbital anesthesia, by an 8 g weight dropped 20 cm onto the exposed dura at T9, with the vertebral body mechanically stabilized. Animals were maintained with specialized care for 4-11 months post injury, then the thoracic spinal cord was isolated *in vitro*. Microelectrode recordings were made from single myelinated axons that conducted across the chronic lesion site, in ventral and lateral tracts.

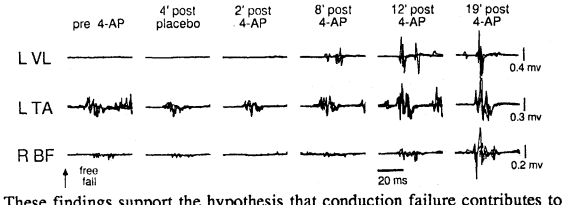
The majority of tested axons (68%) in spinal cords from chronically paralyzed animals were blocked at temperatures below 36°C, compared with only 19% in those animals that showed some recovery of postural and locomotory function. Of 8 axons in which it was possible to demonstrate a conduction block, briefly apply 0.5 mM 4-AP, wait for up to 10 minutes and test the temperature of conduction block again, 2 showed substantial improvement in safety factor, with conduction block raised from 29 and 30°C to >37°C. (Following exposure to 4-AP, the absolute refractory period at 37°C was 4 ms in one of these axons.) Two other fibers showed modest increases in blocking temperature of only 2-3°C, sufficient to produce conduction at 37°C in one axon, but not the other. Four other fibers showed no change in the temperature of conduction block with exposure to 4-AP. Another striking effect of the drug was an increase in spontaneous activity of axons in the white matter, which are usually silent in the isolated preparation. 4-AP produced spontaneous bursting in 64% of axons that failed to conduct across the lesion, and in 35% of those that did conduct at 25°C in chronically paralyzed animals. 18 axons conducting through the lesion in recovering animals were exposed to 4-AP, but none of these became spontaneously active.

It was concluded that 4-AP may be useful in the treatment of chronic spinal cord injuries, both because it improves safety factor in a proportion of axons and because it increases excitability in others, which may compensate to some extent for loss of information in parallel channels. Nonetheless it appears that much of the conduction loss in the chronic lesion is not reversed, even by quite high levels of 4-AP, and further investigation of the physiological and structural basis of those conduction deficits is required. (Supported by contract TC 85-02 from the American Paralysis Association and by grant NS21122 from NIH/NINCDS).

21.16 RECOVERY OF MOTOR FUNCTION IN CHRONIC EXPERIMENTAL SPINAL CORD INJURY ENHANCED BY 4-AMINOPYRIDINE. J.A. Gruner, A.R. Blight, M. Petruziello*. Depts. of Neurosurgery and Physiology & Biophysics, NYU Medical Ctr., NY, NY 10016.

In order to investigate factors underlying recovery of function after chronic spinal cord injury, we have developed a technique for assessing motor function in animal models of spinal cord injury, based on hindlimb EMG free-fall responses (FFR) (Gruner et al., *CNS Trauma* 1: 139, 1984). Standardized contusion injury of cat spinal cord (Blight & DeCrescito, *Neuroscience*, 19: 321, 1986) produces chronic loss of 90-95% of white matter at the injury site, resulting in near complete paraplegia and severe attenuation of FFR. These chronic deficits may result at least partly from demyelination of the lesion site (Blight, *Neuroscience*, 10: 1471, 1983). Recently, 4-aminopyridine (4-AP) has been reported to improve function and axonal conduction in human and experimental demyelinating lesions. We therefore examined the effect of this drug on injured spinal cord.

Seven adult female SPF cats were injured using the weight drop technique. Under pentobarbital anesthesia, the T9 spinal cord was exposed, and an 8 or 10 gm weight dropped 20 cm onto the dura, with the vertebral body rigidly supported. The sterile incision was closed and the animals allowed to recover. They were maintained with specialized care and were tested 3-7 months later, together with 4 uninjured cats. The animals were sedated with 25 mg/kg ketamine i.m. and placed in a harness for the controlled drop. Free-fall lasted 200 ms; the harness was then decelerated by rubber tubing. EMG's were recorded from vastus lateralis (VL), tibialis anterior (TA), biceps femoris (BF), and medial gastrocnemius (MG) bilaterally. Four sets of 8 drops were obtained at the start of testing and at 5', 10', and 15' after i.v. injection of 1 mg/kg 4-AP; in some animals drops were also obtained after placebo injections. The figure shows an example of the effect of 4-AP on FFR in one injured cat. The mean peak-peak FFR amplitude in injured cats increased from 28 to 138 μV over 15 min. from 4-AP injection ($p<0.006$, paired t-test). In the normal cats, there was an apparent increase from 1.86 to 2.89 mV (NS).



These findings support the hypothesis that conduction failure contributes to functional deficits. 4-AP may also act through enhanced synaptic transmission in the lumbar cord; we have noted that FFR in injured cats are sometimes facilitated by limb manipulation or peripheral electrical stimulation.. (Supported by contract from the American Paralysis Association and NIH grants NS21122 & NS10164).

21.17 A NEW PHARMACOLOGIC APPROACH TO SPINAL CORD INJURY: STUDY USING A DYNAMIC INJURY MODEL IN RATS.
S. Tsuyoshi Ohnishi, James K. Barr* and Chika Katagi*. Membrane Research Institute, University City Science Center, Phila, PA 19104.

It is accepted that traumatic spinal cord necrosis takes place in two steps, i.e., primary injury accompanied by an immediate loss of action potential and rapid ion movements (calcium influx and potassium efflux), and secondary injury which, in several hours, leads to cell necrosis. Thus, the rationale for therapy should be to intervene in these steps. We have been studying irreversible membrane damage of sickle red blood cells(1,2), and found close similarity between the mechanism of spinal cord necrosis and that of sickle cell membrane damage. Namely, (a) reactions seem to be initiated by calcium influx, (b) entering calcium ions activate movement of monovalent cations, and (c) this movement causes water transport, leading ultimately to cell necrosis. We found that two types of drugs can prevent damage of sickle cell membranes (2,3), i.e., (i) inhibitors of the calcium-activated potassium efflux (e.g., quinine), and (ii) drugs which interact with calmodulin (such as chlorpromazine; CPZ). Therefore, we hypothesized that these drugs may have a beneficial effect in spinal cord injury. Using Sprague-Dawley rats (200-250 g) and a dynamic weight-drop (10 g x 5 cm) technique(3), we tested the effect of drugs (one bolus addition; i.p. 30 minutes prior to injury). Motor recovery was estimated using the modified Tarlov score(3). Four weeks after injury, the Tarlov score was 2.2±0.5 (n=10) in non-treated group, but the score improved to 4.4±0.7 (n=5) in a quinine-administered group (20 mg/kg body weight) and to 4.0±0.5 (n=5) in a CPZ-administered group (20 mg/kg) Difference between non-treated and drug-treated groups was significant ($P<0.01$). Other potassium channel inhibitors also demonstrated a beneficial effect. Measurement of water content as well as assay of Ca, Na and K contents by atomic absorption method(4) demonstrated that these drugs inhibited movement of water and cations. Morphometric study also confirmed the beneficial effects of these drugs. References: (1) Ohnishi et al.(1986) Biochim. Biophys. Acta 886:119. (2) Ohnishi et al.(1986) Pharmacology 32:248 (3) Wrathall, J.R. et al.(1985) Exp. Neurol. 88:108. (4) Young, W. and Koreh, I. (1986) Brain Res. 365:42.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION I

22.1 MONOSYNAPTIC CONNECTIONS BETWEEN CEREBELLAR GRANULE AND PURKINJE CELLS IN DISSOCIATED CELL CULTURE.
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Synaptic properties between neurons in the mammalian central nervous system can be most thoroughly investigated in dissociated cell cultures, because of good visibility and accessibility. Previous work (Hirano et al., PNAS 83:4957, 1986) has established synaptic formation between identified cerebellar granule and Purkinje cells in dissociated cell culture. Using these identified cells, we are attempting to characterize the transmitter and the receptor on the postsynaptic membrane. It has been suggested that the transmitter is glutamate, and the recent work by Kano and Kato (Nature 325:276, 1987) suggests that the postsynaptic receptor channel is a quisqualate (QUIS) receptor as opposed to a kainate or N-methyl-D-aspartate (NMDA) receptor.

Neurons were dissociated from cerebella of rat prenatal embryos about 18 days of gestation (for Purkinje cells) and from cerebella of 6-8 day old rats (for granule cells). The dissociated cells were mixed and cultured for 4 weeks before experiments. Simultaneous whole cell clamp recordings were performed on the presynaptic granule and the postsynaptic Purkinje cell somata at room temperature (20-22°C). Monosynaptic connections between two cells were identified by a short (2.5-6.5 msec) and constant latency of excitatory postsynaptic current (epsc). In several pairs of cells the granule cell was filled with lucifer yellow and the Purkinje cell with propidium iodide, and their morphological contact was observed under fluorescent light. Kynurenate, an excitatory amino acid antagonist, abolished the monosynaptic epsc at 1 mM without affecting the presynaptic action potential. DL-2-amino-5-phosphonovaleric acid (APV), a specific antagonist for NMDA receptors, had no effect on the monosynaptic epsc at 0.5 mM in the normal external solution containing 1 mM Mg. Kynurenate inhibited both externally applied glutamate and aspartate induced inward current in Purkinje cells, and APV inhibited the aspartate response while having little effect on the glutamate response. The above results indicate that glutamate is more likely to be the transmitter than aspartate and that a non-NMDA receptor is working on the postsynaptic membrane. Finally we examined the effect of QUIS on a Purkinje cell. QUIS decreased the amplitude of the epsc and increased steady inward current in a dose dependent manner, and the amount of decrease of the amplitude of the epsc by QUIS paralleled the amplitude of QUIS induced inward current. This result is consistent with the presence of QUIS receptors on the postsynaptic membrane, and suggests that QUIS binds to the postsynaptic receptors and reduces the number of receptors available for released transmitter.

22.2 LITHIUM BLOCKS PI MEDIATED TRANSMITTER ACTION IN RAT BRAIN: ELECTROPHYSIOLOGICAL STUDIES IN THE HIPPOCAMPAL SLICE.
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Since lithium blocks the re-cycling of inositol phosphate, it has been hypothesized that this effect on the PI system may contribute to its therapeutic action in affective illness. In previous studies using a smooth muscle preparation, we have demonstrated that therapeutic concentrations of lithium alter transmitter induced contractile responses thought to be mediated by the PI system (Menkes et al., PNAS 83:5727, 1986). Using the hippocampal slice preparation, we have recently demonstrated that muscarinic agonists block the inhibitory actions of adenosine by stimulating protein kinase C, via the PI cycle (Worley et al., PNAS, in press). Accordingly, to examine whether lithium affects PI mediated transmitter responses in brain, we have investigated its effects on muscarinic blockade of adenosine.

Field potentials elicited by stimulation of Schaffer collaterals were recorded from the CA1 pyramidal cell layer. As lithium might be expected to affect the PI system after prolonged stimulation, we first assessed the ability of carbachol (50 μM), which was used to drive the PI system, to block adenosine over the course of a one hour incubation. In these preliminary experiments, we found that in about two-thirds of slices tested, carbachol's blockade of adenosine remained intact for one hour, while in the remainder we observed variable degrees of rundown of carbachol's action. To reduce variability in assessing the effects of LiCl on this response, we studied slices in which carbachol's block of adenosine was preserved following a 1 hr incubation. At this point, carbachol was washed off and slices were then treated with either control saline, LiCl (1 or 2 mM) or RbCl (2 mM) for 1 hr and then re-exposed to carbachol. These concentrations of LiCl or RbCl did not alter baseline synaptic responses or adenosine's ability to completely inhibit the population spike. However, in the LiCl treated slices, carbachol's ability to block adenosine fatigues within 15 minutes, whereas in control or RbCl treated slices, it does not. Interestingly, phorbol 12,13-diacetate (1-2 μM), an activator of PKC, still blocks adenosine when carbachol is ineffective, suggesting that fatigue produced by lithium is proximal to activation of PKC and may reflect its action on the PI cycle.

22.3 EFFECTS OF L-VESAMICOL (L-AH5183) ON NEUROMUSCULAR TRANSMISSION R.F.Halliwell*, C.Prior* and I.G.Marshall* (SPON: N.N.Durant) Dept. of Physiol. & Pharmacol., Univ. Strathclyde, Glasgow G1 1XW, Scotland, UK.

Vesamicol (AH5183, 2-(4-phenylpiperidino) cyclohexanol) produces a frequency-dependent block of neuromuscular transmission (Marshall,I.G., Brit.J.Pharmacol., 38: 503, 1970) and an inhibition of the uptake of acetylcholine by isolated synaptic vesicles (Anderson et al., Molec.Pharmacol., 24: 48, 1983). Vesamicol possesses one assymetric center in the C ring, and the L-isomer has been shown to be the active form in isolated vesicles (Bahr,B.A. & Parsons,S.M., P.N.A.S., 83: 2267, 1986). In this study, we have examined the effects of the two isomers of vesamicol on neuromuscular transmission in the isolated contracting rat hemidiaphragm, and the effects of L-vesamicol on miniature endplate potentials (MEPPs) in isolated mouse diaphragm and end-plate currents (EPCs and MEPCs) in snake costocutaneous nerve-muscle preparations.

In the rat hemidiaphragm L-vesamicol was 20 times more potent at reducing twitches elicited at 1Hz than was D-vesamicol. This finding agrees well with results obtained from isolated synaptic vesicles. In contrast, the two isomers were equieffective in producing twitch augmentation in the hemidiaphragm prior to the neuromuscular block. L-vesamicol was examined in more detail on the hemidiaphragm and found to exhibit similar blocking characteristics to those of racemic vesamicol, i.e. a frequency-dependent block not reversed by anticholinesterases or by choline. However, the block produced by L-vesamicol (5 x 10^{-6}M) was partially relieved by 3,4-diaminopyridine (10^{-4}M).

In the stimulated mouse hemidiaphragm preparation (5Hz for 20 min) L-vesamicol (10^{-5}M) reduced the amplitude of MEPPs. In the presence of L-vesamicol there was a wider spread of MEPP amplitudes than in the absence of the drug, with the appearance of apparently normal-sized MEPPs amongst the small MEPPs. In some preparations it was possible to increase the occurence of these normal-sized MEPPs by the addition of lanthanum (2 x 10^{-4}M). In the voltage-clamped snake muscle L-vesamicol (2.5 x 10^{-6}M) produced a marked increase in MEPC frequency and a reduction in EPC amplitude after rapid nerve stimulation. No changes were observed in the rate of decay of EPCs or MEPCs indicating a lack of effect of vesamicol on endplate ion channels. The reversing effect of aminopyridines and the ability to release normal-sized quanta in the presence of vesamicol block may indicate that it is possible to release a store of transmitter not normally released after block by vesamicol.

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22.4 Selective Inhibition of Protein Kinase C mimic the actions of Acetylcholine in "in vivo" hippocampus. N.Agopyan & K.Krnjevic, Departments of Anaesthesia Research and Physiology,McGill University, Montreal, Quebec,H3G 1Y6 Canada.

Tumor-promoting phorbol esters mimic diacylglycerol (Nishizuka,Y 1984,Science,225:1365) by activating protein kinase C. The muscarinic action of ACh in hippocampus is postulated to accelerate the turnover of inositol phospholipids and recently it was reported that phorbol esters mimic some of the actions of muscarinic agonists, such as blocking the slow afterhyperpolarization (Malenka et al.,1986,J. Neurosc.,6:475). This led us to investigate the effects of selective activation and inhibition of protein kinase C on synaptic transmission in the hippocampus in situ.

All experiments were carried out on Sprague Dawley rats anaesthetized with urethane and maintained in a stereotaxic head frame. Extracellular field potentials, induced by fimbrial stimulation (0.6 Hz), were recorded simultaneously in the CA1 stratum pyramidale and stratum radiatum with the central barrels of 5-and 3-barrelled pipettes separated by a vertical inter tip distance of 200-250 um. Peripheral barrels were used for iontophoretic application of phorbol 12-13-diacetate (PDAc:7.43 mM;14-56 nA), 1-2 methylpiperazine dihydrochloride (H-7: 8 mM; 14-56 nA), acetylcholine chloride (ACh 500 mM; 28-56 nA) and NaCl (3M,56-140 nA).

Applications of PDAc in stratum pyramidale increased the amplitude of the somatic population spike and decreased its latency; but unlike ACh (Rovira et al.,1983,Neuroscience,8:97) it never induced multiple spikes. Application of PDAc in the apical (stratum radiatum) dendritic layer increased the amplitude of the negative field and decreased its latency, which in turn caused an increase in the somatic population spike amplitude and a decrease in its latency. If the stimulation intensity was below threshold for evoking a population spike, PDAc application at either somatic or dendritic level induced the appearance of a population spike, which gradually increased in amplitude and decreased in latency.

On the other hand, H-7 which is the most potent and selective protein kinase C inhibitor (Kawamoto S.& Hidaka, H.,1984, Biochem. Biophys.125, 258) when applied in stratum pyramidale increased the amplitude and the number of population spikes induced by fimbrial stimulation. Application of H-7 in dendritic layer increased the amplitude and the duration of the negative field. H-7 also induced the appearance of a population spike at a stimulation intensity which was below threshold for inducing the population spike. The effects induced by H-7 were reversed by PDAc. PDAc and H-7 induced their effects approximately 20-40 s after the start of application. Unlike ACh they had a longer lasting action. Recovery was usually observed 10-15 min after the termination of injection.

These observations provide evidence that ACh may act via inhibition of protein kinase C rather than activation.

Supported by Savoy Foundation and MRC.

22.5 **INFLUENCE OF CYTOCHALASIN B ON SPONTANEOUS AND DRIVEN ACTIVITY IN RAT SOMATIC SENSORY CORTEX.** H.-M. F. Hwang, S.M. Lee* and F.F. Ebner, Center for Neural Science, Brown University, Providence, R.I. 02912.

Extrinsic inputs to the cerebral cortex terminate mainly on dendritic spines of cortical neurons. Dendritic spines contain a high percentage of the actin found in cortex (Matus, *et al.*, P.N.A.S., 1982). Our experiments tested the hypothesis that transient depolymerization of actin would produce reversible decreases in synaptic efficacy at axospinous synapses. The test consisted of ventricular or local injection of the actin polymerization blocking agent, cytochalasin B (CB), while recording the spontaneous activity and responses to whisker movement in PMBSF SI neurons. Since CB requires DMSO to go into solution, experimental and control injections were made simultaneously in the two hemispheres; the effect of vehicle alone in the left hemisphere was compared to the effect of injecting vehicle plus CB in the right hemisphere for 10-12 hours after injection.

Glass micropipettes, 1.2 mm O.D. for recording and 2.0 mm O.D. for injection were assembled so that the tips were separated by 1.5 mm. Two pairs were placed symmetrically in the part of the barrelfield representing usually the D5 whisker in 5 adult Long-Evans rats under urethane anesthesia. The depth was fixed so that the injection was into layer IV and the recording was from layer V neurons. 4 ul of Ringers solution with 1% DMSO was injected into the left hemisphere and 4 ul this vehicle plus 10^{-4} M CB into the right hemisphere.

A predictable sequence of changes in spontaneous activity and stimulus evoked neuronal discharges occured following injection of CB, that were never observed with the vehicle alone. Normal cell activity followed both injections for approximately 30 min before the CB side started to show a slowly progressing diminution in spontaneous bursting of layer V pyramidal cells. Near total absence of spontaneous activity lasted for less than 1 hour on the CB side, during which time no stimulus-evoked action potentials could be elicited by whisker movement. Eventually in all animals an altered type of spontaneous activity returned on the CB side. The alteration took the form of: 1) a decrease in the number of spikes per burst (without altered interspike interval), 2) an increased interburst interval, 3) an increase in epileptiform activity and spindling, 4) a decrease in the amplitude of spontaneous spikes and 5) a decrease in the number and amplitude of spikes per stimulus-evoked response. These alterations persisted for at least 8 hours before the responses showed a trend toward a return to normal. We are investigating whether complete recovery occurs at very long survival times and the possible mechanisms that may be responsible for the changes in synaptic properties induced by CB. (supported by ONR grant #NOOO-14-811-K-0041)

22.6 GONADAL STEROID EFFECTS ON ATP-STIMULATED SODIUM TRANSPORT IN PIG BRAIN SYNAPTOSOMES. C. Rollin*, A. Arieff*, C. Fraser*, J. Kucharczyk, D. Norman* and P. Sarnacki*. Departments of Medicine and Radiology, University of California, San Francisco, CA 94143.

The principal female gonadal hormones, estrogen and progesterone, have been shown to elicit rapid changes in brain cell membrane permeability and conductance, leading to modifications of membrane functions. The subcellular basis for some of these changes was investigated in synaptosomes isolated from different regions of the brain of prepubertal micropigs. Synaptosomes are resealed vesicles from nerve endings isolated by homogenization and differential centrifugation on a discontinuous Ficoll gradient, and are morphologically intact and metabolically active. ATP stimulated sodium uptake was measured in inverted vesicles in the presence and absence of steroid hormones. Either estradiol-17B or progesterone was added to the incubation media at concentrations of 0.01-100 ng/ml and 1-1000 ng/ml, respectively. Sodium uptake was then measured at 5 minutes. All concentrations of estradiol resulted in a significant ($p < 0.001$) decrease in sodium uptake with the maximum inhibitory effect exerted at a physiological concentration of 100 pg/ml. The average sodium uptake decreased from 6.3 ± 0.3 to 5.4 ± 0.3 nmoles/mg synaptosomal proteins. Similarly, progesterone at all concentrations inhibited sodium uptake with the maximum inhibitory effect observed at 1000 ng/ml. All areas of the brain (cerebral and cerebellar cortex, pons, basal ganglia, septum) showed significant ($p < 0.001$) progesterone-induced reduction in sodium uptake (6.6 ± 0.3 to 4.7 ± 0.5 nmoles/mg protein). Previous studies have demonstrated an inhibitory interaction between progesterone and Na-K-ATPase in cardiac muscle cells and erythrocytes. Sex differences have also been observed in Na-K-ATPase activity in human erythrocytes. The results of the present study suggest that both estrogen and progesterone also significantly inhibit ATP stimulated sodium uptake in pig brain synaptosomes.

22.7 IS VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) THE VASODILATOR TRANSMITTER IN CEREBRAL ARTERIES? T.J-F. Lee, Y. Shirasaki*, S.D. Shillcutt*, and S. Sarwinski*, Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

Dense VIP-like immunoreactive fibers have been shown to be present in pial vessels from several species. Ultrastructural immunocytochemical studies demonstrate that the VIP-like immunoreactivity is present in the neuronal granular vesicles of the nonsympathetic nerves. Release of the immunoreactive VIP upon excitation of these nonsympathetic nerves has been demonstrated in cat pial vessels. Furthermore, VIP induces vasodilation, which is independent of endothelial cells, and antibodies against VIP block the transmural nerve stimulation (TNS)-induced vasodilation. These results suggest that VIP is the potential vasodilator transmitter in cerebral blood vessels, although the direct evidence for demonstrating the vasodilator responses to the endogenously released VIP has not been presented. Therefore, the present study was designed to further investigate the potential role of VIP as the cerebral vasodilator transmitter. The results from in vitro tissue bath study indicate that in cat cerebral arteries VIP receptor antagonists such as (N-Ac-Tyr^1,D-Phe^2)-GRF$_{(1-29)}$-NH$_2$ and (D-P-chloro-Phe^6, Leu^{17}) VIP, blocked the exogenous VIP-induced relaxation but did not affect the TNS-induced relaxation. Hemoglobin and methylene blue, which are known to inhibit the cyclic GMP synthesis, blocked the TNS-induced vasodilation, suggesting that the TNS-induced relaxation is mediated by cyclic GMP system. Exogenously applied VIP, however, enhanced cyclic AMP levels but did not affect those of cyclic GMP. These results are inconsistent with the hypothesis that VIP is directly involved in the TNS-induced vasodilation in cerebral arteries. (Supported by NIH HL 27763 and AHA/IHA 83-1040.)

22.8 LINDANE BUT NOT DELTAMETHRIN BLOCKS A COMPONENT OF GABA-ACTIVATED CHLORIDE CHANNELS. N. Ogata*, S.M. Vogel* and T. Narahashi (SPON: J. Frey). Department of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

The pyrethroid insecticide, deltamethrin, has been shown to inhibit the binding of a radioligand for the picrotoxinin-recognition site in rat brain synaptic membranes (Lawrence, L.J. and J.E. Casida, Science, 221:1399, 1983), suggesting a possible interaction between pyrethroids and the $GABA_A$ receptor-ionophore complex. In addition, another class of insecticides, lindane (the gamma isomer of hexachlorocyclohexane), produces CNS hyperexcitability similar to that caused by known GABA antagonists in mammals. We, therefore, examined direct interactions between these insecticides and the GABA-activated ionic channels, using cultured neurons of the newborn rat dorsal root ganglia (DRG). The membrane of the DRG cell was voltage-clamped using the whole cell patch clamp technique. GABA (10^{-5}M) produced an inward current of about 0.5 nA which comprised an initial peak and sustained components. The initial peak component decayed rapidly, whereas the sustained component persisted throughout the superfusion of GABA. An increase in GABA concentration after the decay of the initial component or repeated application of a single concentration of GABA within short intervals produced only the sustained component. Therefore, the initial peak and sustained components appeared to reflect desensitizing and non-desensitizing components, respectively. Both components had the same reversal potential of about -50 mV when internal and external concentrations of Cl^- were 20 mM and 130 mM, respectively. The reversal potential was shifted in a manner predicted by the Nernst potential for Cl^- when external Cl^- concentrations were altered. Co^{2+} (2 mM) and tetrodotoxin (2 x 10^{-6}M) had no effect on the GABA-induced inward current. Both desensitizing and non-desensitizing components were readily blocked by bicuculline (10^{-6}M), indicating that the inward current induced by GABA was $GABA_A$ receptor-mediated. Lindane (10^{-5}M), which had no effect on any of the voltage-gated sodium, potassium, or calcium channels, readily suppressed the initial peak component of the GABA-induced Cl^- current, without affecting the sustained component. On the contrary, deltamethrin, which markedly modified the sodium channel kinetics, had no effect on the GABA-induced Cl^- current. These results indicate that the action of lindane, but not of deltamethrin, appears to involve interaction with the $GABA_A$ receptor-ionophore complex. Our results that the initial peak component was preferentially blocked by lindane might indicate that there are two types of $GABA_A$ receptor-Cl^- channel complexes in rat DRG cells.

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22.9 ETHANOL HAS NO EFFECT ON GABA-INDUCED MEMBRANE CURRENT IN CELLS CULTURED FROM DISSOCIATED EMBRYONIC RAT HIPPOCAMPUS S.Huck, R.Gratzl+ and F.Grießmayer+, Dept.of Neuropharmacology, University of Vienna, A-1090 Vienna, Austria.

Recent studies indicate that ethanol stimulates GABA-mediated $^{36}Cl^-$ transport in rat brain synaptosomes (Suzdak et al., PNAS 83, 4071, 1986). The ethanol-mediated $^{36}Cl^-$ influx is selectively blocked by the imidabenzodiazepine Ro 15-4513 (Suzdak et al., Science 234, 1243, 1986).

Electrophysiological data of ethanol effects on the GABA system are, however, conflicting. We therefore investigated the GABA-ethanol interrelationship by means of the patch clamp technique in cultured hippocampal cells.

The hippocampal tissue of 18 day old embryos was dissociated and maintained in culture by conventional procedures. Recordings were performed within 2 to 7 days after plating. Patch pipettes were filled with (mM): CsCl (104), TEA-Cl (16), $CaCl_2$ (0.19), EGTA (4), glucose (8), HEPES (8), pH 7.3. The bathing solution was NaCl (120), KCl (3), $CaCl_2$ (2), $MgCl_2$ (2), glucose (20), HEPES (10), pH 7.3. Substances were applied close to the cells by means of a 5-barrel needle device, capped by a glass capillary with a tip diameter between 100 and 150µ. The fluid, ejected by hydrostatic pressure, was stopped or allowed to flow by pneumatic pressure-controlled cylinders developed in our laboratory.

The application of GABA induced dose-dependant, rapidly inactivating inward currents in cells clamped at negative holdings potentials. In the presence of 100µM pentobarbital, GABA responses were markedly enhanced. Pharmacologically relevant concentrations of ethanol, ranging from 10 to 100mM, neither induced changes in membrane conductance when the substance was given alone, nor did they potentiate or inhibit the effect of 5µm GABA when applied in combination.

At the present, we do not know why ethanol stimulates the influx of $^{36}Cl^-$ in synaptosomes and potentiates the action of GABA-ergic substances in this preparation, whereas it is devoid of such effects when investigated in cultured hippocampal cells with electrophysiological techniques.

22.10 SODIUM VALPROATE DECREASES EXCITATORY SYNAPTIC POTENTIATION IN THE IN VITRO HIPPOCAMPUS. W.H. Griffith and L. Taylor*. Department of Medical Pharmacology and Toxicology, Texas A&M University, College Station, Texas 77843

Evidence is available to suggest a significant involvement of excitatory synaptic transmission in the generation and spread of epileptiform activity in the hippocampus. One mechanism responsible for short term synaptic enhancement is post-tetanic potentiation (PTP); and under certain conditions, potentiation within a network of cells will result in an epileptiform event or paroxysmal depolarizing shift (PDS). We studied the effects of the anticonvulsant sodium valproate (NaVP) on, first, the magnitude and time course of PTP and, second, the ability of NaVP to inhibit PDS generation. We report that NaVP (30-200 µM) decreases the magnitude but not the time course of PTP of the dendritic CA1 field excitatory postsynaptic potential (EPSP); and 100 µM NaVP reduces PDS generation in CA3 pyramidal cells.

Transverse slices of hippocampus from adult rats (175-200 gms) were cut using a McIlwain tissue chopper and transferred to a holding chamber where they were maintained for 1-2 hours before use. NaVP was first dissolved in distilled water and then diluted to a final concentration in the physiological solution. Extracellularly recorded field EPSPs were recorded from the apical dendrites of CA1 pyramidal cells following Schaffer-collateral stimulation. Intracellularly recorded EPSPs and excitatory postsynaptic currents (EPSCs) were recorded in CA3 pyramidal cells following mossy fiber stimulation. Reproducible episodes of PTP could be recorded in the same preparation using a conditioning paradigm of 100 Hz stimulation for 1 sec. Every slice served as its own control and results were analyzed using a two-tailed paired T test. Under control conditions, PTP of the dendritic EPSP exhibited increases of 200-250% of control and declined with a time constant of decay 50-100 ms (N=31). NaVP at concentrations of 30-200 µM significantly ($P<0.05$) decreased the amplitude of PTP but did not change its time course (N=8 at each concentration).

A second series of experiments studied EPSCs of the mossy fiber to CA3 synapse. In disinhibited slices (addition of 10-20 µM picrotoxinin), enhancement of excitatory synaptic responses can lead to the network-evoked PDS. Under voltage clamp recording conditions, the underlying synaptic current can be studied at different potentials and the synaptic conductance quantified. NaVP (100 µM) depressed the generation of the PDS (N=5) and reduced the synaptic conductance during the network event. One anticonvulsant action of NaVP may be to reduce excitatory synaptic potentiation resulting in a reduction in the ability of nerve networks to generate epileptiform episodes (Supported by NIH grant NS22456).

22.11 DIFFERENTIAL EFFECTS OF FOUR-AMINOPYRIDINE ON SYNAPTIC TRANSMISSION IN BULLFROG SPINAL CORD IN VITRO . N.C. Tkacs* and R.D. Wurster . Department of Physiology, Loyola University Medical Center, Maywood, IL 60153.

The potassium channel blocking drug, 4-aminopyridine (4-AP), is thought to potentiate synaptic transmission by prolonging the action potential at synaptic terminals, thus facilitating calcium entry. We have studied the effects of this compound on ventral root responses (VRRs) to lateral column (LC) and dorsal root (DR) stimulation in the isolated bullfrog hemicord.

Spinal cords were removed from four bullfrogs by standard methods. After hemisection, the caudal hemicord is placed in the superfusion chamber and eighth or ninth dorsal and ventral roots are led into adjacent mineral oil pools for stimulation and recording. Tungsten wire electrodes are advanced into the lateral funiculus to stimulate descending motor pathways. The cord is continually superfused with oxygenated bicarbonate-phosphate buffered Ringer's solution. Temperature is maintained at 12 C.

The stimulation protocol for the two experimental preparations and time- and stimulation-matched controls was as follows: Period I - 50 minutes, stimulation every two minutes, alternating LC and DR supramaximal stimuli; Period II - 50 minutes, stimulation every two minutes in the presence or absence of 5 uM 4-AP; Period III - 60 minutes, stimulation every two minutes, then less frequently for 3 to 5 hours.

4-AP appeared to affect only the DR-VRR. During Period III the average DR-VRR in the 4-AP group was 75% larger than control. In contrast, the LC-VRR during the same period was 1.5% smaller in the 4-AP group compared to control. Data from the remaining three hours of washout show a similar augmentation of the DR-VRR in the 4-AP group of 94% over controls, while the LC-VRR was 16% smaller in the 4-AP group.

One possible explanation for this difference is that the pool of motoneurons is closer to being maximally excited by the LC stimulus, which always produces a 3- to 5-fold greater response than DR stimulation. Alternatively, the 4-AP effect on DR vs LC VRRs may reflect some difference in modulation of the two synaptic inputs, which also differ in bouton location and interneuron involvement. Further studies will be required to characterize the effective dose range of 4-AP, and the effect of altering stimulation patterns on 4-AP potentiation.

22.12 **4-AMINOPYRIDINE INDUCED RHYTHMIC SPONTANEOUS DISCHARGE IN THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE RAT.** ***Karim A. Alkadhi.*** Department of Pharmacology, College of Pharmacy, University of Houston, Houston, TX 77004.

It is known that 4-aminopyridine can induce marked spontaneous discharge in mammalian ganglia (Goto and Watanabe, Japan. J. Pharmacol. 32: 607, 1982). The discharge varies in amplitude and frequency from ganglion to ganglion. In about 70% of the preparations I have observed that 4-aminopyridine induced rhythmic spontaneous bursts of discharge. Superior cervical ganglia isolated from rats were desheathed and placed in oxygenated Locke's solution (1.1. mM Ca^{2+}, pH 7.4) in a constant temperature (31±1°C) chamber. Potentials were recorded by a capillary suction electrode from the postganglionic (internal carotid) nerve. Addition of 50 μM 4-aminopyridine caused spontaneous discharge to appear within 5 min. The discharge increased in frequency and amplitude with time and usually stabilized in about 30 min. The rhythmic bursts may appear initially (within 5-10 min) and later become fused as the frequency of discharge increases. In some experiments the rhythmic bursts persisted throughout. The rhythmic bursts may appear suddenly after full development of the discharge in which case the potentials in a burst are usually much larger (2-3 times) in amplitude than regular discharge. The bursts occurred regularly every 1.43±0.01 s. Each burst is typically composed of 4-5 potentials, the first of which is usually the largest in amplitude (102.6±2.2, 63.6±4.4, 72.2±3.5, 69.7±1.8, μV, potentials 1 through 4 respectively). The potentials within a burst occur at regular intervals of 0.12±0.001 s. The discharge and bursts were completely blocked by hexamethonium (500 μM) indicating involvement of acetylcholine receptors. Preliminary experiments suggest that rhythmic discharge may be independent of the 4-aminopyridine concentration.

22.13 INHIBITION OF CENTRAL NEUROTRANSMITTER RELEASE BY ω-CONOTOXIN GVIA, A PEPTIDE MODULATOR OF THE N-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL. D.J. Dooley[1], A. Lupp*[2], G. Hertting*[2] and H. Osswald*[1]. [1]Gödecke Research Institute, D-7800 Freiburg, F.R.G. and [2]Institute of Pharmacology, University of Freiburg, D-7800 Freiburg, F.R.G.

ω-Conotoxin GVIA (ω-CT), a 27 amino acid peptide (Olivera et al., Science, 230:1338, 1985), binds to the neuronal N-type voltage-sensitive calcium channel (N-VSCC) (Cruz et al., Biochemistry, 26:820, 1987), and, thereby, reduces the depolarization-induced calcium influx required for vesicular release of neurotransmitter (Hirning et al., Neurosci. Abstr., 12:28, 1986; Reynolds et al., Proc. Natl. Acad. Sci. USA, 83:8804, 1986; Rivier et al., J. Biol. Chem., 262:1194, 1987). In the present study, we indirectly investigated the generality of this phenomenon across different neurotransmitter systems by measuring stimulation-evoked neurotransmitter release from slices prepared from discrete regions of the rabbit central nervous system.

Electrical stimulation (2 min; rectangular pulses of 2 msec, 3 Hz, 5 V/cm, and 24 mA) of superfused slices containing [^{3}H]-dopamine, [^{3}H]-noradrenaline, [^{3}H]-5-hydroxytryptamine, or [^{3}H]-acetylcholine produced tritium overflows which were decreased by 40-50 % by 5 nM ω-CT (30-min tissue exposure time). Additional experiments using 5 nM ω-CT and hippocampal slices labeled with [^{3}H]-noradrenaline indicated the following: (1) the time dependence of ω-CT-induced inhibition, (2) the competitive antagonism between buffer calcium concentration and ω-CT, (3) the lack of effect of stimulation frequency (15 min; 0.4 Hz) on ω-CT-induced inhibition, (4) the ineffectiveness of the L-type VSCC modulators (+)- and (-)-202-791 (1 μM) either in the presence or absence of ω-CT, (5) the additive effect of the N-VSCC antagonist cadmium (40 μM) and ω-CT, (6) the antagonism between the protein kinase C activator 4-β-phorbol-12,13-dibutyrate (0.1 μM) and ω-CT, and (7) the antagonism between the potassium channel antagonist 4-aminopyridine (10 μM) and ω-CT. In all cases, the employed substances did not alter basal tritium outflow.

ω-CT appears to be a useful and potent pharmacologic tool in studying the involvement of the N-VSCC in synaptic function. The comparable ω-CT-induced attenuation of stimulation-evoked release of the classical neurotransmitters (viz., dopamine, noradrenaline, 5-hydroxytryptamine, and acetylcholine) suggests that the molecular nature of the N-VSCC is similar or identical across central neurotransmitter systems. Moreover, pharmacologic modulation of the N-VSCC conceivably alters both intraneuronal and multisynaptic events.

22.14 EFFECTS OF GABA ON CA3 PYRAMIDAL CELL DENDRITES IN THE MATURE RABBIT HIPPOCAMPUS. D. Janigro and P.A. Schwartzkroin, Dept. of Neurological Surgery, Univ. of Washington, RI-20, Seattle, WA 98195

It is generally agreed that gamma-aminobutyric acid (GABA) acts as an inhibitory neurotransmitter in various regions in the CNS. Its inhibitory effects are thought to be mediated through at least two different receptor subtypes. The GABA-A receptor is sensitive to blockade by micromolar concentrations of the convulsant agent bicuculline, and is associated with chloride channel. The GABA-B receptor is not sensitive to bicuculline and is thought to mediate an increase in K^+ conductance. GABA effects in the CA1 region of the hippocampus have been shown to depend on the site of application; somatic application generally leads to cell hyperpolarization, whereas application of GABA in the dendritic region depolarizes the cells. We now report the results of investigations on the effects of GABA applied on CA3 pyramidal cell dendrites.

Slices from mature (30-35 days postnatal) rabbits were examined in vitro using standard electrophysiological techniques. GABA was either pressure ejected (1 mM) or iontophoretically applied (1 M, pH 4) onto the distal portion of basal or apical dendrites. The predominant response of the pyramidal cells tested with dendritic GABA application was a 5-20 mV hyperpolarization, although depolarizing responses were obtained in 25% of the 18 cells tested. Both hyperpolarizing and depolarizing responses were associated with a decrease in cell input resistance. Bicuculline methiodide (BMI, 40 uM, bath application) abolished the early component of the afferent-induced IPSP in the CA3 region and led to afterdischarge activity following both orthodromic and antidromic stimulation. The hyperpolarizing response to dendritic GABA application was either unaffected or increased by this BMI treatment, whereas the depolarizing response was abolished (within 30), unmasking an underlying hyperpolarization. Perfusion of the tissue with medium containing 0.5 mM $CaCl_2$ and 8.0 mM $MgSO_4$ did not affect the response to GABA, suggesting that GABA was having a direct post-synaptic effect. Intracellular infusion of Cl^- into CA3 pyramidal cells (KCl-filled pipettes) did not alter the hyperpolarization produced by dendritic GABA application.

Our data show that the predominant response of CA3 pyramidal cells to dendritic GABA application is a hyperpolarization. This response contrasts with the widespread depolarization produced by dendritic GABA application in the CA1 region. We suggest that the CA3 hyperpolarizing response is mediated through a GABA-B receptor since it is insensitive to BMI and appears to be unaffected by altering the intracellular Cl^- concentration. In contrast, the depolarization following dendritic GABA application is sensitive to BMI, and is likely to be mediated via a GABA-A receptor subtype. In some cells, the GABA-A mediated depolarization may mask an underlying GABA-B mediated hyperplarization.

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22.15 ANOXIA IN HIPPOCAMPAL SLICES: COMPARISON OF FIELD POTENTIAL RECORDINGS FROM SUBMERGED AND SUPERFUSED PREPARATIONS.
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Synaptically evoked field potentials recorded from submerged hippocampal slices exhibit considerably smaller amplitudes (approx. 2 mV) compared with responses from either superfused (gas/liquid interface) or *in vivo* preparations (10 to 30 mV). A possible explanation for this decreased amplitude is the enhanced "grounding" produced using the submerged preparation. Transmembrane current fluxes which underlie extracellularly recorded field potentials could be shunted more readily to bath ground when slices are completely surrounded by the conductive medium (artificial cerebral spinal fluid; ACSF). Alternatively, it is possible that the viability of neurons is compromised using submerged preparations and reduced field potential amplitudes could reflect a smaller number of cells contributing to synaptically evoked responses. The present study examined which of these two possible factors contribute most to reduced extracellular voltage responses by comparing field potential amplitudes recorded before and after hippocampal slices were submerged. Particular care was taken to ensure that stimulating and recording sites were maintained as slices were gradually covered by ACSF solution (5 - 10 min). The temperature of the solution was continually monitored and maintained at 35°C in the recording chamber. Response amplitudes and the size of stimulus artifacts did not change (< 10%) for the first hour after slices were completely submerged; however, a gradual decrement in amplitudes were observed over the following 2 to 3 hours. This reduction in amplitude resulted in field potentials of 0.5 to 5.0 mV and was sometimes accompanied by the appearance of two or more population spikes, suggesting that depression of postsynaptic excitability did not contribute to the observed depression of response amplitudes. When slices were returned to the superfused condition, response amplitudes did not recover, or showed only marginal improvement. The decrement in field potential amplitudes was markedly enhanced by decreasing the pO_2 in the recording chamber. Thus, the smaller field potential responses from submerged preparations appear to result from neuronal depression, secondary to a lack of oxygen, rather than from differences in the electrical characteristics between superfused and submerged preparations.

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22.16 OXIDATIVE DAMAGE IN GUINEA PIG HIPPOCAMPAL SLICE.
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Previous studies have shown that free radicals, generated by radiation and through the Fenton reaction, produce both synaptic and postsynaptic damage in the hippocampal brain slice (Pellmar, Brain Res. 364:377-381, 1986; Pellmar and Tolliver, in Brain Slices, Karger, Basel, 1987). The possible mechanisms underlying free radical damage include lipid peroxidation and oxidative attack. To evaluate the contribution of oxidation to the observed damage, chloramine T, a specific oxidant of methionine and cysteine residues of proteins, was studied.

Hippocampal slices were prepared from euthanized male Hartley guinea pigs. Stimulation of *stratum radiatum* (0.2 Hz, 0.02-0.5 mA, 200 μs) with stainless steel electrodes evoked a population spike (pop spike) recorded in *stratum pyramidale* of CA1 and a dendritic response (pop PSP) and afferent volley (AV) recorded in the *stratum radiatum* of CA1. From these orthodromically evoked potentials, three input-output (I/O) curves were generated before and after application of chloramine T. Pop spike vs AV demonstrated the net effect of the oxidant, pop PSP vs AV revealed synaptic mechanisms, and pop spike vs pop PSP showed postsynaptic actions. A dose response curve for chloramine T was generated: the pop spike was evoked at half maximal amplitude in control solution and the decrease produced by chloramine T was evaluated. Only one dose was applied to each slice.

Chloramine T (25-500 μM) produced a dose-dependent decrease in the orthodromic response (ED_{50} ≃150 μM). A dose of at least 50 μM was required to produce an effect. The actions of chloramine T were usually reversible with wash. The I/O curves revealed that both the pop spike and the pop PSP were significantly reduced. However, the ability of the pop PSP to produce a pop spike was not impaired by chloramine T. Chloramine T was specific for synaptic damage (100 μM, $n = 8$, $p < 0.05$; 250 μM, $n = 7$, $p < 0.05$). These studies suggest that oxidation reactions can account for the synaptic component of the damage produced by free radicals but may not underlie all the effects. This is consistent with the mechanisms suggested by the radiation experiments. Studies with another oxidizing agent are in progress to further test this conclusion.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

23.1 PRESYNAPTIC ROLE OF INOSITOL 1,4,5-TRISPHOSPHATE AND DIACYLGLYCEROL IN BRADYKININ-INDUCED ACETYLCHOLINE RELEASE AT NG108-15 NEUROBLASTOMA HYBRID-MYOTUBE SYNAPSES.
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Bradykinin (BK) causes rapid conversion of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG) in NG108-15 neuroblastoma x glioma hybrid cells. Diffusional BK application onto NG108-15 cells facilitates acetylcholine release from the hybrid cells: miniature end-plate potential (m.e.p.p.) frequency in a cultured rat muscle cell which had been synaptically connected to the NG108-15 cells were increased by BK application (Yano et al., J. Biol. Chem. 259, 10201, 1984). Simultaneous electrophysiological recordings from a synapse pair of hybrid and muscle cells revealed that the frequency of m.e.p.p.s. was increased during both membrane hyperpolarization and depolarization of the hybrid cell in response to iontophoretic application (+30 nA for 1-5 s) of BK (0.1 mM in 0.1 mM HCl in pipettes). The early BK-induced facilitation of ACh release during the hyperpolarization of the hybrid cells was mimicked by intracellular injections (10-50 nC) of Ca^{2+} (0.5 M in pipettes) or $InsP_3$ (0.25 mM in pipettes). The mean m.e.p.p. frequency increased by 6.9, 3.9 and 3.7 fold in BK, Ca^{2+} and $InsP_3$ application, respectively, from the control values (0.3-4 events/s).

Within 3-5 min after perfusion with 1-2 μM phorboldibutyrate (PDBu), the frequency of m.e.p.p.s. started to increase, and reached a plateau after 8 min with a mean increase of 3.0 fold during a depolarization of the hybrid cells which is due to the inhibition of the M-current (Higashida & Brown, Nature, 323, 333, 1986). Facilitation by BK application or injection of $InsP_3$ was observed even in hybrid cells which had been treated with PDBu or 4 mM Ba^{2+}, an M-current inactivator, or 30 μM H-7, an inhibitor of protein kinase C. The results suggest that these two intermediate substances ($InsP_3$ and DAG) mediate BK-induced presynaptic facilitation: ACh release is inducible by $InsP_3$-dependent release of Ca^{2+} from intracellular storage sites and protein phosphorylation by DAG-activated protein kinase C.

23.2 TYPICAL AND ATYPICAL ANTIPSYCHOTICS: DIFFERENTIAL ACTIONS AT DOPAMINE (DA) RECEPTOR SUBTYPES.
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The actions of typical (e.g., chlorpromazine, fluphenazine, haloperidol) and atypical (i.e., clozapine and fluperlapine) antipsychotic drugs at D-1 and D-2 DA receptors in rat striatum were examined. The affinities of these compounds at D-1 DA receptors, which are positively coupled to adenylate cyclase, were measured using ^{3}H-SCH 23390 binding, and their affinities at D-2 receptors, which are negatively linked to adenylate cyclase in rat striatum, were measured using ^{3}H-spiperone binding. The stimulation of adenylate cyclase by the D-1 agonist SKF 38393 and the K^+-evoked release of ^{3}H-acetylcholine (Ach) from striatal tissue slices were measured as functional indices of activity at D-1 and D-2 receptors, respectively (Saller and Salama, J. Pharmacol. Exp. Ther. 236:714, 1986).

In vitro, the typical antipsychotics possessed considerably greater affinities for D-2 binding sites than for D-1 sites. The affinities of clozapine and fluperlapine at D-1 sites relative to D-2 sites were much greater than those of the typical antipsychotics. In this regard, the in vitro D-1/D-2 affinity ratio of D-butaclamol was similar to clozapine and fluperlapine, but in ex vivo binding studies, it resembled the typicals.

A high positive correlation was found between the abilities of all of the compounds that were examined to inhibit SKF 38393-stimulated adenylate cyclase activity and their affinities at ^{3}H-SCH 23390-labeled sites. This observation somewhat contrasts with the report that clozapine and fluperlapine appeared to possess greater potency at blocking DA-stimulated adenylate cyclase activity than at displacing ^{3}H-SCH 23390 binding relative to the typical antipsychotics (Andersen and Braerstrup, J. Neurochem. 47:1822, 1986). However, DA is a mixed agonist that also stimulates D-2 receptors which inhibit adenylate cyclase activity, and this might partially explain this discrepancy.

Clozapine and fluperlapine also differed from the typicals in their effects on the K^+-evoked release of ^{3}H-Ach. The typicals all enhanced the K^+-evoked release of ^{3}H-Ach, presumably by blocking D-2 receptors, whereas clozapine and fluperlapine tended to lower ^{3}H-Ach release. The observations with clozapine and fluperlapine, which block D-1 and D-2 receptors, are reminiscent of our previous findings that D-1 blockade with SCH 23390 can attenuate the enhancement of ^{3}H-Ach release by haloperidol (Saller and Salama, ibid.). Thus, differences in the effects of typical and atypical antipsychotics may be at least partially attributable to their differential actions at D-1 and D-2 receptors.

23.3 SYNERGISTIC ACTIONS OF NE AND PROTEIN KINASE C: ELECTROPHYSIOLOGICAL STUDIES IN THE DENTATE GYRUS. P.F. Worley and J.M. Baraban, Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Both norepinephrine (NE) and protein kinase C (PKC) have been implicated in long-term potentiation (LTP) of synaptic responses in the dentate gyrus elicited by stimulation of the perforant pathway. To assess the interactions between these two modulators of synaptic activity, we have examined the acute effects of phorbol esters, activators of PKC, and NE on synaptic responses in the dentate. In a previous study, we demonstrated that a prominent action of phorbol esters is their ability to block adenosine's inhibitory effects on synaptic transmission (Worley et al., PNAS, in press). In the present study, we have found that in the dentate gyrus, NE (1-10 μM) greatly potentiates the ability of phorbol 12,13-dibutyrate (PDA) to block adenosine. NE's effect is mimicked by the application of forskolin (10 μM), an activator of adenylate cyclase. Neither 50 μM forskolin nor 100 μM NE alone alter adenosine's inhibitory action. These findings suggest that PKC and adenylate cyclase systems act synergistically to modulate synaptic responses in the dentate. Others have demonstrated the selective involvement of NE in LTP in the dentate but not in the CA1 region of the hippocampus. We have also found that NE and forskolin do not potentiate PKC's block of adenosine in CA1. Thus, the interactions between cyclase and PKC systems appear to be particularly important in modulating synaptic responses in the dentate and may reflect the extremely high levels of G_s-cyclase complex in granule cells detected by [^{3}H]forskolin autoradiography (Worley, PNAS 83:4053, 1986).

23.4 ALPHA-ADRENOCEPTOR MODULATION OF TRANSMITTER RELEASE AT MAMMALIAN MOTOR NERVE TERMINALS.
H. Chen* and W.F. Dryden, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Miyamoto and Breckenridge (J. Gen. Physiol. 63: 609-624, 1974) showed that both epinephrine and norepinephrine could increase MEPP frequency in rat phrenic nerve neuromuscular junction, and attributed this to an action on presynaptic adrenoceptors which increased nerve terminal cyclic-AMP level. This contrasted with an earlier report (Kuba, J. Physiol., 211: 551-570, 1970) in which the effects of the catecholamines on EPC were blocked by phentolamine but not by pronethalol. Other experiments, using reversal of tubocurarine blockade, indicated indirectly that the presynaptic effect of norepinephrine and epinephrine were mediated by alpha-adrenoceptors (Bowman and Nott, Pharmacol. Res., 21: 27-72, 1969; Malta et al., Br. J. Pharmacol., 65: 249-256, 1979). The present work was undertaken to compare the effects of specific agonists and antagonists of adrenoceptor subclasses on spontaneous and accelerated transmitter release at the mouse phrenic nerve terminals.

Mouse hemidiaphragm preparations with residual phrenic nerve stump were pinned on a Sylgard base and perfused at room temperature with Bretag's solution (Life Sci., 8: 319-329, 1969). Using conventional intracellular microelectrodes MEPP frequency was recorded for periods up to 30 min from the same muscle endplate under control conditions, and during perfusion of agonist drug. Similar experiments were performed in which $[K]_0$ was increased to 15 mM with a corresponding reduction in $[Na]_0$. Resting MEPP frequency was not affected by epinephrine (10 μM), norepinephrine (1-10 μM) or phenylephrine (1-10 μM). Exposures >15 min to isoproterenol (0.1-10 μM), dobutamine (1-10 μM) or salbutamol (0.1-10 μM) depressed spontaneous M.E.P.P. frequency in a concentration dependent manner that was unaffected by propranolol (10 μM). Accelerated release, using 15 mM $[K]_0$ was increased by epinephrine (1-10 μM), norepinephrine (0.1-10 μM) and phenylephrine (1-10 μM). This effect was not sustained >15 min and was blocked by prazosin (1-10 μM) but not by yohimbine or nadolol. Isoproterenol (1-100 μM) had no effect. Catecholamine induced increases in transmitter release at the motor nerve terminal are mediated through alpha-1 adrenoceptors. No evidence for the presence of other adrenoceptor subclasses was found. The effect is only seen under conditions of accelerated release, in contrast to cyclic-AMP mediated effects, which are also seen in resting MEPP frequency.

23.5 ETHANOL POTENTIATES PRIMARY AFFERENT DEPOLARIZATION IN FROG SPINAL CORD. M.O. Poulter* and A.L.Padjen, (SPON.B.Harvey) Department of Pharmacology and Therapeutics, McGill Univ., Montreal, Quebec, H3G 1Y6

The effect of ethanol on primary afferent depolarization (PAD, a GABA-mediated synaptic potential) was studied using intracellular recording from large myelinated primary afferent axons penetrated at the dorsal root (DR) entry zone. In agreement with the previous results obtained from whole population recording (sucrose gap technique) DR-PAD was increased in amplitude and decreased in duration (+25% $p<<.001$ and 25% $p<.02$ n=8 by paired t-test, respectively). The decrease in effective resistance (R_e) accompanying PAD (cf. Padjen & Hashiguchi, 1983; obtained by "current-clamp" technique) was further decreased by ETOH (60% with 100 mM), without any change in estimated reversal potential. In the presence of tetrodotoxin (0.25 μM, used to block indirect responses), ETOH did not reproducibly potentiate GABA (1 - 2 mM) mediated change in membrane resistance unlike responses on dorsal root ganglia (GABA 10 - 50 μM) which were regularly increased (cf. Padjen et al. 1987). ETOH increased R_e (+25% n=5 $p<.03$ by paired t-test) and usually depolarized the primary afferents (1-2 mV; reversed on washout). In view of the possiblilty that ETOH increases intracellular Ca^{2+} ($[Ca^{2+}]_i$; cf. Carlen et al. 1985) we examined the effects of caffeine (5 mM) (which raises $[Ca^{2+}]_i$). The results were qualitatively similar to those of ETOH: an increase in the R_e (+38% n=3 $p<.01$ by paired t-test) and a small depolarization (3-4 mV).

Our results suggest that ETOH: 1. increases PAD by, at least in part, potentiating GABA-mediated chloride conductance; contribution of presynaptic effects could not be excluded. 2) The increase in R_e is caused by the blockade of a resting conductance, possibly gK_s (slow K conductance of Dubois, 1983) which is active at resting membrane potential (result of an increase in $[Ca^{2+}]_i$?).

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23.6 CHOLINERGIC TRANSMISSION IN PANCREATIC GANGLIA OF THE CAT.
J. A. Love* and J. H. Szurszewski. Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905.

The intrapancreatic neural mechanisms involved in the control of pancreatic secretion are unknown. To better understand the neural control of pancreatic function as well as the neurophysiology of parasympathetic ganglia, we examined synaptic transmission in intrapancreatic ganglia of the cat. Cats were anesthetized with ketamine and pentobarbital and the neck of the pancreas removed. Pancreatic ganglia with their attached nerve trunks were dissected free from the parenchyma and pinned to the floor of a tissue bath. Ganglia were perfused with oxygenated Krebs solution warmed to 35-37°C. Intracellular recordings were made using 3M KCl-filled glass microelectrodes and nerve trunks were stimulated with bipolar steel electrodes. Cholinergic agonists dissolved in Krebs solution were applied to the ganglia by pressure ejection from a pipette positioned close to the recording electrode. Cholinergic antagonists were applied by superfusion. In all cells tested nerve stimulation evoked fast excitatory postsynaptic potentials (EPSPs) which were abolished by hexamethonium. Slow EPSPs were observed in most cells following repetitive nerve stimulation at frequencies as low as 1 Hz. Frequently, slow EPSPs were resistant to cholinergic blockade with hexamethonium and atropine. Exogenous acetylcholine (ACh) evoked 3 types of responses. Most frequently a fast initial depolarization was followed by a slowly developing, long-lasting depolarization. The fast ACH depolarization was accompanied by a decrease in input resistance, enhanced by hyperpolarization, diminished and then abolished by depolarization to -20 to -10 mV, and abolished by hexamethonium. The slow ACh depolarization was accompanied by an increase in input resistance and excitability, enhanced by depolarization, diminished and abolished but not reversed by hyperpolarization to -60 to -70 mV, and abolished by atropine. In some cells a rapid hyperpolarization with a decrease in input resistance was observed between the fast depolarization and the slow depolarization. This potential decreased with hyperpolarization and appeared to be enhanced by an increased amplitude of the preceding fast depolarization. Other cells responded to acetylcholine with only a fast depolarization. The nicotinic agonist DMPP mimicked the fast ACh potential while muscarine mimicked the slow ACh potential. Thus, pancreatic ganglia exhibit both fast nicotinic and slow muscarinic cholinergic potentials which allow for the integration of central and possibly peripheral input to the pancreas. The ACh potentials resemble those observed in sympathetic ganglia. (Supported by DK 17632 and HLO 7111.)

23.7 THE RELATIONSHIP OF AGONIST PROPERTIES TO THE ACTIVATION KINETICS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. R.L. Papke, G.L. Millhauser, Z. Lieberman* and R.E. Oswald. Department of Pharmacology, Cornell University, Ithaca, N.Y. 14853 USA.

A series of five systematically varying derivatives of acetylcholine (ACh) were used to study the activation kinetics of nicotinic acetylcholine receptors (nAChRs) from BC_3H-1 cells. The goal of the study was to relate the structural characteristics of the agonist to the microscopic kinetics of agonist binding and channel activation as deduced from the opening and closing of single AChR channels recorded in the cell attached configuration. The derivatives were substituted on the acetyl moiety with either H, F, Br, Cl or CH_3. All five derivatives were potent agonists which gave rise to single channel events having conductances of 50 pS. In all cases, the open channel lifetimes (and burst durations) were double exponentially distributed with the shorter time constant varying between 0.2 and 0.35 ms and the longer time constant varying between 8 and 19 ms, depending upon the agonist (PrCh>ClACh>ACh>BrACh> FACh). At 100 nM, at least four exponentials were present in the closed time distribution for all five agonists. Because of the variation of the shortest time constant with the nature of the agonist but not the concentration of agonist, it was assumed to represent dwells in the A_2R state (see scheme below). By examining only the bursts of openings represented by the longer exponential of the open time and burst duration histograms, the scheme shown below can adequately explain the observed data. Based on the above data, estimates were made of the opening (β) and closing (α) rates of the channel and the dissociation (k_-) rate of agonist binding. In conjunction with estimates of the number of AChRs per patch (170 ± 50) and the number of bursts per second, the association rate (k_+) was measured, assuming no cooperativity in binding (*i.e.*, $k_+=k_1=2k_2$ and $k_-=2k_{-1}=k_{-2}$). The association and dissociation rates for the agonists were highly correlated with the bond length at the substituted position (rates decreased with increasing bond length); and the opening and closing rates were correlated with the ionic character of the bond (opening rates decreased and closing rates increased with increasing ionicity of the bond). This suggests that the energy barrier for binding is increased by increasing the bond length (BrACh>ClACh>PrACh>FACh>ACh), and the doubly liganded open state is destabilized by the ionic character of the substituent bond.

$$A + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A + AR \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^*$$

A = ACh, **R** = AChR, **AR** = singly liganded AChR, $\mathbf{A_2R}$ = doubly liganded AChR (closed channel), $\mathbf{A_2R^*}$ = doubly liganded AChR (channel open).

23.8 EFFECTS OF SOMAN ON TRANSMISSION THROUGH BULLFROG SYMPATHETIC GANGLIA. T.J. Heppner* and J.F. Fiekers. Dept. Anat. and Neurobiol., Univ. of Vermont Coll. Med., Burlington, VT 05405.

The concentration-dependent effects of the acetylcholinesterase inhibitor soman were examined on transmission through the isolated bullfrog sympathetic chain. Suction electrodes were used to (1) stimulate the sympathetic chain anterior to the 8th ganglion and (2) record the compound action potential (CAP) from the 9th and 10th spinal nerves. The CAP was recorded in normal saline and at concentrations of soman ranging from 0.01-10 uM. Soman at concentrations < 10 uM had no detectable effects on the CAP evoked with 0.1 Hz supramaximal stimulation. In 10 uM soman, there was a minimal decrease in the CAP amplitude while the duration showed a slight increase after 10 min of continuous stimulation at 0.1 Hz.

The concentration-dependent effects of soman (0.1-10 uM) on the CAP were also examined using train stimulation (maximum stimulating rate = 1 train/5 minutes) at frequencies of 10,20 and 30 Hz (2 second duration). The response to train stimulation was analyzed by comparing the amplitude of the initial spikes in the train with the mean amplitude of the last three spikes. The percent decrease in amplitude in normal saline at 20 and 30 Hz train stimulation was 11% and 19.5%, respectively. In the presence of 10 uM soman, the percent rundown was increased to 18.2% and 51.5% at train frequencies of 20 and 30 Hz, respectively. Spike amplitudes were not significantly changed in soman concentrations < 10 uM.

Intracellular responses were also obtained from the 9th and 10th ganglia during single and train stimulation before and after the addition of soman. The amplitude and duration of the synaptically induced depolarization following a spike were increased 300% and 240%, respectively, 3 minutes after soman addition (1.0 uM). At 10 Hz train stimulation in normal saline, intracellular recordings often showed no sustained depolarization. However, train stimulation in the presence of 0.1 uM soman induced a sustained depolarization that continued for several minutes after stimulation was stopped. Trains of 30 Hz increased the amplitude of the sustained depolarization approximately 20 mV.

Additional studies with soman have shown that (1) antidromic spikes are not changed (1.0 uM) and (2) cells voltage clamped at -50 showed a prolonged decay phase of the EPSC in 1.0 uM soman. The above results are consistent with a localized effect of soman on ganglionic transmission. This work supported in part by the US Army Medical Research and Development Command, Contract No. DAMD17-86-C-6031.

23.9 HALOTHANE BLOCKS LONG-TERM POTENTIATION BUT NOT PAIRED-PULSE POTENTIATION IN HIPPOCAMPUS. J. J. Kendig and M. B. MacIver, Department of Anesthesia, Stanford University School of Medicine, Stanford CA 94305-5117

Previous studies from this laboratory have shown that the halogenated ether anesthetic, methoxyflurane, blocks paired-pulse potentiation (PP), but not long-term potentiation (LTP) of synaptic transmission in hippocampal slices (Tauck and Kendig, 1985, Soc. Neuroscience Abs., vol. 11, p. 780). This is in contrast to the effects of dissociative anesthetics (e.g. ketamine and PCP) which effectively block LTP via depression of responses mediated by NMDA excitatory amino acid receptors. These results suggest that the structurally different volatile and intravenous anesthetics may alter the integrative properties of hippocampal synaptic transmission in a differential manner, involving distinct, structurally selective, membrane sites of action for each agent. The present study examined whether volatile agents differ among themselves in this respect by examining the effects of a halogenated alkane anesthetic (halothane) on PP and LTP for synaptically evoked responses from Schaffer-collateral inputs to hippocampal CA1 neurons. Clinical concentrations of halothane (0.5 to 1.0 vol %) produced a reversible depression of evoked field potentials (50 to 20% of control); however, PP and LTP were not blocked. Higher concentrations (>1.2 vol %) produced further depression (<10% of control) of field potential amplitudes and blocked LTP, but PP potentiation was not altered. The effects of all concentrations studied (up to 2.0 vol %) were readily reversible. The block of LTP and lack of effect on PP potentiation produced by halothane contrasts with the effects of methoxyflurane. The differential effects of these two volatile anesthetics suggest that their mechanisms of action may be fundamentally different at the synaptic level and/or their membrane sites of action differ. Furthermore, these results provide additional support for the concept that the mechanisms underlying PP and LTP in hippocampal synaptic responses are separable.

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23.10 THE EFFECT OF PHENCYCLIDINE ON THE PHYSICAL STATE OF MODEL MEMBRANES. A FLUORESCENCE AND ELECTRON SPIN RESONANCE STUDY. M.M. Sarasua*, W.O. Boggan, K.A. Koehler*, S.L. Masters*. Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC 29425 and Departments of Biochemistry and Surgery, Case Western Reserve University School of Medicine, Cleveland, OH 44109.

To characterize the membrane depth-dependent effects of the drug Phencyclidine (PCP) in dipalmitoylphosphatidylcholine: dipalmitoylphosphatidylglycerol (8:2) sonicated bilayer vesicles, a series of substituted (9-anthroyloxyl) stearic acid fluorescence and doxyl stearic acid spin resonance probes are employed. The PCP concentration-dependence of the response of probes located at C-2, 3, 9 and 12 reveal marked regio-specific differences in the effects of PCP on the fluorescence polarization values for four fluorescence probes. These differences are manifest in the PCP concentration-dependencies of fluorescence polarization both above and below the phospholipid phase transition temperature, T_m, and in the width of the phase transition, ΔT. The 5-doxylstearic acid probe was utilized for determination of S, the order parameter, of the probe as a function of PCP concentration. The biphasic behavior of PCP in negatively charged PC/PG membranes are interpreted in terms of selective interactions of PCP with different regions or phases of the phospholipid bilayer.

23.11 KETAMINE INCREASES DISCHARGE RATE BUT REDUCES SENSORY RESPONSES OF SINGLE CORTICAL NEURONS RECORDED IN BEHAVING RATS I.M. Patel*, and J.K. Chapin, (Spon: G.C. Salmoiraghi), Hahnemann University, Philadelphia, PA 19102.

The dissociative general anesthetic ketamine is widely used in the clinic and in research, and it is functionally related to the drug of abuse phencyclidine, yet its effects on neural circuits are poorly understood. The aim of this study was to define the effect of subanesthetic doses (5-10 mg/Kg body weight) of ketamine on discharge rates and sensory responses of single neurons recorded in the somatosensory (SI) cortex of awake, behaving rats. Units were recorded either with drivable microelectrodes or with floating microwires. In the latter, up to eight single units could be simultaneously recorded for time periods up to 21 days. The behavioral paradigm generally involved running the animals on a treadmill which was switched on and off in a regular cycle. In some animals it was also possible to directly test drug induced changes in neuronal sensory responsiveness by stimulating through electrodes chronically implanted in the forepaw.

The initial behavioral effects of these subanesthetic doses involved: 1- a 10-20 minute induction phase characterized by confusion and marked hyperactivity, and 2- a subsequent 20-40 minute period before recovery in which the drug effects clearly remained, but the animals were able to perform correctly in the treadmill paradigm.

Neurophysiologically, it was found that these doses of ketamine increased the discharge rates of single somatosensory cortical neurons from 100-300%. These increases were greatest during the initial induction period, but continued for the full 40-50 minute duration of the drug effect. During resting as well as treadmill running, these increases were far greater than could be accounted for by concomitant increases in motor activity. Paradoxically, the same ketamine doses strongly suppressed the short latency sensory responses of these neurons, whether the stimulation was delivered through electrodes implanted in the paw, or was the result of the natural stimulation of skin receptors which occurs at the start of treadmill movement. Nevertheless, some cells exhibited an increase in long latency (200 msec) responses to the same sensory stimuli. It is possible that this increase in cortical activity, combined with the suppression of sensory input, may produce some of the disorienting behavioral effects of this drug. Supported by AA-06965, AA-00089, and BNS-8519579 to JKC.

23.12 PATCH CLAMP RECORDING FROM CORPUS STRIATUM NEURONS OF ADULT RATS. Jonathan E. Freedman and Forrest F. Weight. Electrophysiology Section, Lab. of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Fully differentiated neurons from the corpus striatum (caudate and putamen) of adult rats were acutely dissociated by a modification of the PIPES-trypsin method of Kay and Wong (*J. Neurosci. Methods* **16**: 227). This method rapidly and consistently yielded intact cell soma with truncated neurites and clean membrane surfaces suitable for recording with patch electrodes. Patch recording from mammalian CNS was thus possible without resort to embryonic tissue.

In whole-cell recordings, patch pipettes contained (in mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 2; EGTA 11; HEPES, 10 (pH 7.4, 320 mosm). The extracellular bath contained: NaCl, 150; KCl, 5; $CaCl_2$, 2.5; $MgCl_2$, 1; *d*-glucose, 10; HEPES, 10 (pH 7.4, adjusted with sucrose to 340 mosm).

Current clamp recording indicated that the cells had resting membrane potentials of -50 to -80 mV and apparent input resistances of 300 - 500 MΩ. Cells fired action potentials of 90 mV spike amplitude, and the firing rate increased with depolarization. This indicated that the cells remained electrically excitable, with high input resistance presumably resulting from shortened neurites and GΩ seal recording conditions. In whole-cell voltage clamp, step depolarization from -90 mV elicited fast inward and delayed outward currents. Total inward current had a threshold around -60 mV and an amplitude of at least 3 nA at -50 mV. Total outward current had a threshold around -45 mV and an amplitude of 1 - 4 nA at +30 mV.

Single channel recording was performed in the cell-attached configuration. Whereas few spontaneous channel openings were observed when the pipette contained a solution similar to the extracellular bath, several distinct types of channels were expressed when the electrode contained the high KCl patch pipette solution described above.

The striatum is a major postsynaptic target of dopaminergic neurons in mammalian brain. We hope to utilize this preparation to study the effects of dopamine at the single channel level, so as to better understand the actions of this neurotransmitter as well as the actions of neuroleptic drugs.

23.13 INHIBITION BY DOPAMINE OF BASAL INOSITOL PHOSPHATE FORMATION IN RAT STRIATAL SLICES. Pizzi,M.*, Da Prada,M.*, Spano, P.F., and Haefely W.E*(SPON: M.Motta). Inst. Pharm. Exp. Ther. School of Medicine Brescia Univ., Italy. Dept.Pharm. Res. Hoffmann - La Roche , Basle Switzerland.

Dopamine (DA) has been shown to act in the nervous system through the stimulation of specific receptors, designated as D-1 and D-2 , which stimulate and inhibit adenylate cyclase respectively.
However many of cerebral dopamine functions seem to be uncoupled to any modification of cyclic AMP neuronal content. For other neurotransmitter such as Ach, NE, 5-HT ,it has been demonstrated that receptor activation could stimulate polyphosphoinositide (PPI) breackdown with the formation of diacylglicerol inositol monophosphate (InsP), inositol bisphosphate ($InsP_2$) and inositol trisphosphate ($InsP_3$). The aim of our study was to identify whether DA too might affect the PPI turnover.
Rat striatal slices ,after a 20 min preincubation in a Krebs-Ringer buffer (KRb) at 37° C under an atmosphere of O_2/CO_2, were labelled wuth myo-(3H)inositol (1.35 uM) for 2 hours. Labelled slices were rinsed and than incubated in a KRb in the presence of 10 mM Li^+, 5 uM scopolamine (muscarinic antagonist), 5 uM kynurenic acid (glutamate antagonist) and test drugs (final volume 500 ul). The reaction was stopped after 20 min by 2min boiling , and by adding 750 ul $HClO_4$ 0.4 N. Inositol polyphosphates were separeted by ion exchange chromatography. DA dose-dependently decreased the level of $InsP_3$ and $InsP_2$ with an IC_{50} of about 180 nM. DA effect has been shown to be time-dependent. It reached its maximal value at 20 min incubation (over 50 % decrease). Dopamine effect was more potently reproduced by the D-2 selective agonists RU 24213 (IC_{50} nM), quinpirole (IC_{50} 10 nM) and lisuride (IC_{50} 10 nM). On the contrary fenoldopam which selectively stimulates the D-1 receptor , did not affect the inositol phosphate content even at hight concentrations (up to 1uM). Norpropylapomorphine (NPA), effect tested at 1 uM concentration was prevented by the D-2 receptor antagonist domperidone and by haloperidol (both 5 uM), but not by the D-1 antagonist SCH 23390 (100 nM). In summary our results indicate that DA affects the PPI turnover in brain as revealed by the decrease of inositol polyphosphate content. This phenomenon is linked to the activation of a D-2 type receptor , and is probably independent of any modification of endgenous Ach/glutamate release.

23.14 EFFECTS OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANTS ON 5-HYDROXY TRYPTAMINE UPTAKE AND PAROXETINE BINDING. Y. Minatogawa*, Y. Watanabe*, A. Moriya*, T. Higuchi, Y. Igarashi*, T. Yamauchi* and M. Mikuni°. Dept. of Psychiatry, Saitama Medical School, Moroyama, Saitama, 350-04 Japan, °Div. of Mental Disorder Research, Natl. Institute of Neuroscience, Natl. Center of Neurology and Psychiatry, Kodaira, Tokyo, 187 Japan.

It is well known that acute administration of antidepressant(AD) inhibits the uptake of 5-hydroxytryptamine(5-HT) at presynaptic membranes. However, there were few reports on the chronic effects of AD on 5-HT uptake sites. Recently, high affinity[^{3}H]paroxetine binding sites have been demonstrated in mammarian brain and these binding sites are considered to be associated with 5-HT uptake transporter complex. However, there is no report on the chronic effect of AD on [^{3}H]paroxetine binding sites.

To investigate the effects of chronic AD administration on 5-HT uptake sites and [^{3}H]paroxetine binding sites, following experiments were carried out. Rats were administered (i.p.) either chlorimipramin (CMI, 10mg/kg), desmethylimipramin (DMI, 10mg/kg) or mianserin (MIA, 5mg/kg) for 2 weeks. For kinetic studies, cerebral cortex was homogenized with 100 vol. of Krebs-Ringer bicarbonate (KRB,pH 7.4) and after centrifugation, the pellet was resuspended with 50 vol. of KRB. The suspension was incubated with 5-HT ($1x10^{-8}$ ~$4x10^{-7}$M) containing[^{3}H]5-HT($1x10^{-8}$M) for 2 min at 37°C. The data obtained were analyzed by Lineweaver-Burk method. For uptake inhibition studies, the crude membrane suspension was incubated with CMI ($1x10^{-4}$~$1x10^{-10}$M) and[^{3}H]5HT ($5x10^{-8}$M). The data obtained were analyzed by conventional graphical techniques. Specific 5-HT uptake was defined as the difference between the uptake value obtained at 37°C and that at 0°C. Paroxetine binding studies were performed by incubating the crude membrane (4 mg/tube) with[^{3}H] paroxetine ($0.2x10^{-9}$~$5x10^{-9}$M) for 60 min at 25°C in Tris-HCl buffer-solution (pH 7.4) containing 120 mM NaCl and 5 mM KCl. Specific binding was defined as the difference between the binding in the presence and absence of fluoxetine ($1x10^{-5}$M).

In kinetic study of 5-HT uptake, the Km and Vmax values of control rats were 0.15μM, 4.98pmole/mg protein·min, respectively. No changes were observed on the Km values following CMI, DMI or MIA chronic administration. Only chronic MIA administration induced slight decrease of the Vmax value and increase of the Ki value. Scatchard analysis of[^{3}H]paroxetine binding revealed a Kd of 41.4 pM and the Bmax of 32.0 pmoles/g tissue, and no obvious changes were observed in chronic AD administration.

These data suggest that chronic AD administration except MIA may induce no adaptive change in 5-HT uptake transporter complex.

24.1 ALTERATIONS OF THE CENTRAL ADRENERGIC SYSTEM IN CHRONIC MPTP-INDUCED MONKEY PARKINSONISM. R. Miyoshi, S. Kito, M. Shimizu*, and Y. Yamamura. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

In 1983, Langston described 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated human parkinsonism, and it was discovered that MPTP was selectively toxic to the dopamine neurons of the substantia nigra. Recent studies have shown that not only the nigro-striatal dopamine but also locus coeruleus noradrenaline neuronal systems are susceptible. In the present paper, the authors examined alterations of the central adrenergic system in chronic MPTP-induced monkey parkinsonism. Adult Japanese monkeys were used in this experiment. MPTP was administrated intravenously to monkeys in doses of 0.16 to 0.27 mg/kg body weight/shot repeatedly. Animals were feeded for 6 to 18 months. After deep anesthesia, brains were rapidly removed and dissected into hemispheres. One hemisphere was used for conventional neuropathological observations and the other one for receptor binding assay of adrenergic receptors. The frontal, parietal, temporal and occipital cortices and cerebellum were dissected and P_2 fractions were prepared. For observation of α_1 and α_2 adrenoceptors, ^{3}H-WB 4101 and ^{3}H-clonidine were used as radioactive ligand, respectively. In the substantia nigra of all MPTP-induced monkey parkinsonism, nerve cells were remarkably reduced and those surviving were atrophic as previously reported. In addition, loss of nerve cells and gliosis were considerably observed in the locus coeruleus, though not so severely affected as in the substantia nigra. There were no notable changes in other brain areas. In the brain of these parkinsonian monkeys, ^{3}H-clonidine binding sites were significantly decreased in the temporal and parietal cortices and also had a tendency to decrease in other cortex areas. As for ^{3}H-WB 4101 binding sites, they were reduced as half as normal controls in the temporal and parietal cortices and tended to decrease in other areas. Saturation experiments revealed that the decrease of both ^{3}H-clonidine and ^{3}H-WB 4101 binidng sites was due to a reduction of maximal receptor number. Above-mentioned results strongly suggest that the adrenergic neuron system is damaged in addition to the dopaminergic one in the brain of MPTP-induced monkey parkinsonism.

24.2 DA RECEPTOR BINDING IN MPTP TREATED MONKEYS: CORRELATION WITH NEUROCHEMISTRY AND BEHAVIOR . K.A. Steece, M.W. Tayrien*, T.J. Collier, R. Loy, R.H. Roth[1], D.E. Redmond,Jr.[2], & J.R. Sladek, Jr.. Dept. of Neurobiol. & Anat., Univ. of Rochester School of Medicine, Rochester, NY, and Depts. of Pharmacology[1] and Psychiatry[2], Yale Univ. School of Medicine, New Haven, CT.

The degeneration of the nigrostriatal DA system associated with Parkinson's disease reveals a complex regulation of D2 DA receptors. Intially there is an apparent compensatory increase in the number of receptors. In contrast, the more disabled patients who no longer respond to levo-dopa therapy have a low number of striatal D2 receptors. Both an increase (Joyce *et al.*, 1986, Brain Res 382:360) and a decrease (Hantraye *et al.*, 1986, Life Sci 39:1375) in D2 receptor number have been reported in MPTP-induced parkinsonian monkeys.

Morphological and neurochemical studies in our laboratories with MPTP treated monkeys have confirmed the destruction of nigrostriatal neurons by this neurotoxin. These data have been correlated with the behavioral status of individual monkeys. It was thus of interest to examine D2 receptor status in monkeys with various degrees of experimental parkinsonism.

Ongoing experiments in our laboratories make analysis of receptor changes in 4% paraformaldehyde perfused tissue imperative. Therefore, it was neccessary to determine whether striatal D2 receptors were still viable using this fixation protocol. Biochemical experiments were performed to determine binding characteristics of sulpiride displacement of 3H-spiperone to slide mounted striatal tissue sections in 0.1% (control) and 4% paraformaldehyde perfused rats. Equilibration time was 60 minutes in control tissue and 120 minutes in 4% tissue. This may be attributable to a delay in penetration rate of the ligand into the tissue related to an increase in cross-linking associated with the stronger degree of fixation in the 4% animals. A maximum ratio of specific to non-specific binding (3:2) was obtained for both preparations with two 15 minute washes in cold buffer. Scatchard analysis revealed no difference in affinity between 0.1% (K_D=80$\pm$12pM($\pm$SEM)) and 4% (K_D=73$\pm$11pM) fixed tissue. These data indicate a slight decrease in receptor density from 0.1% (B_{MAX}= 50$\pm$1 fmol/mg tissue) to 4% (B_{MAX}=38$\pm$5) fixation. These initial studies in perfused rat brain indicate our ability to assess changes in a population of receptors that satisfy many of the criteria which have been described for D2 receptor populations in other more extensively characterized systems.

Binding studies in 3 control African green monkeys indicate D2 receptors in this system also survive the 4% fixation protocol. Preliminary results in 2 MPTP treated monkeys revealed a marked decrease in specific D2 receptor binding in these severely parkinsonian monkeys compared to intact animals. Autoradiography is currently being examined in several MPTP treated monkeys to investigate whether various receptor changes might be regionally specific as well as correlated to the degree of experimental parkinsonism. This modification of the approach for receptor binding analysis as performed in concert with ongoing behavioral, neurochemical and immunohistochemical analyses will permit application of this technique to transplant recipients to better correlate improvements seen following fetal nerve cell grafts in parkinsonian monkeys.

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24.3 BIOTRANSFORMATION OF MPTP GENERATES SUPEROXIDE RADICAL IN VITRO. Z.L. Rossetti*, A. Sotgiu, D.E. Sharp, M. Hadjiconstantinou and N.H. Neff. Department of Pharmacology, The Ohio State University, College of Medicine, Columbus, OH 43210.

The chemical mechanism(s) for the destruction of neurons by MPTP is still unknown. It has been suggested that during the biotransformation of MPTP cytotoxic intermediates are formed. Recently, the formation of oxygen free radical intermediates has been detected from the enzymatic reduction of the oxidation product of MPTP, 1-methyl-4-phenyl-pyridinium ion (MPP^+). We report now that incubation of MPTP with a mouse brain mitochondrial preparation results in oxygen-dependent formation of free radicals, which can be trapped and observed by electron spin resonance (ESR). The spin adducts with 1,1-dimethylpyrrolidine-N-oxide (DMPO) of hydroxyl radical and of an unidentified carbon-based radical were observed. Radical formation could be suppressed by superoxide dismutase (SOD), indicating the production of superoxide radical. Pretreatment of animals with deprenyl, a monoamine oxidase (MAO) inhibitor, before the preparation of mitochondria or the inclusion of deprenyl in a mitochondrial preparation from control animals inhibited the appearance of the ESR signal, suggesting that free radical generation is MAO dependent. In the absence of mitochondria, an ESR spectrum was obtained when 1-methyl-4-phenyl-2,3-dihydropyridinium perchlorate ($MPTP^+$, the intermediate metabolite of MPTP) and MPP^+ were incubated together, but not when they were incubated independently. We conclude from these observation that MPP^+ causes the isomerization of $MPDP^+$ to 1,2 or 1,4 isomers which are oxidized to MPP^+ with the formation of superoxide. ^{13}C NMR studies of the reaction mixtures support this explanation.It is possible that generation of free radicals contributes to MPTP toxicity in vivo.

24.4 SYNTHESIS AND BIOLOGICAL ACTIVITY OF METHYL SUBSTITUTED ANALOGS OF THE NEUROTOXIN MPTP AND ITS METABOLITES $MPDP^+$ AND MPP^+ E. A. Johnson, A. Trevor, and N. Castagnoli Jr. (SPON: J. Lavail) Division of Toxicology, University of California, San Francisco, CA 94143-0446

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has recently been shown to cause selective destruction of the substantia nigra of humans and non human primates, resulting in a Parkinsonian syndrome which is clinically indistinguishable from idiopathic Parkinsons disease. The neurotoxic effects of MPTP are dependent upon its bioactivation by monoamine oxidase (MAO) to produce the two electron oxidation product $MPDP^+$. This unstable metabolite is rapidly further oxidized to the stable pyridinium species MPP^+. The neurotoxicity of MPTP is currently ascribed to the action of one or both of these metabolites. The possibility that idiopathic Parkinsons disease might result from exposure to an environmental toxin similar to MPTP has been suggested. Such a toxin may also require activation by MAO. Elucidation of the structure activity relationship of substituted tetrahydropyridines as substrates of MAO is an important step in the process of elimination of potential candidate neurotoxins. Therefore, we undertook the synthesis and biological evaluation of methyl substituted analogs of the tetrahydropyridine and its two metabolites. Compound **1**, 1,2-dimethyl-4-phenyl-1,2,3,6-tetrahydropyridine, and the corresponding 1,2-dimethyl-4-phenylpyrdinium iodide (**2**) were obtained via disproportionation of 1,2-dimethyl-4-phenyl-1,2-dihydropyridine. Compound **3**, 1,6-dimethyl-4-phenyl-1,2,3,6-tetrahydropyridine was obtained via a Grignard reaction of MeMgBr on 1-methyl-6-cyano-4-phenyl-1,2,3,6-tetrahydropyridine. The methyl substituted $MPDP^+$ analog, **4**, (1,2-dimethyl-4-phenyl-2,3-dihydropyridinium perchlorate), was obtained via a Grignard reaction of MeMgBr on MPP+. The interactions of compounds **1** and **3** with MAO and their striatal dopamine depleting properties in the mouse have been examined. The oxidation of **1** and **3** proceeded extremely slowly. Compound **1**, however, competitively inhibited the MAO B catalysed oxidation of MPTP (Ki .378 mM) suggesting that it does have access to the active site of the enzyme. IV administration of **1** to C57 black mice caused only 8.4% depletion of straital dopamine compared to an 81% depletion with MPTP. On the other hand, the pyridinium compound **2**, a potential metabolite of **1** and **3**, was as effective as MPP^+ in inhibiting mitochondrial respiration and even more toxic than MPP^+ to freshly prepared hepatocytes. It appears, therefore, that although the structural requirements of MPTP type MAO substrates may be severe, the corresponding requirements for molecules displaying toxic properties related to the metabolite MPP+ may be less restricive.

24.5 TOXICITY OF COMPOUNDS RELATED TO MPP+ FOR DOPAMINERGIC NEURONS IN CULTURE. P.P. Michel*, J. Sanchez-Ramos*, B. Pressman*, S. Efange* and F. Hefti (SPON:J.D. Potter). Dept. of Neurology, University of Miami, Miami FL 33101.

MPTP is a selective neurotoxin for dopaminergic neurons. Several reports indicate that the selectivity of the action of MPTP is due to the uptake of its metabolite, MPP+, into the dopaminergic neurons. After uptake, MPP+ is believed to inhibit mitochondrial energy metabolism resulting in cell death. We therefore hypothesized that other compounds satisfying these two criteria (selective uptake and mitochondrial toxicity) are selective toxins for dopaminergic neurons. We previously reported that MPP+ selectively destroys dopaminergic neurons in cultures of dissociated cells from the rat ventral mesencephalon. This culture system was used to test for the effects of potential toxins.

Cultures were prepared from rat embryos (E15) as described earlier (Sanchez-Ramos et al., Neurosci. Lett. 72:215,1986). They were grown for 7 days and then exposed to test compounds for 24 h. Thereafter, they were analyzed for selective and non-selective toxicity. Selective toxicity for dopaminergic neurons was assessed by staining the cultures for tyrosine hydroxylase (TH) and counting the number of positively stained neurons. Non-specific toxicity was assessed by counting cells visible in phase contrast microscopy.

We tested different guanidine derivatives which are ionized cations at physiological pH. The alkylguanidines (C=4,5,7 or 8), previously described as electron transport inhibitors, induced a non-specific degeneration of all the cultured cells. The effective concentration 50 (EC50) decreased with increasing length of the alkyl side chain and ranged from 45.8 to 1.9 uM. Under the same conditions, MPP+ selectively destroyed TH-positive cells with an EC50 of 0.3 uM. Benzyl guanidine and guanethidine produced only non-specific toxic effects ressembling those of alkylguanidines. The EC50 of benzyl guanidine was 20.9 uM. Guanethidine which destroys peripheral catecholaminergic neurons in vivo, was least effective (EC50 64.3uM). Compounds under investigation include homo-MPP+, isoquinolinium, and triazines.

Since human Parkinson's disease is associated with a selective degeneration of dopaminergic neurons, these studies might lead to the identification of novel compounds possibly involved in the its pathogenesis.

24.6 **IN VITRO EFFECT OF CYPERQUAT (MPP^+), PARAQUAT AND DIQUAT ON ^{45}Ca TRANSPORT IN MITOCHONDRIA FROM RAT STRIATUM AND LIVER.**

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1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) is a neurotoxic compound, which produces parkinsonism in man and several animal species. MPTP is metabolized to MPP^+ by the mitochondrial enzyme monoamine oxidase. Studies from our laboratory (Abstracts Society for Neuroscience #350.4, 1985 and #28.7, 1986) and those from other groups have shown that MPTP per se does not affect energy production in mitochondria. However, MPP^+ inhibits the NAD^+-linked substrate oxidation in mitochondria from various sources. Since calcium plays an important role in the release of neurotransmitters and since mitochindria have a cardinal role in the regulation of intracellular calcium we have evaluated the effects of MPP^+ and two structurally related herbicides paraquat and diquat on calcium transport in mitochondria from rat striatum and liver.

Mitochondria were isolated at 0-4°C, from neostriatum (pooled from at least four animals) and liver of adult male Sprague-Dawley rats. The homogenizing medium consisted of 0.23M mannitol, 0.07M sucrose, 0.1mM EDTA and bovine serum albumin at pH 7.4. The homogenates were centrifuged at 2,000g for 10 min to remove debris. The supernatants were centrifuged at 10,000g for 10 min to obtain mitochondrial pellets. Calcium transport was studied by incubating the organelles in the presence of 0.1mM $^{45}CaCl_2$, 0.23M mannitol, 0.07M sucrose, 5mM K_2HPO_4. Tris-HCl pH 7.4, plus pyruvate (5mM) - malate (1mM) as substrate at 30°C, in the presence or absence of 5mM $MgCl_2$ + 30μM ADP. The organelles were preincubated with MPP^+ (0.5mM), paraquat (5mM) and diquat (5mM) for 3 min. The uptake was started by adding ^{45}Ca and stopped by filtering the mixture through GF/B glass microfiber filters.

In the absence of Mg.ADP, mitochondria from striatum exhibited a linear uptake of Ca over 10 min and this was inhibited by MPP^+, paraquat and diquat. Liver organelles under this condition showed a small and non-linear accumulation of ^{45}Ca. Addition of Mg.ADP markedly increased Ca accumulation in striatal and liver preparations. In striatal preparations, in the presence of Mg.ADP, the inhibition of Ca uptake by MPP^+, paraquat and diquat was attenuated. In liver mitochondria diquat exhibited a marked inhibition of Ca uptake, whereas cyperquat and paraquat showed only marginal inhibition. These findings indicate that cyperquat and related compounds can perturb mitochondrial transport.

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24.7 MPTP INDUCES DEGENERATION IN NUCLEUS SAGULUM, THALAMUS, DORSAL RAPHE, AND OF NON-CORTICAL PROJECTING DOPAMINERGIC BRAINSTEM NEURONS. R.C. Switzer and S.K. Campbell*, University of Tennessee, Depts. Pathology and Medical Biology, Knoxville, TN 37920

For an MPTP-treated animal to be a valid model of Parkinson's disease in humans, the populations of neurons induced to degenerate by MPTP in an animal must be congruent with those observed to be affected in Parkinsonism. To detect and characterize the population of degenerating neurons in MPTP-treated C57B6 mice, we applied the degeneration-sensitive cupric-silver stain of de Olmos. Previously (Neurosci. Abst. 1985), we had shown that within 48-72 hours after MPTP was administered, degeneration of neurons occurred in the substantia nigra (SN) and ventral tegmental area (VTA) in cell groups A8,9, and 10. In addition to these populations, we have also found degenerating neurons in the brainstem within the nucleus sagulum, in the rostral dorsal raphe nucleus, and in the lateral part of the medial thalamic nucleus (the region embraced by the interal medullary lamina of the thalamus). These three areas have far fewer neurons affected than in SN and VTA. The nucleus sagulum is a little known cell group situated laterally near the surface of the brainstem just caudal to the lateral lemniscus and dorsal to the middle cerebellar peduncle. A first impression is that these neurons are not part of the catecholamine population but part of the distribution of the noradrenergic A7 cell group which, as found in the rat, seems to overlap the location of the nucleus sagulum. A localization of dopamine beta hydroxylase in the C57 mouse is necessary to resolve this issue. The few but nevertheless distinct degenerating thalamic neurons do not appear to be part of any catecholaminergic cell groups. The raphe neurons affected are restricted to the rostral limits of this system at a coronal level that includes the oculomotor nucleus.

Although the number of degenerating neurons revealed by the cupric-silver stain is quite spectacular, it is clear that not all of the neurons are affected. Since the areas to which the components of the cell groups A8, 9, and 10 project are well established, we can infer which subsets of neurons are not affected by examining the areas to which the axons project for degenerating synaptic terminals. The striatum, nucleus accumbens, and ventral striatum exhibit dense terminal degeneration while we found lesser densities of terminals in the habenula. None of the cortical areas that receive dopamine input displayed any degeneration. This feature could be an important clue in determining why only some of the dopamine neurons in the CNS are affected by MPTP.

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24.8 FLUOXETINE PRETREATMENT ATTENUATES THE MPTP-INDUCED DECREASE IN DIHYDROXYPHENYLACETIC ACID (DOPAC). W.J Brooks* and G.C. Wagner. Psychology Dept., Rutgers Univ. New Brunswick, NJ 08903

In order for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to exert neurotoxic effects on dopaminergic neurons, it must first be metabolized to 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase 'B' (MAO-B). Since MAO-B is located in serotonergic neurons and astrocytes, the conversion of MPTP to MPP+ must take place outside the dopaminergic neuron. It was previously demonstrated that pretreatment with a serotonin transport inhibitor, fluoxetine, protected dopaminergic neurons against the toxic action of MPTP. In addition to its action as a neurotoxin, MPTP has also been shown to acutely inhibit monoamine oxidase, thereby causing a decrease in the concentration of DOPAC in the striatum. The following study was conducted in order to determine if fluoxetine pretreatment would attenuate the MPTP-induced decrease in DOPAC.

Adult male Swiss-Webster mice were fluoxetine (10 mg/kg, IP) 16-h prior to MPTP (10 mg/kg, IP) or with an equal volume of saline 16-h prior to the MPTP. Control mice received fluoxetine 16-h prior to a second injection of saline or two injections of saline. All mice were sacrificed 2 h after the second injection, brains removed, striatum dissected and assayed for dopamine, DOPAC and serotonin by HPLC.

It was observed that control mice (saline/saline) had 13.04 ug dopamine/g tissue (± .87), 1.99 ug DOPAC/g tissue (± .15) and 0.77 ug serotonin/g tissue (± .16). Fluoxetine alone produced no significant change in any of these values when administered 18 h prior to sacrifice. MPTP alone caused no significant change in dopamine (11.16 ug/g ± .74) or serotonin (0.71 ± .05) levels but produced a significant decrease in DOPAC (0.87 ± .10) levels. Fluoxetine, administered as a pretreatment to MPTP, produced no significant change in the concentration of dopamine (12.36 ug/g ± .62) or serontonin (1.06 ug/g ± .18) but significantly reversed the MPTP-induced decrease in DOPAC (1.31 ug/g ± .08).

These data indicate that the decrease in the concentration of DOPAC caused by MPTP is occurring outside the dopaminergic neuron as a result of the inhibition of monoamine oxidase in serotonergic neurons or astrocytes.

24.9 AGE-DEPENDENT EFFECTS OF MPTP: CORRELATION WITH MAO-B ACTIVITY. S.L. Walsh, S. Auerbach and G.C. Wagner. Depts. of Psychology and Biology, Rutgers University, New Brunswick, NJ 08903.

Systemic administration of MPTP, a potent neurotoxin, results in selective destruction of the dopaminergic neurons in humans, subhuman primates and rodents. It has been demonstrated that this toxin induces a permanent parkinsonian-like syndrome in humans and subhuman primates. These toxic actions are, in part, dependent upon MPTP's conversion to MPP^+ by monoamine oxidase B (MAO-B), and the subsequent uptake of MPP^+ by the dopaminergic uptake pump. Pretreatment with the MAO inhibitors, pargyline and deprenyl protect against the toxic effects of MPTP in vivo. Since MAO activity increases significantly as aging occurs in humans, subhuman primates and rodents, there may be an enhanced sensitivity to the toxic effects of MPTP in developing animals. In addition, it would be predicted that this increased sensitivity to MPTP would correlate with the increasing MAO activity.

Swiss-Webster male mice aged 1, 2, 4, 8, and 16 weeks and 1 year of age were treated with four injections (subcutaneous) of 12.5 mg/kg of MPTP hydrochloride with two hours between each injection. For each age group, one group of controls were treated with saline vehicle under the same injection regimen, and a second group of controls were untreated and sacrificed at the age of treatment for the first two groups. One week after treatment, the mice were sacrificed, brains dissected and divided into right and left halves. For all three groups, the right brain halves were assayed for monamine oxidase B activity using a colorimetric assay with benzylamine as the substrate. The left halves were assayed for dopamine and serotonin content utilizing high performance liquid chromatography.

It was observed that there was a significant age-dependent increase in MAO-B activity. There was also a significant age-dependent effect of MPTP toxicity on dopamine levels, with the youngest age points showing no depletions and the one year-old mice suffering a 68% depletion of whole brain dopamine. No significant effect was observed on serotonin levels. The age-dependent increase in MAO-B activity was highly correlated with the extent of the lesion incurred at various age points. It was also observed that MPTP administration resulted in a significant increase in MAO-B activity in comparison to controls one week after treatment, with the youngest age groups exhibiting a greater increase in activity than older animals.

24.10 THE AGE-RELATED ENHANCEMENT OF MPTP TOXICICITY: TOXICOKINETIC VERSUS TOXICODYNAMIC FACTORS. I. Irwin*, G.A. Ricaurte*, L.E. DeLanney, J.W. Langston. (SPON: J.W. Tetrud) Inst. Med. Res., San Jose, CA; Stanford Univ. Sch. of Med., Stanford, CA.

Recent observations indicating that the neurotoxic effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) increase with age suggest that this neurotoxin may provide a useful tool for the study of the relationship between aging and Parkinson's disease (Lewin, 1985; Langston, 1987). To clarify the relative contribution of toxicokinetic factors (changes in biodisposition of MPTP or its putative toxic metabolite MPP+) vs toxicodynamic factors (changes in the sensitivity of neurons with age) several experiments were done. The first of these was designed to determine (1) if MPP+ is further converted to other metabolites in the CNS and (2) if the elimination of MPP+ from the CNS changes with age. To circumvent questions relating to the alterations in the peripheral biodisposition of MPTP and MPP+ with age, MPP+ was given directly into the central nervous system (CNS) via the intracerebroventricular (ICV) route. C57Bl/6 mice of two different ages (6 wk vs 8 mos old) were compared in all experiments. ^{3}H-labelled MPP+ (20 μg) was administered ICV to mice from each age group (n=30/group). Five animals from each group were killed 2, 4, and 8 hrs later, their brains removed, and the total radioactivity measured using liquid scintillation counting. By comparing these results with concentrations determined by GC/MS, virtually all of the radioactivity could be accounted for as MPP+. Thus, it appears that MPP+ is not further metabolized in brain. MPP+ was eliminated from the brain in a time-dependent manner in both young and older animals, but no significant differences were detected between the two age groups (MPP+ values [μg/g] for young vs old were: 2 hr - 5.6 ± 0.5 vs 5.5 ± 0.9; 4 hr - 3.5 ± 0.3 vs 3.4 ± 0.4; 8 hr - 1.5 ± 0.3 vs 1.2 ± 0.1).

Since no differences in the CNS biotransformation or elimination of MPP+ between the two groups were found, we could now directly assess the relative sensitivity of the two age groups to the DA-depleting effects of MPP+ by administering it directly to the brain via the IVC route. MPP+ (10 or 20 ug) was administered to each group (n=5/dosage group). Animals were killed after 5 days and striatal DA measured. DA depletions (μg/g) were equivalent in old vs young (Control - 11.3 ± 0.2 vs 11.1 ± 0.4; 10 μg - 11.4 ± 0.5 vs 10.5 ± 0.51; 20 μg - 7.3 ± 1.7 vs 8.1 ± 2.1). Hence, there appears to be no difference in sensitivity between the two age groups studied in regard to the DA-depleting effects of MPP+. These results suggest that toxicokinetic factors may underly the age-related increase in the striatal dopamine-depleting effects of MPTP.

24.11 THE EFFECT OF DIETHYLDITHIOCARBAMATE (DDC) ON THE BIODISPOSITION OF MPTP: AN EXPLANATION FOR ENHANCED NEUROTOXICITY. E. Y. Wu*, I. Irwin*, L. S., Forno, L. E. DeLanney, A. Trevor*, J. W. Langston. Univ. Calif., San Francisco, CA; Inst. Med. Res., San Jose, CA; Vet. Adm. Med. Ctr., Palo Alto, CA.

DDC has been reported to exacerbate the dopamine-depleting effects of MPTP in mice (Corsini et al, 1985). We have confirmed these observations in 6 to 8 wk old C57Bl/6 mice. Two animals given 400 mg/kg of DDC 1/2 hr prior to a 30 mg/kg IP dose of MPTP and studied 10 days later showed severe neuronal cell loss in the substantia nigra (SN), accompanied by glial-microglial reactions. Lower doses of MPTP (10 or 20 mg/kg) in combination with DDC produced definite but less severe cell loss. In contrast, when mice were given MPTP alone (30 mg/kg/dose IP) (even when repeated on a daily basis for up to 10 days) or DDC alone (400 mg/kg), no SN cell loss was seen. Histologically, these changes are quite similar to those seen in primates after MPTP administration.

To determine the mechanism underlying the DDC-induced exacerbation of MPTP-induced neurotoxicity, groups of animals (n=15/group) were given either MPTP (30 mg/kg) alone, DDC (400 mg/kg) alone, or DDC (400 mg/kg) 1/2 hour prior to MPTP (30 mg/kg). Mice were killed after 0.5, 1.0 and 2.0 hr, and striatum (STR), ventral mesencephalon (VME), and frontal cortex (FC) analyzed for MPTP and MPP+ using GC/MS. MPTP (detected only at 30 min) was much higher in mice given DDC + MPTP vs MPTP alone (STR - 4.7 ± 0.6 vs. 1.9 ± 0.2; VME - 1.4 ± 0.1 vs. 4.3 ± 0.7 vs. 1.4 ± 0.1; FC - 5.5 ± 0.6 vs 1.6 ± 0.3 μg/g). Pre-administered DDC also increased MPP+ concentrations in these regions (STR - 11.3 ± 0.2 vs 10.6 ± 0.7; VME - 9.1 ± 1.1 vs 6.6 ± 0.4; FC - 12.7 ± 1.0 vs 7.9 ± 0.5 μg/g). The molecular mechanisms underlying this effect were further investigated in vitro. In a partially-purified MAO B preparation, DDC enhanced the biotransformation of MPTP in a concentration-dependent manner. Bovine liver MAO B (6-8 mU) incubated with 3.3 mM MPTP produced MPDP+ at a rate of 38.7 ± 4.0 nmole/30 min. The addition of DDC (100 or 500 μM) significantly increased this rate to 52.6 ± 5.7, and 58 ± 3.3 nmole/30 minutes respectively. DDC (12.5, 25, 100, or 500 μM) also enhanced MPP+ production (19.9, 24.6, 33.6, 53.5% respectively) in mouse brain homogenates incubated with MPTP (100 μM).

These results suggest that DDC enhances MPTP-induced neurotoxicity by increasing brain concentrations of MPP+. Factors contributing to this increase appear to include greater delivery of MPTP to the central nervous system, increased MAO-mediated biotransformation of MPTP to MPP+, and possibly reduced elimination of MPP+ from the brain.

24.12 EFFECTS OF THE COMBINED TREATMENT OF DIETHYLDITHIOCARBAMATE AND MPTP ON BEHAVIOR IN THE RODENT. D.M. Yurek, A.Y. Deutch[1], R.H. Roth[1], and J.R. Sladek, Jr. Dept. of Neurobiology and Anatomy, Univ. of Rochester Medical School, Rochester, NY 14642 and [1]Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510.

The dopamine-depleting effects of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are enhanced when administered in combination with diethyldithiocarbamate (DDC) in mice (Corsini *et al.*, *Eur. J. Pharmacol.*, 119:127-128). This study examined the behavioral correlates associated with this treatment. Male C57 BL mice were pretreated with 400 mg/kg (i.p.) of DDC one-half hour prior to the administration of either vehicle, 15.0, 22.5, 26.5, or 30.0 mg/kg (i.p.) of MPTP. The same treatment was repeated twelve hours later. Initially, weight loss was observed for the two highest doses of MPTP-treated mice and maximal weight loss occurred at 3 and 5 days after the treatment. Mice with >15% weight loss displayed dorsal spinal flexion and were hyper-responsive to tactile stimuli. However, 14 days following MPTP treatment we observed that body weights returned to pre-MPTP levels for all doses, although postural abnormalities were still evident.

The effects of the combined treatment of DDC and MPTP on diurnal spontaneous locomotor activity varied. Locomotor activity was assessed by monitoring the number of infrared light beam interruptions/hour that occurred during either the 'light' or 'dark' phase of the animal's diurnal cycle. Pre-MPTP activity monitoring determined that dark phase activity was 15-fold greater than light phase activity. At 3 and 5 days post-MPTP, dark phase activity was found to be significantly lower than pre-MPTP dark activity for the two highest MPTP doses. Conversely, during this same post-MPTP period, light phase activity was found to be greater than pre-MPTP light phase activity for the same MPTP doses. These data suggest that the combined treatment of DDC and MPTP (1) attenuates locomotor activity associated with the active phase of the diurnal cycle and (2) activates mice during the inactive phase of their diurnal cycle.

One week after the MPTP administration these same mice were treated with either vehicle, 0.2, or 2.0 mg/kg (i.p.) apomorphine. The low dose of apomorphine (0.2) enhanced locomotor activity for animals treated with two highest doses of MPTP. Mice treated with the two highest doses of MPTP and administered 2.0 mg/kg (i.p.) apomorphine exhibited enhanced stereotypic behavior. These data most likely reflect post synaptic receptor supersensitization.

Behavioral correlates for MPTP-induced dopamine depletion exist 1 week following MPTP treatment. However, the recovery of feeding behavior, as expressed by body weight changes, which occurred after the first week is indicative of compensatory changes in dopamine function. Furthermore, the hyper-responsivity associated with mice treated with the two highest doses of MPTP may indicate that lateral caudate innervation may have been spared, thus we may be observing the behavioral effects associated with an incomplete dopaminergic lesion.

These data will also be discussed in relation to changes in striatal dopamine content and nigral tyrosine hydroxylase levels. This research was supported by T32 MH 18260 (DMY) and AG 00847 (JRS).

24.13 EFFECTS OF DIETHYLDITHIOCARBAMIC ACID ON THE DISTRIBUTION AND TOXICITY OF 1-METHYL-4-PHENYLPYRIDINIUM IN C57/BL MICE. D.B. Miller, J.F. Reinhard[1] and J.P. O'Callaghan. U.S. EPA, RTP, NC 27711 and [1]The Wellcome Research Laboratories, Burroughs Wellcome Co., RTP, NC 27709

Diethyldithiocarbamic acid (DDC) potentiates the toxicity of the dopaminergic neurotoxicant 1-methyl-4-phenyl-1 2,3,6-tetrahydropyridine (MPTP) based on increased lethality, enhanced catecholamine depletions and elevated glial fibrillary acidic protein (GFAP) levels in striatum and hippocampus as well as more pronounced motor dysfunction (Miller et al. Toxicologist 7:154, 1986). Because DDC and related dithiocarbamates can cause a redistribution of quartenary compounds (O'Callaghan and Miller, Br. Research 370:354, 1986) it may effect this increase in toxicity by a redistribution of 1-methyl-4-phenylpyridinium (MPP^+) the toxic metabolite of MPTP or it may increase the toxicity of MPP^+ itself. Here we characterize the effects in the C57/Bl mouse of DDC on the distribution and toxicity of MPP^+. Tritiated MPP^+ was used to determine the distribution of MPP^+ in brain, heart and liver following DDC. Brain and heart catecholamine levels were determined by liquid chromatograph and electrochemical detection. RIA of the astrocyte-localized protein GFAP was used to assess possible CNS injury following DDC/MPP^+.

Mice were given (n=6) s.c. sal or DDC (400 mg/kg) followed 0.5 hr later by an i.p. injection of tritiated MPP^+ and unlabeled MPP^+ at a dosage of 5.0 mg/kg. At 0.25, 0.5, 1.0 or 4 hrs after dosing mice were anesthetized with pentobarbital, perfused with 0.9% sal, and brain, heart and liver were removed. Tissues were dissolved and radioactivity determined by liquid scintillation spectrometry. Other groups (n=5/gp) received s.c. sal or DDC (400 mg/kg) followed 0.5 hr later by i.p. MPP^+ (5.0 or 10.0 mg/kg) daily for 4 days. Two days after the last injection brain and heart were removed for biochemical assay. DDC did not affect the distribution of MPP^+. DDC did increase, however, the general toxicity of MPP^+ as indicated by 70% mortality in the DDC/10.0 mg/kg MPP^+ group after the first injection. No decreases in dopamine or elevations in GFAP were observed in striatum after either MPP^+ or DDC/MPP^+ However, slight elevations in GFAP were seen in hippocampus in the DDC/MPP^+ group and indicate the combination may result in damage to this brain area.

24.14 RELATIVE EFFICACY OF BETA-CARBOLINE ANALOGS OF MPP+ AS INHIBITORS OF STRIATAL DOPAMINE UPTAKE. M. A. Collins, G. E. Drucker*, E. J. Neafsey and K. Raikoff.*
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N-Methyl-phenylpyridinium ion (MPP+) is the oxidized active form of the synthetic dopaminergic neurotoxin, MPTP. It is of interest that N-methylated beta-carbolines (BCs), which can form in peripheral and nerve tissues via carbonyl condensations of indoleamines (tryptamine, serotonin, etc.) followed by enzymic oxidation/methylation, structurally resemble MPP+. We have proposed (Collins, Neafsey, Neurosci. Lett. 1985) that the nigrostriatal dopaminergic loss underlying Parkinson's disease could be due to "MPP+-like" alkaloidal BCs which arise in vivo. Since avid uptake of MPP+ into dopamine cells appears to be a prerequisite for its neurotoxicity, it was reasoned that a potential endogenous toxin also would exhibit the same requirement. Thus, we have synthesized or obtained BCs and 3,4-dihydro-beta-carbolines (DHBCs) of various families, both N-methylated and normethylated, and have initiated a comparative study of their ability to inhibit 3H-dopamine uptake by rat striatal synaptosomes. By analogy with MPP+, inhibition of dopamine uptake is assumed to be due to competitive uptake of BCs and DHBCs into dopamine terminals. To date, certain structure-activity relationships have emerged. Overall, DHBCs are nearly equipotent with BCs as dopamine uptake inhibitors. The most effective alkaloidal derivatives are the 7-oxygenated (methoxy, hydroxy) BCs and DHBCs, with IC50's in the range of 4-12 uM, whereas the 6-methoxylated analogs are 15- to 25-fold less potent. N-methylation to form a quaternary nitrogen (beta-carbolinium ion) generally appears to increase potency, with the exception of the 7-hydroxy-substituted compounds. This might reflect the ability of the hydroxyl to delocalize the quaternary positive charge into the indole ring. Although an order of magnitude less potent that MPP+ in terms of assumed uptake, selected BCs (or DHBCs) apparently would be accumulated to a significant extent by dopaminergic cells over an prolonged period of time. Whether such BCs can induce neurotoxicity in those cells remains to be investigated in cultures and in vivo models. Supported by NS 23891 and the LUMC Dean's Fund.

24.15 COMBINED TREATMENT WITH ETHANOL OR ACETALDEHYDE AND MPTP: A MODEL OF SELECTIVE DESTRUCTION OF SUBSTANTIA NIGRA DA NEURONS IN YOUNG MATURE MICE. A. Zuddas, S. Schinelli, U. Bonucelli, G.U. Corsini, I.J. Kopin, NINCDS, National Institutes of Health, Bethesda, MD.

In humans and monkeys MPTP induces parkinsonism due to selective destruction of nigra striatal DA neurons. Long lasting neurochemical changes, but not cell loss, have been reported in brain of young mature mice treated with high doses of MPTP. We have recently reported that ethanol (ETOH), acetaldehyde (ACE) as well as diethyldithiocarbamate (an inhibitor of super oxide dismutase and aldehyde dehydrogenase) potentiate MPTP toxicity in mice, (Corsini, et al., Life Sci. 40:827, 1987) and causing an extensive destruction of DA neurons in substantia nigra pars compacta (SNc) (Zuddas, et al., Neurology 37 Suppl 1:333, 1987). To determine whether such enhancement of neurotoxicity was specific, we studied the effects of these combined treatments on mice of different ages, by measuring the content of DA, NE and 5HT in different brain areas. We also studied the recovery of striatal DA 5 weeks after the treatments. In addition, the striatal DA was evaluated in aged rats treated with ethanol and MPTP. Male C57 black mice, 5 and 8 weeks old were treated with ACE (250 mg/kg, i.p.) 10 min before, 10 and 30 min after MPTP (30 mg/kg, i.p.). Five week old mice were sacrificed seven days after the treatments; 8 week old animals were sacrificed either seven or 35 days after the injections. ACE treatment of 5 week old mice did not enhance DA depletion seen 7 days after MPTP treatment (50% depletion vs 43% depletion by MPTP alone). In 8 week old mice, however, ACE given with MPTP decreased striatal DA by 93% compared with 51% depletion when given alone. One month later, striatal DA content recovered to 64% of controls in the older mice treated with MPTP alone, whereas in ACE-MPTP treated animals, no significant recovery occurred. In the ACE-MPTP treated mice changes in DA levels were observed only in the striatum. DA content in hypothalamus, olfactory bulb and frontal cortex was unmodified in comparison to controls. Contents of NE and 5HT in hypothalamus, cerebral cortex, olfactory bulb and striatum were not affected by any of the treatments. In addition, marked loss of tyrosine hydroxylase immunoreactivity was observed in SNc but not in ventral tegmental area nor in locus coeruleus. The same biochemical and histological findings were observed in 8 week old mice treated twice with combined ETOH (1 g/kg, i.p.) and MPTP (30 mg/kg, i.p.). On the other hand, this treatment was not effective in the rat. One year old Sprague Dawley rats treated 5 days with ETOH (1 g/kg,i.p.) and MPTP (30 mg/kg, i.p.) or with MPTP alone showed no change in striatal DA content. Out data show that enhancement of MPTP neurotoxicity by acetaldehyde is specific for nigra striatal DA pathway without significant recovery by 5 weeks. Rats and very young mice are not sensitive to this enhancement. This result suggests that in 8 week old mice, ETOH or ACE and MPTP treatment is a more useful model of parkinsonism than MPTP treatment alone.

24.16 INTERACTION OF BRAIN-DERIVED MITOGENS AND ETHANOL ON ASTROCYTIC CELL KINETICS. D. L. Davies and T. Jang, Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.

Injury of the brain stimulates the presence of tissue mitogens which may contribute to reactive gliosis (Nieto-Sampedro, M., et al., Brain Res. 343:320, 1985), and similar mitogens have been implicated in developmental proliferative growth of the brain (Guilian, D., et al., J. Cell Biol. 102:803, 1986). Moreover, ethanol-intoxication is a frequent predisposing factor for traumatic injury in adults and prenatal ethanol exposure may result in cerebral hypocellularity. The present study was undertaken to assess the astrocytic response to tissue mitogens and the vulnerability of this response to ethanol toxicity.

An extract of brain was used as a source of mitogens. The extract was prepared by homogenation of adult rat brains in phosphate buffered saline (1:2 ratio) followed by centrifugation at 10,000G for 30 min; the supernatant was sterilized by filtration. Astrocytic cell cultures were used as an assay system.

Primary cultures were prepared from the cerebra of rats between 1 and 3 days postnatal age. The medium was DMEM fortified with 10% FBS. Confluent cultures were shaken vigorously for 5 sec to remove process bearing cells; the remaining adherent cells were trypsinized and subcultivated into 35 mm culture dishes.

On culture day 6, eight experimental groups were designated. Half of the cultures received brain extract (150 mg protein/ml nutrient medium); ethanol was administered at 4 dose levels: 0.0 g%, 0.1 g%, 0.2 g%, or 0.5 g% (i.e. 0.0, 21.7, 43.4, or 108.5 mM). Cultures were incubated in closed vapor chambers designed to maintain consistent ethanol concentrations with 5% CO_2 at 37° C. The total number of cells in these cultures was determined on culture days 8, 10 and 12. For analysis of cell-cycle parameters cultures were stained with propidium iodide (Burns, E. R. et al., Cytometry 4:150, 1983) and the DNA content of individual cells was assessed by flow cytometry (Ortho Cytofluorograft IIS). The astrocytic identity of the cultures was verified by immunolocalization of GFA (the antibody was a gift from Dr. D. Dahl). The ethanol concentration of the medium was measured enzymatically.

Ethanol content of the medium after 72 hr of incubation was at least 94% of the initial concentrations. Brain extract slightly increased total cell number, however, statistically significant differences were not achieved with this dosage of the extract. The flow cytometric data indicated that treatment with brain extract marginally increased the number of cells in the S and G_2/M phases of the cell-cycle. Constant exposure to 0.5 g% ethanol for 6 days suppressed total cell number relative to untreated controls. Thusfar, ethanol-related changes in the cell-cycle have not been detected. Supported by USPHS Grant #AA07145.

24.17 STEREOLOGICAL STUDY OF THE RAT HIPPOCAMPAL MOSSY FIBER-CA3 SYNAPSES AFTER LONG-TERM ALCOHOL CONSUMPTION AND WITHDRAWAL.M.M. Paula-Barbosa, M.A.Tavares*, M.M.Pacheco*, M.C.Pinho* and A.Cadete-Leite*
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Neuronal loss was found to occur in the adult rat hippocampal formation (HF) after 5 months of chronic alcohol consumption (CAC) (Walker,D.W., Science, 209:711,1980). As we observed that in the cerebellar cortex alcohol induced changes were progressive, we thought it would be of interest to study, using recently described stereological techniques, the effects of CAC in the HF after longer periods of treatment and withdrawal.Attention was paid to the synaptic system between granule cell mossy fibers and CA3 pyramids (MF-CA3) due to its relevant role within the HF trisynaptic circuitry.

This study was undertaken in 5 groups of 6 rats:alcohol treated with an 20% aqueous ethanol solution for 6 and 18 months starting at 2-month age, respective age-matched pair-fed controls and in an alcohol-treated group for 12 months, rehabilitated ad libitum for 6 months (recovery group). The disector method (Sterio,D.C., J. Microsc., 134: 127,1984) was applied for the determination of numerical densities (Nv) of neurons and synapses. For the neurons, serial semithin sections from 3 different blocks per animal were used (6 disectors per block). For the synapses, serial ultrathin sections from 3 blocks per animal were used (10 disectors per block).

After 6 and 18 months of CAC the Nvs of granule cells and CA3 pyramids were significantly reduced. No significant differences were found in the number of synapses between MF-CA3 after 6 months of experiment, conversely to what happens after 18 months of CAC where MF-CA3 synapses were significantly reduced. The fraction of MF endings plasmalemma occupied by active zones was significantly increased in alcohol-fed groups. In the recovery group a significant reduction of neurons was found when compared both with the 18 months alcohol-fed and control groups. The Nv of MF-CA3 synapses was significantly reduced when compared with the 18-month control group; no significant differences were found when compared with the alcohol-treated group. The fraction of MF endings plasmalemma occupied by active zones was significantly increased when compared with controls; no significant differences were found when comparison was made with alcohol-treated group.

We conclude that: 1) the HF is remarkably sensitive to CAC ; 2) there are signs of synaptic remodelling in alcohol-treated and recovery groups and 3) alcohol withdrawal doesn't reverse the alcohol induced degenerative changes.

SENSORY SYSTEMS: SOMATOSENSORY

25.1 RESPONSIVENESS OF SURVIVING SOMATIC SENSORY CORTEX FOLLOWING LESIONS OF SI OR SII IN ADULT AND INFANT CATS AND PRIMATES. H. Burton, K. Sathian*, K. Alloway, S. Warren and M. Carlson.
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Lesions of either the first (SI) or second (SII) somatosensory cortical area impair the capacity of adult macaques to discriminate textured surfaces, whereas infant macaques recover this capacity if either SI or SII is spared (Burton et al., '87). An important issue is whether the lesions interrupt "serial" or "parallel" interactions between SI and SII. Thalamocortical connections may be crucial to this problem as the extent of dual projections to SI and SII from VPL may change with age or species. We are studying these issues by recording neuronal activity in SI or SII after lesions of the other area made at various ages in 3 species: cats, galagos and macaques.

Following removal of SI bilaterally and SII unilaterally at 3 days or 4 weeks of age in cats, we found that at maturity the remaining SII mapped normally with cutaneous stimuli. Local anesthetization of SI with lidocaine in adult cats (Burton & Robinson, Somato Res. 4:215-236,1987) or lesions of SI in adult galago also failed to alter SII responsiveness.

We have also made SI and SII lesions in the hand and digit areas of juvenile and infant macaques. Multi-unit recordings, to date, from the SI hand area show no changes in responsiveness or topographic organization after lesions of SII. Recordings have also been obtained from SII following an 8 month old SI lesion made in a juvenile. In this case responses to somatic stimuli could be recorded in the depths of the lateral sulcus. In contrast to the normal SII, most of the responses were not evoked by low threshold cutaneous stimuli and well-defined receptive fields were not found. Effective stimuli consisted of slow stroking, squeezes, or crude joint manipulations. Von Frey filament forces of >300mg, and frequently of >1000mg, were needed to activate neurons. Some pockets of low threshold (<300mg) responses were found amidst the higher threshold activity. Despite the generally increased difficulty in evoking responses, the expected radial to ulnar digit sequence was recorded along the parietal operculum. Recordings in SII obtained from outside the hand and digit area responded to low threshold stimuli. No selective enlargement of these representations have been noted. Results from SII in animals with infant SI lesions will be reported subsequently. (Funds from NS15070, NS07071, MH14677)

25.2 POTENTIAL ROLE OF THALAMOCORTICAL CONNECTIONS IN RECOVERY OF TACTILE FUNCTION FOLLOWING SOMATIC SENSORY CORTEX LESIONS INFANT PRIMATES. M. Carlson, D.D.M.O'Leary & H. Burton.
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Electrophysiological studies of tactile input to primary (SI) and secondary (SII) somatic sensory cortices have allowed comparison of the organization and function of SI and SII hand areas in the Old World anthropoid, Macaca, and prosimian, Galago. Our recent behavioral studies of infant Macaca have shown that, although SI and SII are necessary for normal tactile discrimination in the adult, that either SI or SII alone can mediate texture discrimination in the infant.

We have found texture discrimination capacity of the normal adult Galago to be less than that of adult Macaca, possibly due to the smaller and less complex SI hand area in Galago. Removal of SI or SII in adult Galago reduced tactile performance below normal levels. Ongoing studies of infant Galago show that, as in infant Macaca, tactile function recovers following either SI or SII removals.

We have proposed that the recovery of tactile function in the infant primate may depend upon an exuberant pattern of thalamocortical projections from the ventroposterior (VP) nucleus in the infant. Such projections might be stabilized by removal of either SI or SII and, thereby, serve as a basis for the equipotentiality of function seen in SI and SII in infant primates. To examine this issue in normal animals, we have injected the retrogradely transported fluorescent dyes, Fast Blue and Diamidino Yellow, into SI and SII, respectively. Dye injections into adult Galago confirm our earlier findings using HRP of separate projections to SI and SII from VP. Similar dye injections in infant Galago reveal a less segregated pattern in VP, and double-labeled cells are more common. Injections in SI- or SII-lesioned infants and adults are planned to search for maintained projections in the infant-lesioned animal.

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25.3 GABA, NEUROPEPTIDE AND ChAT EXPRESSION IN NEURONS OF THE FETAL MONKEY SENSORY-MOTOR CORTEX. G.W. Huntley*, S.H.C. Hendry, E.G. Jones, L.M. Chalupa and H.B. Killackey (SPON: M.A. Kirby). Depts. of Anatomy and Neurobiology and Psychobiology, University of California, Irvine, CA 92717 and Dept. of Psychology, University of California, Davis, CA 95616.

Immunocytochemical methods were used to examine the morphology and distribution of GABAergic, peptidergic and cholinergic neurons in the sensory-motor cortex of monkeys (Macaca mulatta) during the latter third of gestation (E110, 121, 135 and 150). Areal and laminar boundaries were determined by specific patterns of histochemical staining for cytochrome oxidase and acetylcholinesterase and by staining with thionin. In each of the fetuses, GABA-immunoreactive neurons are found through the thickness of the cortex and in the underlying white matter but were concentrated in two bands that included developing layers I-III and layer VI and the subjacent white matter. All GABA neurons are non-pyramidal cells. Co-localization experiments demonstrate that sub-populations of the GABA neurons are immunoreactive for cholecystokinin octapeptide (CCK), substance P (SP), somatostatin (SRIF) and neuropeptide (NPY) from the earliest ages examined. CCK somata occupy layers II and III; the cells give rise to processes that descend and form a dense plexus in layers V and VI. SP somata are concentrated in a thin band corresponding to layer V; they give rise to processes that ascend and form a dense plexus in layers I and II. The SP somata are particularly dense in the first somatic sensory area (SI) and less dense in the precentral motor area (area 4). Both SRIF and NPY somata are densely packed in layer VI and the subcortical white matter; their processes make up a broad plexus in layer V and a thin plexus in layer I. The layer I plexus is absent from area 3 of SI. At the four ages examined, none of the GABA or neuropeptide neurons is retrogradely labeled by injections of Fast Blue, Nuclear Yellow or rhodamine latex beads in the contralateral sensory-motor cortex. The described patterns are seen for each of the fetuses, although the numbers of neuropeptide immunoreactive neurons and the intensity of their staining declines in the older fetuses. Non-pyramidal neurons immunoreactive for choline acetyltransferase (ChAT) are also present in the sensory-motor cortex of fetal monkeys. A low density of ChAT-positive somata are diffusely scattered in layers V and VI and more densely packed in the subcortical white matter; their processes ascend to the middle layers of the cortex. The immunostaining patterns in fetal monkeys are unlike those seen in adult monkeys in the density of somata that are stained in the white matter (GABA, NPY and SRIF), in the restricted laminar distribution of somata and processes (GABA, CCK and SP), in the morphology of neurons (SP) and in the existence intrinsic ChAT-immunoreactive neurons.

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25.4 **DOUBLE LABELLED NEURONS IN PRIMARY SOMATOSENSORY CORTEX (AREA 3B) OF THE FETAL RHESUS MONKEY.** L.M. Chalupa and H.P. Killackey, Dept. of Psychology and Calif. Primate Research Center, Univ. of California, Davis, 95616 and Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

In a previous study, we provided evidence that callosal projection neurons in the postcentral gyrus of the rhesus monkey undergo a marked change in areal distribution between embryonic days (E) 119 and 133. Initially, callosal projection neurons are evenly and continuously distributed throughout the postcentral gyrus although located in the appropriate cortical layers. At E133 and later, their areal distribution is not continuous, the density of callosal projection neurons varying both within and across cytoarchitectonic areas (Killackey & Chalupa, J. Comp. Neurol. 244:331, 1986). In the present study, we investigated the possibility that some callosal projection neurons may have multiple projections during the period of widespread distribution.

The cerebral cortices of four fetal rhesus monkeys (2 at E110, and 2 at E135) were injected with a combination of fluorescent tracers. Multiple injections of fast blue were made throughout the postcentral gyrus of one hemisphere and more restricted injections of rhodamine labelled microspheres were made into the posterior portion of the crown of the postcentral gyrus of the other hemisphere. The fetuses were sacrificed at E138 or later, after the discontinuous distribution of callosal projections is established. The brains were removed, sectioned and examined for the distribution of labelled cells with fluorescent microscopy.

In the animals injected at the younger age (E110), when callosal neurons were widespread, a continuous pattern of fast blue labelled neurons could still be detected in the postcentral gyrus. Further, in area 3b pockets of neurons which were labelled with fast blue were also labelled with rhodamine microspheres. As expected in the animals injected at the older age (E135), the overall distribution of fast blue neurons was discontinuous. In area 3b, individual neurons were labelled with either fast blue or rhodamine microspheres, but there were very few, if any, double labelled cells. These results indicate that the discontinuous distribution of callosal projection neurons is achieved in the primate cortex by mechanisms involving process elimination.

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25.5 NEONATAL THALAMIC LESIONS ALTER THE PATTERN OF CALLOSAL CONNECTIONS IN THE PARIETAL CORTEX OF THE RAT. K.A. Koralek and H.P. Killackey. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

In neonatal rats, callosal projection neurons are widely and uniformly distributed across the parietal cortex. During early postnatal development, many neurons eliminate their callosal processes, and a well-defined pattern of callosal projections emerges (Ivy and Killackey, J. Neurosci., '82). We have recently shown that neonatal damage to the somatosensory periphery alters the tangential distribution of callosal cells and terminations in the parietal cortex (Koralek and Killackey, Soc. Neurosci. Abs., '86). These data suggest that the input from the thalamus shapes the distribution of callosal cells and terminations. In this study we directly test this hypothesis.

We removed the thalamus of rats on the day of birth, a time prior to the growth of thalamocortical axons into the somatosensory cortex. Large unilateral thalamic lesions were made by aspiration from a posterior approach in neonatal Long-Evans rats. At one month of age, multiple injections of horseradish peroxidase were placed into the cortex contralateral to the lesion, so as to be evenly distributed throughout parietal cortex. After a 24-36 hour survival period, the animals were perfused and the hemisphere ipsilateral to the lesion was flattened and sectioned tangentially. The thalamus was sectioned in the coronal plane. Both series of sections were processed with tetramethyl benzidine. Normal rats of the same age were used as control subjects.

Animals subjected to thalamic lesions at birth present a severely altered pattern of callosal connections. Aberrant patches of labelled cells and terminations are interspersed with zones containing little or no label. In all cases examined so far, the tangential distributions of callosal projection neurons and of terminations are the same. Features which characterize the normal adult callosal pattern cannot be recognized within the aberrant patchwork.

We conclude that input from the thalamus plays a crucial role in shaping the normal pattern of callosal connections during development of parietal cortex.

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25.6 NEONATAL DEAFFERENTATION-PRODUCED METABOLIC REORGANIZATION IN SI BARREL CORTEX OF RAT: A COMBINED (14C)-2 DEOXYGLUCOSE (2DG) AND CYTOCHROME OXIDASE (CO) STUDY. R.L. Craik, C.L. Hand and P.J. Hand. Physical Therapy, Beaver College, Glenside, PA; Anim. Biol., Sch. of Vet. Med., and Dept. of Neurol., Sch. of Med., Univ. of PA, Phila., PA 19104.

The metabolic (functional) reorganization of rat SI cortex following neonatal vibrissa follicle deafferentation which spared #3 vibrissa of row C (SC3) consisted of an enlarged and diffuse pattern of SC3-activated metabolic labeling in contralateral SI compared to that associated with activation of the control C3 vibrissa (CC3; Sheu and Hand, 1982). The current study combines use of the 2DG and CO techniques to quantify (1) areal extent, (2) density, and (3) localization of SC3 metabolic labeling in laminae IIIb-IV of SI cortex of Sprague-Dawley rats (n=8) 90 days postneonatal vibrissa follicle deafferentation (postnatal day 2 or 3; PND 0=birthdate).

Quantitative areal analysis of C3-related metabolic activity in serial tangential sections through lam. IIIb-IV revealed the mean areal extent of labeling on the SC3 side was 1.7mm^2 compared to .32mm^2 on the CC3 side, representing a 5.3-fold increase on the SC3 side. Regarding density of metabolic labeling related to activated SC3 and CC3 barrels, quantitative densitometric analysis indicated an average increase of 9.4% for SC3 barrel compared to CC3 (p=.01). The 2DG labeling associated with CC3 activation was localized to the CO-labeled CC3 barrel and its surrounding septum. In contrast, 2DG labeling associated with SC3 activation was enlarged, diffuse, and extended over CO-labeled rows B, C, and D, with densest metabolic labeling confined to row C and its septa. Compared to the control side, boundaries of the CO-labeled barrels on the SC3 side were not as well-defined. Extent of the alteration of CO-labeled barrels on the SC3 side was variable in nature, ranging from ill-defined to totally disrupted barrels.

In conclusion, CO-labeled SI barrels were altered variably by neonatal vibrissa deafferentation, with some exhibiting severe disruption while others were reasonably distinct. The 2DG pattern of labeling associated with SC3 activation, however, was consistently diffuse and extended well beyond the CO-labeled SC3 barrel limits. If CO-labeled activity in SI barrel cortex is primarily in dendritic mitochondria of barrel neurons (Wong-Riley and Welt, 1980), then our results of a less-disrupted CO-pattern of labeling compared to the 2DG pattern suggest that factors such as reorganization of thalamocortical and local corticocortical circuitry may be operating in addition to some reorientation of barrel neuron dendrites to produce the enlarged and diffuse SC3-activated 2DG labeling. (Supported by NIH grant NS-22283-01A2)

25.7 THE DEVELOPMENT OF THE VIBRISSAE RELATED NEOCORTICAL AFFERENTS TO SOMATOSENSORY CORTEX OF THE RAT. Karl. F. Jensen, Neurotoxicology Division, Health Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC 27711

The clustered pattern of terminal arbors of thalamocortical axons projecting to the vibrissae region of the rat somatosensory cortex mimics the pattern of whiskers on the face of the rat. Furthermore, the immunohistochemical labeling pattern of an antibody to p38, an intrinsic protein of small synaptic vesicles (Jahn et al., 1985, PNAS 82:4137-4141), corresponds to the vibrissae related pattern of thalamocortical afferents in the developing somatosensory cortex (Jensen et al. 1986, Soc. Neurosci. Abstr. 12:1051). To further characterize the development of the vibrissae related neocortical map the pattern of p38 immunoreactivity was compared to the vibrissae related pattern of cytochrome oxidase activity.

Long-Evans rat pups were sacrificed at various times between birth and 21 days of age by perfusion with 4% paraformaldehyde under barbiturate anaesthesia. Tangential sections of flattened neocortex were incubated with a monoclonal antibody to p38 and processed for either indirect flourescein or PAP immunohistochemstry. Adjacent sections were processed to demonstrate the vibrissae related pattern of cytochrome oxidase activity.

While both p38 immunoreactivity and cytochrome oxidase activity demonstrate the vibrissae related pattern, two significant differences were observed in the development. First, patterned p38 immunoreactivity is apparent on pnd-2 prior to the appearance of patterned cytochrome oxidase activity on pnd-3. Second, while the vibrissae related pattern of cytochrome oxidase activity persists into adulthood, p38 immunoreactivity becomes uniformly distributed throughout the vibrissae region from pnd-12 to pnd-14. These results suggest that while p38 and cytochrome oxidase are not colocalized throughout development, the vibrissae related pattern of cytochrome oxidase is intimately linked to the ingrowth of neocortical afferents during early postnatal development.

25.8 THE EFFECTS OF SENSORY DEPRIVATION ON GLUTAMIC ACID DECARBOXYLASE IMMUNOREACTIVITY IN THE RAT SmI BARREL CORTEX. N.D. Akhtar* and P.W. Land. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261

The somatosensory cortex (SmI) of adult rats contains neuronal aggregates, called barrels, each of which is functionally linked to the corresponding vibrissa (the "columnar" vibrissa or whisker) on the contralateral face. Electrophysiological studies (Simons and Land, Nature 326:694-697, 1987) indicate that tactile deprivation by chronic whisker trimming from birth results in abnormalities in responses of barrel neurons to stimulation of the regrown whiskers. These abnormalities include enlarged receptive fields, reduced selectivity for whisker deflection at particular angles, increased responsiveness and altered patterns of stimulus evoked discharges compared with cells in normal barrels. Such changes suggest diminished effectiveness of intracortical inhibition in neonatally deprived barrels.

Immunocytochemical methods were used to examine the distribution of glutamic acid decarboxylase (GAD) in SmI of rats that had one or more rows of whiskers chronically trimmed for 2 months beginning at birth or during adulthood. Whisker trimming begun in adulthood results in a marked but reversible decrease in GAD staining in barrels corresponding to the trimmed whiskers. This presumably reflects down- and up-regulation of GAD synthesis following changes in sensory input. By contrast, GAD staining in SmI of animals trimmed from birth is similar to that in normal animals. Deprived and non-deprived barrels contain equivalent numbers of GAD+ neurons and a dense accumulation of GAD+ terminals. The trimmed vibrissae of some neonatally deprived animals were allowed to grow to normal lengths for 6-8 weeks, after which time those whiskers were again trimmed for 2 months. The second trimming, in adulthood, had no effect on GAD staining in the corresponding barrels.

Our results indicate that GAD neurons and terminals in SmI barrels develop without normal stimulation of the corresponding whiskers. However, GAD regulation in neonatally deprived barrels becomes refractory to subsequent changes in vibrissal input, whereas maintenance of GAD levels in normal barrels is critically dependent on stimulation of the columnar whisker. The finding that sensory input from neonatally trimmed vibrissae has little or no influence on the metabolism of GAD containing cells is surprising, given the unusually vigorous response of many deprived neurons to whisker deflection. One possibility is that GAD cells in neonatally deprived barrels become dominated by non-columnar inputs. This would be compatible with recent electrophysiological data and would further point to abnormalities in intracortical inhibitory mechanisms following tactile deprivation early in life. Supported by NIH grant NS 23047.

25.9 DENDRITIC OVERPRODUCTION AND ORIENTED DENDRITIC LOSS IN THE DEVELOPMENT OF NEURONS IN THE POSTEROMEDIAL BARREL SUBFIELD OF MOUSE SOMATOSENSORY CORTEX. W. T. Greenough, F.-L. F. Chang, I. R. Niesman, and E. P. Loeb. Depts. Psychol. and Anat. Sci., and Neur. & Behav. Biol. Prog., Univ. Illinois, Champaign, IL 61820.

Axonal proliferation and loss, in the generation of patterned arrays in the CNS, are well established developmental phenomena. Dendritic overproduction and regression has been reported, and in some cases there is evidence for its modulation by neuronal activity. However, the notion that initial dendritic outgrowth may in some cases be relatively nonsystematic and that pattern is subsequently imposed through selective dendrite elimination has not been widely demonstrated. The highly ordered dendritic arrangement of the vibrissae-associated somatosensory cortical barrel system, in which dendrites of cells in barrel walls are oriented towards the hollow, provides a unique opportunity to study oriented dendritic field development in cerebral cortex. We studied a total of 143 Golgi-stained (methylene blue counterstained) spiny stellate neurons in the walls and hollows of cortical barrels of mice at postnatal ages 10, 15, 20, 25, and 30 days. Only cells in approximately the inner 1/3 of the cell-dense barrel wall were examined. Projection tracings of the dendritic fields of these cells were made with the aid of a camera lucida, and the relative location of the barrel hollow was recorded. Dendritic length was quantified in terms of the number of intersections between dendrites and a series of concentric rings at 10 micron intervals around the soma. For quantification of growth oriented towards or away from the hollow, the rings were separated into quadrants, and only intersections in the quadrant centered towards the hollow and the quadrant opposite, away from the hollow, were included. The overall growth of dendritic fields was progressive across these ages, dendritic length increasing with increasing age. The number of primary branches decreased with increasing age, while the remaining branches proliferated. However, the pattern of growth was different for branches extending towards the hollow or away from it: for branches growing towards the hollow, dendritic growth increased smoothly across the ages examined; for branches growing away from the hollow, dendritic length decreased with increasing age. While primary branches decreased across age both on the side of the soma towards the hollow and on the side opposite the hollow, the decrease was substantially greater on the side away from the hollow. These data indicate that oriented dendritic fields in this system arise, at least in part, through elimination of branches that have grown in the improper direction. —Supported by ONR and NIMH.

25.10 INVARIANCE IN THE OVERALL PATCHY ORGANIZATION OF TACTILE PROJECTIONS TO CEREBELLAR CORTEX FOLLOWING PERIPHERAL NERVE LESIONS MADE EARLY IN CEREBELLAR DEVELOPMENT. J. B. Schlottman* and J. M. Bower. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

The organization of mossy fiber tactile projections to the granule cell layer of the cerebellar hemispheres results in a patchy somatotopic map representing selective parts of the body surface (Shambes et al., B.B.E. 15: 94, 1978). The patches are defined by sharp boundaries between projections from different body regions (Woolston et al., Brain Res. 209: 255, 1981). Comparisons of these maps between individuals of one species (rat) or even between closely related species (rat-mouse) reveal a remarkably invariant pattern of patches in any one cerebellar folium. For example, the map in crus IIa of the rat or mouse is characterized by a large ipsilateral upper lip patch surrounded by smaller patches representing other peri-oral surfaces (e.g., lower lip, lower incisor, upper incisor). This invariance suggests that the general organization of this patchy pattern may be somewhat inflexibly specified during development. This suggestion is in keeping with our recent Purkinje cell lineage experiments showing, in chimeric mice, that cells are grouped in lineage domains comparable in size and distribution to the physiologically defined patches (K. Herrup & J. Bower, Soc. Neurosci. Abstr. 12: 769, 1986).

To investigate further the establishment of this patchy organization during development, we have disrupted the normal patterns of innervation by lesioning specific peripheral nerves at a time before cerebellar connections are fully established. Specifically, we severed (under anesthesia) trigeminal nerve branches innervating the upper lip of 9-day-old rats. After these rats reached adulthood, we mapped the area of crus IIa usually containing a large representation of the now denervated upper lip. The resulting maps reveal that the overall patchy organization was virtually unchanged, i.e, there remained a very large central patch similar in shape and dimension to the one normally receiving upper lip projections. This large patch was now completely occupied by projections from two small areas bordering the upper lip, namely the corner of the mouth and an area directly below the nose. The outlying small patches representing other body surfaces in these animals were in all respects normal. These results are consistent with the view that the boundaries between patches in the tactile projection to cerebellar granule cell layer may be established inflexibly during development, for example by Purkinje cell lineage.

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25.11 TRANSGANGLIONIC HRP TRANSPORT FROM PREVIOUSLY LESIONED AND UNLESIONED FELINE TEETH. M.A. Henry[1], L.E. Westrum[2], Lonnie R. Johnson[3]* and R.C. Canfield[4]. Facial Pain Center[2], Univ. of Florida, Gainesville, FL 32610, Depts. of Neurosurg.[2] and Rest. Dent.[4], Univ. of Wash., Seattle, WA 98195 and Dept. of Surg. Dent.[3], Univ. of Colo., Denver, CO 80262

Previous studies have established that transganglionic degeneration occurs following peripheral dental lesions. The present study investigates the differences and/or similarities in HRP transport following pulpal injections in previously lesioned and unlesioned teeth within the same subject. Immature feline subjects, 5-7 weeks of age, were used in the study. Coronal pulpal tissue was removed (pulpotomy) from the ipsilateral maxillary and mandiblular cuspid. One week to 10 days later, 1 µl of a 40% HRP solution was injected into each of the previously lesioned, ipsilateral cuspids and into the contralateral (unlesioned) maxillary and mandibular cuspids. The HRP injection procedure was repeated on the second day and the subjects transcardially perfused with mixed aldehydes on the third day. The trigeminal ganglia, brain stem and cervical spinal cord to the C_3 level were removed, sectioned and processed for HRP light microscopy by the TMB method of Mesulam. Examination revealed approximately the same number of labeled cells within the ipsilateral (lesioned side) and contralateral (unlesioned side) ganglia. Examination of the cervical spinal cord revealed sparse terminal labeling within the substantia gelantinosa layer of the dorsal horn from C_1 - C_3. Within caudal pars caudalis (PC), terminal and fiber labeling increased noticeably on both sides of the brain stem. A difference was detected in the relative density of labeled elements within mid PC, with the unlesioned side containing more label. This difference was maintained rostrally through to mid pars interpolaris (PI). Within mid PI and continuing rostrally through pars oralis, the densest label appeared, but there were no apparent differences in the relative density of label when one side was compared to the other. Even though small volumes of HRP solution were passively injected into the preparation, HRP still appeared to leak out the apex and label periodontal ligament receptors as a few labeled mesencephalic nucleus cell bodies were identified on both sides.

In conclusion, this study demonstrates less HRP label within the periobex region of the spinal trigeminal nucleus on the side that was previously lesioned. This is the same area where synaptic terminals and small axons undergoing electron-dense degeneration have been described following dental lesions. Other regions of the trigeminal brain stem nuclear complex appear unaffected. Supported by NIH grants DE04942 and NS20482.

25.12 **ONTOGENY OF CARBOHYDRATE DIFFERENTIATION ANTIGENS IN CHICK DORSAL ROOT GANGLIA AND SPINAL CORD** S. A. Scott, J. Dodd† and T. M. Jessell††. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794, †Dept. of Physiology and Cell Biophysics and ††Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, NY, NY 10032.

Dorsal root ganglia (DRG) contain a diversity of sensory neurons, which make specific connections in the periphery and CNS. Little is known about the development and early connectivity patterns of these functional subsets of DRG neurons. To investigate these problems it would be useful to have markers for various classes of sensory neurons that project to different targets. To this end we are examining the ontogeny and specificity of expression of carbohydrate differentiation antigens [Dodd *et al.* (1984); Dodd & Jessell (1985); Dodd (1986)] in chick DRG and spinal cord.

Cryostat sections of lumbosacral spinal cord and DRG of embryos from St18 (3d) through hatchlings have been labeled with monoclonal antibodies AC4, anti-SSEA-1 or 1B2/1B7, which recognize different lactoseries glycoconjugates. To identify the targets of immunoreactive cells, sensory neurons projecting in selected cutaneous or muscle nerves have been retrogradely labeled with fluorescent tracers. The AC4 antigen first appears in chick neural tissue between St22 (3½d) and St26 (5d). By St27 (5½d) the dorsal half of the spinal cord and a few ventrolateral DRG neurons stain intensely. The dorsal expression of this antigen in the cord is transient; by St32 (7½d) AC4 immunoreactivity forms a uniform meshwork throughout the gray matter, but is essentially absent from cell bodies. In hatchlings the AC4 antigen also weakly delineates dorsal horn laminae 1-3. The number of AC4-positive sensory neurons increases during development, with approximately 30-40% of sensory neurons being labeled throughout DRG in hatchlings. SSEA-1-reactive neurons appear in DRG on a roughly similar time course, but this antigen is not expressed transiently in dorsal cord. In contrast, the 1B2/1B7 antigen appears relatively late in embryonic development. Weakly labeled sensory neurons can first be detected in embryos near hatching; however, labeling is virtually absent from the spinal cord at all stages studied. Preliminary observations of retrogradely labeled DRG suggest that AC4- and SSEA-1-positive sensory neurons project to targets in both skin and muscle in hatchlings. Further studies are underway to determine whether these subpopulations of neurons also project to diverse targets in younger embryos.

25.13 **DEVELOPMENT OF THE MUSCLE AFFERENT-MOTONEURON PATHWAY IN THE CHICK EMBRYO: PHYSIOLOGY.** M. T. Lee and M. J. O'Donovan. Dept. Physiol. & Biophys., U. of Iowa, Iowa City, IA 52242.

We have examined the development and properties of synaptic connections between muscle afferents and motoneurons in the spinal cord of the chick embryo *in vitro*. Responses to muscle nerve stimulation were recorded intracellularly in hindlimb motoneurons and extracellularly in ventral roots (VRs) and muscle nerves.

In relatively mature embryos (stages 38-39), muscle afferent activation produces depolarizing synaptic potentials in the motoneurons. The earliest of these potentials can be elicited with low-intensity stimulation and appears 4-5 msec after the arrival of the afferent volley in the cord. With more intense stimulation, this early PSP merges with later depolarizations of variable amplitude and duration, including those produced by the central pattern generator for locomotion. The early PSP persists in the presence of 200 µ*M* 2-amino-5-phosphonovaleric acid (APV), a specific antagonist of NMDA receptors, but is greatly reduced in amplitude by 1.25 m*M* kynurenic acid, a general antagonist of excitatory amino acids. Both of these agents completely block the later depolarizations. The excitatory nature of the early PSP is indicated by its ability to fire motoneurons and by its persistence in saline containing 100 µ*M* picrotoxin and 10 µ*M* strychnine, blockers of IPSPs mediated by GABA and glycine, respectively. Solutions that inhibit chemical synaptic transmission (zero-Ca + 2m*M* Mn) eliminate both the early PSP and later depolarizations. Taken together, these observations suggest that the early PSP is the monosynaptic EPSP produced by Ia muscle afferents.

We have used VR recordings to monitor the development of connectivity between muscle afferents and motoneurons. The monosynaptic EPSP is detectable in the VRs by stages 31-32 (8-9 days), when afferents begin to grow into the vicinity of motoneuron dendrites. Longer-latency depolarizations can be elicited by stimulation of muscle afferents at least 1-2 days earlier, by stage 28. The pharmacological distinction between the monosynaptic EPSP and later depolarizations exists at all stages where both inputs are present. Even at the earliest period in the formation of sensory-motor synapses, we have found no evidence for electrical transmission in this pathway. The first monosynaptic connections between muscle afferents and motoneurons develop at a time when both are decreasing in number through cell death, raising the possibility that cell death may be involved in the generation of sensory-motor specificity.

In order to characterize the patterns of sensory-motor specificity in this system, we have developed a procedure for recording motoneuron synaptic potentials in identified muscle nerves. In the presence of APV, picrotoxin, and strychnine, the afferent-evoked, monosynaptic EPSP can be isolated and is detectable in muscle nerves. Moreover, the various nerves can be distinguished on the basis of their responses to stimulation of different muscle afferents. This procedure offers the opportunity to determine the connectivity patterns of large populations of afferents and motoneurons in a manner that is rapid and sensitive.

25.14 ANATOMICAL STUDIES OF AFFERENT PROJECTIONS TO MOTONEURONS IN THE LUMBOSACRAL CORD OF THE CHICK EMBRYO. M.J. Koebbe* and M.J. O'Donovan (SPON: Gary Dutton) Dept. of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

We have examined the development of sensory projections to lumbosacral motoneurons in the chick spinal cord between stage 28 and stage 40. The spinal projection of sensory axons was visualized by anterograde labelling with horseradish peroxidase (HRP). Contralateral motoneurons in the same segments were retrogradely labelled with HRP. HRP was injected into the dorsal root ganglia (DRG) and ventral roots (VR) of 1-3 lumbosacral segments in each embryo using an in vitro preparation of the spinal cord. Transport of HRP was allowed to proceed for 5-9 hours. The spinal cords were processed for the HRP reaction product using either tetramethyl benzidine (TMB) or diaminobenzidine chloride (DAB). Spinal cords processed with DAB were paraffin embedded and sectioned at 10 µM. Best demonstration of HRP in sensory axons and motoneurons was attained with 40 µM frozen sections using a concentration of 0.005% TMB in an ammonium-molybdate buffer at pH 6.0.

Sensory axons first penetrated the dorsal gray matter at stage 29. Prior to this stage labelling was confined to a dorsolateral band of the white matter. By stage 32 afferent fibers had reached the vicinity of motoneuron dendrites which extended into the dorsal third of the spinal cord. The sensory projection increased in density and projected more ventrally with further development so that by stage 36 sensory axons had reached motoneuron somata, corresponding to an average growth rate of approximately 100 µM/day. By stage 39 sensory fibers were widely distributed throughout the spinal gray matter and distinct terminal fields were evident in the dorsal horn and in the lateral motor column. These results indicate that afferent fibers first reach motoneuron dendrites between stages 31 and 32. This time corresponds to the earliest stage that monosynaptic connections between muscle afferents and motoneurons can be detected physiologically (Lee and O'Donovan, this meeting) and indicates that the first sensory-motor connections are axo-dendritic and are located in the dorsal 1/3 of the spinal cord. Supported by NIH NS22559.

26.1 NEONATAL COCHLEAR LESIONS: INFLUENCE UPON 2-DEOXYGLUCOSE UPTAKE BY CENTRAL AUDITORY STRUCTURES IN ADULTHOOD. A.F. Ryan* and N.K. Woolf. Division of Otolaryngology, UCSD School of Medicine and VA Medical Center, La Jolla, CA 92093.

The destruction of all or part of the cochlea deprives a portion of the central auditory system of afferent innervation. In the adult, this deafferentation results in a permanent loss of inputs to the central neurons, and substantial bilateral asymmetry in evoked 2-deoxyglucose (2-DG) uptake by many auditory nuclei. The present study was designed to evaluate the degree to which developmental plasticity might allow the functional re-innervation of central auditory neurons following neonatal damage to the cochlea.

Neonatal mongolian gerbils had one cochlea surgically ablated at either 0, 2, 6, or 10 days after birth (DAB). After survival to young adulthood (45-60 DAB), the animals were injected with 16.7 uCi of 14C-2-deoxyglucose and placed in a sound-attenuated chamber. They were stimulated bilaterally at 85 dB SPL with either wide band noise or a 3.0 kHz pure tone for one hour, after which their brains were processed for autoradiography. Uptake of 2-DG into brain structures was assessed by densitometry. The results obtained in neonatal animals were compared to those observed in gerbils which received cochlear ablations as adults.

Responses of the cochlear nucleus and inferior colliculus innervated by the intact ear of all subjects were similar to those seen in gerbils with two normal cochleas.

In all successful lesions, 2-DG uptake in the cochlear nucleus on the side of the ablated cochlea was extremely low, consistent with complete deafferentation. The volume of this cochlear nucleus was severely reduced in neonatally ablated subjects, and especially in those lesioned at 0-6 DAB.

In the inferior colliculus associated with the lesioned cochlea of all subjects, regardless of age, 2-DG uptake was markedly lower than in the colliculus innervated by the intact cochlea. In animals stimulated with the 3.0 kHz tone, the inferior colliculus innervated by the intact cochlea showed a restricted band of elevated 2-DG uptake in the appropriate tonotopic location. In contrast, the opposite inferior colliculus showed a band of reduced 2-DG uptake at the same tonotopic location, consistent with inhibitory input from the intact cochlea. This response is is similar to that seen in a normal animal stimulated monaurally.

These results are inconsistent with extensive functional reorganization of the innervation to the inferior colliculus following neonatal cochlear ablation.

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26.2 DENDRITIC ARCHITECTURE IN THE LATERAL SUPERIOR OLIVARY NUCLEUS OF ADULT AND DEVELOPING FERRET. Craig K. Henkel, Mary Wells and Judy K. Brunso-Bechtold. Dept. of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

In order for the developing auditory system to assemble neural circuits for sound localization, central neurons with binaural afferents must form appropriate connections. Presumably this development will be reflected in the maturation of dendritic processes. In this study the normal dendritic architecture of neurons in the lateral superior olivary nucleus (LSO) was studied in the ferret. A modification of the Golgi-Kopsch technique was used and observations are reported from adult material and a postnatal series including ages P0, P4, P6, P7, P8, P12, P24, P21, P22, P24, and P56. In the adult the most commonly observed neurons in LSO distributed dendrites in one of two patterns, fusiform or multipolar. Fusiform cells had two to three thick, smooth, primary dendrites that were obviously oriented across the tonotopic axis of the nucleus. These primary dendrites exhibited very little branching proximally whereas distally they were characterized by a profusion of thin tendrils. The tendrils extended up to 35 µm from the ends of the parent processes. The second pattern was that of multipolar cells with as many as five primary dendrites radiating from the soma and with no apparent orientation to the tonotopic axis. The dendrites of multipolar cells were thinner than those of fusiform cells and exhibited three to four orders of branching. Long, delicate dendritic extensions were frequently observed, but elaboration of tendrils at the ends of these dendrites was uncommon. By the end of the first postnatal week cells with clearly oriented dendrites were observed. More frequently the cells were multipolar and resembled the stellate cells in mature animals. Through postnatal day 24 numerous dendritic appendages and thin filamentous processes with bulbous swellings persisted on all regions of the dendrites and somata. By postnatal day 56 the dendrites and surface of the somata were smooth or exhibited only occasional dendritic spines. The formation of tendrils was not observed to be initiated until the fourth postnatal week. In conclusion, in the ferret at least two cell types were observed in LSO. Other experiments will be required to determine whether or not both types are principal cells with axons projecting to the inferior colliculus. The separate time courses for dendritic orientation and initiation and proliferation of tendril outgrowth suggest that these features of dendritic maturation are correlated with different events and will be explored further.

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26.3 ULTRASTRUCTURE OF THE DEVELOPING MEDIAL SUPERIOR OLIVE IN THE FERRET. Judy K. Brunso-Bechtold, Constance Linville, and Craig K. Henkel. Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

Because of the importance of the medial superior olive (MSO) in mammalian binaural interaction, we have undertaken a detailed study of the development of that nucleus. As the ear canals in the ferret do not open until one postnatal month, the ferret is an excellent model in which to study MSO development. Ferrets were sacrificed on postnatal days (P) 0, 2, 4, 8, 16, 20, and 34 as well as at maturity. All animals were perfused with a fixative of 2% glutaraldehyde/2% paraformaldehyde in .15M cacodylate buffer and the brain was stored overnight in fixative. The brainstem was then sectioned at 200 µm in the coronal plane. The tissue was blocked using a lightfield/darkfield dissecting microscope, postfixed in 2% osmium with 1.5% potassium ferricyanide, dehydrated, and embedded in araldite. Thin sections were mounted on formvar-coated slot grids and viewed with a Zeiss 10-CA electron microscope. On P0 the neurons are extremely immature in appearance with relatively scant cytoplasm and no appendages. There are few synaptic terminals present and those that can be seen are small with rounded vesicles and are most commonly found in the neuropil. Glia can be distinguished at this stage, but there are few of the glial processes which are prevalent at later ages. At P2, P4 and P8, appendages and synapses are present more frequently, although they are still quite sparse. Glial processes are becoming more apparent and often lie adjacent to the somata. At P8, the somata contain more cytoplasm and are more mature in appearance. By P16 and P20 synaptic terminals containing flattened vesicles are common. Terminals are prevalent on the cell body and are considerably larger than at earlier ages although there do not appear to be as many contacts as in the adult. In addition, the terminals are beginning to be encapsulated by glial processes. Numerous appendages are present on the somata and proximal dendrites and myelin figures can be seen in the neuropil. By P34 the nucleus appears quite mature. The synaptic terminals are often large, containing either flattened or rounded vesicles, are found around the somata and proximal dendrites of the oriented cells, and are heavily encapsulated by glial processes. Thus, features of the mature synaptology are beginning to develop in the two weeks preceding opening of the ear canal. Supported by NIH grant NS 23092.

26.4 RAPID CHANGES IN GERBIL ANTEROVENTRAL COCHLEAR NUCLEUS CELL SIZE FOLLOWING TETRODOTOXIN BLOCKADE OF EIGHTH NERVE ACTIVITY. T.R. Pasic* and E.W Rubel. Depts. of Otolaryngology and Physiology/Biophysics, Univ. of Washington Sch. of Medicine. Seattle, WA 98195.

Large spherical cells of the mammalian anteroventral cochlear nucleus (AVCN) receive direct excitatory input from eighth nerve axons. Cochlear ablation has revealed transsynaptic regulation of large spherical cells by eighth nerve axons in neonates of several vertebrate species, including the gerbil. Such changes may be due to loss of eighth nerve electrical activity or loss of activity-independent trophic factors. We have developed a method to chronically, yet reversibly, block eighth nerve electrical activity without violating the integrity of the inner ear. Tetrodotoxin (TTX) was embedded in an ethylene-vinyl acetate copolymer resin (Elvax). A small piece of Elvax containing 250, 500 or 750 ng of TTX was then placed on the round window membrane, allowing the TTX to diffuse into the inner ear. The onset, duration and magnitude of the auditory threshold shift was measured by auditory brain stem response. For all doses, the sound-evoked response was abolished within ten minutes of placement of TTX on the round window membrane. Neural blockade lasted 24 to 46 hours. Implants of Elvax without TTX did not produce a significant threshold shift. TTX, which blocks voltage-gated sodium channels, did not abolish the potassium-dependent cochlear microphonic response.

The regulatory role of afferent electrical activity on gerbil AVCN large spherical cells was examined by measuring their cross-sectional area after one of three manipulations: unilateral eighth nerve action potential blockade with TTX in Elvax, unilateral surgical cochlear ablation or unilateral sham operation (Elvax without TTX). Cell size decreases 21% ipsilateral to TTX placement ($p < 0.01$), 25% ipsilateral to cochlear ablation ($p < 0.01$) and increases 2% ipsilateral to sham operation ($p > 0.50$) by 48 hours after the manipulation. These findings confirm previous work in the avian auditory system (Born, D.E. and E.W Rubel, J. Neurosci., submitted) and support the hypothesis that electrical activity is a major factor in transneuronal regulation. Support was provided by PHS grants T32-NS07246 and R01-NS24518.

26.5 THE ROLE OF MONAURAL AND BINAURAL INPUTS IN THE DEVELOPMENT OF THE AUDITORY SPACE MAP IN THE FERRET SUPERIOR COLLICULUS

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(SPON: European Neuroscience Association)

The superior colliculus (SC) contains topographically aligned maps of auditory and visual space. Both binaural and monaural cues contribute to the generation of the auditory receptive fields. Thus, acute, unilateral ear occlusion or cochlear destruction result in a loss of spatial tuning to white-noise stimuli more than 20 dB above threshold, but have no effect on the sensitivity of SC cells to sound location for near threshold intensities. We have previously demonstrated that the neural mechanisms by which these cells achieve their spatial selectivity can be adjusted by early acoustic experience. Raising ferrets with one ear occluded from before the onset of hearing until adulthood leads to essentially normal spatial receptive fields and map of auditory space in the SC ipsilateral to the occlusion for all sound levels tested (Hutchings, King & Moore, 1986, J. Physiol. 381, 49P). In order to assess the role of the monaural input from the intact, contralateral ear in the maturation of the auditory space map, we have examined the responses of SC cells in adult ferrets subjected to unilateral cochlear ablation at 21 days of age.

Recordings were made in an anechoic room from paralysed, anaesthetized animals when they were 4-5 months old. Within 15 dB of threshold, all cells (n = 28) examined exhibited sharply tuned receptive fields with clearly defined best positions in both azimuth and elevation. The best positions of these units varied in azimuth from the anterior to the posterior field along the rostro-caudal axis of the SC and were, in general, closely aligned with the centres of the visual receptive fields of cells recorded in the overlying superficial layers. However, at higher sound levels, no topography was evident since nearly all cells either gave omnidirectional responses or were sharply tuned to a restricted region of space behind the animal, irrespective of their location within the SC.

The presence of threshold spatial tuning in neonatally cochlear ablated ferrets indicates that this is a response to monaural cues. However, this mechanism appears to be insufficient to maintain tuning at higher sound levels, which is consistent with the effects of acute cochlear destruction. These results therefore suggest that the normal representation of auditory space observed in animals with one ear chronically occluded results from a change in the binaural cues to which the cells respond.

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26.6 DEVELOPMENT OF CROSSED AND UNCROSSED PROJECTIONS FROM THE COCHLEAR NUCLEUS TO THE INFERIOR COLLICULUS IN THE FERRET.

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In a series of studies we have demonstrated plasticity in the number of neurons in each cochlear nucleus (CN) projecting directly to the inferior colliculus (IC) of the ferret. Normally, the ratio of uncrossed to crossed neurons in the adult CN-IC projection is I:C=1:45. Neonatal, unilateral hearing loss (conductive and/or sensorineural) results in a significant change in this ratio, principally if not exclusively because of an increase in the number of ipsilaterally projecting CN neurons. To investigate some possible mechanisms underlying this plasticity we have studied the number and laterality of neurons comprising the CN-IC projection in normal, neonatal ferrets.

Ferrets were produced from timed matings and pregnancies. At ages ranging from postnatal day (P) 9 to P80 multiple injections of 10% WGA-HRP (200-300nl) were made into the left IC. Animals were perfused 48hrs. later and the brains were reacted using TMB histochemistry. For every third, 40μm frontal section the position of retrogradely labelled neurons in each CN was drawn on a camera lucida. Counts were made for each division of the CN, as defined by the neutral red counterstain, and corrected for missing sections.

In the youngest ferrets (P9) it was not possible to perform reliable quantitative analysis. Nevertheless, there appeared to be more labelled CN neurons ipsilateral to the injected IC than in adults. In older animals (P18, P26) quantitative analysis showed a significant ($p < 0.01$) increase in both the number of ipsilaterally labelled CN neurons and the ratio (P18: I:C=1:33, P26: I:C=1:29) of ipsi- to contralateral labelling, relative to adults. These increases were similar in magnitude to those observed following neonatal cochlear lesions (I:C=31). At older ages (P39, P57, P77) the ratio (I:C) did not differ significantly from the adult ratio. No age-related changes in the contralateral projection were observed.

These results show that, in common with the retinotectal system, there is an initial exuberance in the number and/or terminal arborization of ipsilaterally-projecting CN neurons in the auditory brainstem.

26.7 VISUAL DEPRIVATION DEGRADES THE AUDITORY MAP OF SPACE IN THE OPTIC TECTUM OF DEVELOPING BARN OWLS.

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In barn owls reared under normal conditions, auditory neurons in the optic tectum are sharply tuned for sound source location and are systematically organized according to their spatial tuning to form a map of space. In addition, these neurons respond to visual stimuli and the visual and auditory maps of space in the tectum are in register.

Auditory spatial tuning derives from the central processing of localization cues such as frequency-dependent, interaural differences in timing and intensity of sound; the auditory space-map results from a systematic variation in the tuning of neurons for these cues. Does the development of this "computational" map depend upon vision? In part, it does.

Two barn owls were raised with both eyelids sutured shut. When full grown, the eyelids were opened and the owls were prepared for tectal recording. Auditory-visual units in the optic tectum were tested for location of auditory and visual receptive fields and sharpness of auditory spatial tuning. The data show that the auditory spatial tuning of single units was normal in sharpness (based on size of the spatial region from which the unit responds with greater than 50% of maximum response) and that spatial tuning varied approximately normally across the tectum. Thus, vision is not necessary for the development of a basic auditory map of space.

However, the alignment of auditory spatial fields with visual receptive fields of units was abnormally poor. Whereas, visual receptive field locations progressed systematically across the tectum, auditory spatial tuning changed relatively erratically. The data support the hypothesis that visually-guided experience during early life is responsible for the precision of the auditory map of space observed in the tectum of normal adults.

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26.8 RAPID PROLIFERATION OF GLIAL PROCESSES FOLLOWING DEAFFERENTATION OF N. MAGNOCELLULARIS IN NEONATAL CHICKENS.

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The brain stem auditory system of neonatal chickens has proven to be a useful preparation in which to study afferent regulation of neuronal elements in the central nervous system. Previous studies have shown rapid changes in cell size, protein systhesis and metabolic properties in n. magnocellularis (NM) neurons following removal of the cochlea. Neuronal changes are evident within 1 hour of cochlea removal and well defined within 6 hours. Recent experiments indicate that an important regulatory signal underlying these transneuronal events is the activity of 8th nerve afferents to NM.

We have begun to examine changes in non-neuronal elements that may be regulated by neuronal activity. Changes in glial fibrillary acidic protein (GFAP) immunoreactivity were studied in astrocytes of NM following unilateral removal of the cochlea. The cochlea was removed in neonatal chickens (5-14 days posthatch) in such a way as to spare eighth nerve ganglion cells. Following survival times ranging from 1-24 hours, the chicks were anesthetized and perfused with a fixative containing paraformaldehyde, lysine and sodium meta-periodate. Serial sections through NM were cut on a vibratome or from paraffin embedded tissue and reacted with a rabbit antiserum against bovine GFAP (Dako, Inc.) at concentrations of 1:400 or 1:800. Control sections were incubated in nonimmune purified rabbit IgG.

GFAP staining in NM ipsilateral to the cochlea removal was compared to that observed in the contralateral, normally innervated, NM in the same tissue section. One hour following cochlea removal there was no obvious difference in staining for GFAP on the two sides of the brain. However, in every subject sacrificed 3 hours after cochlea removal there was an increase in the intensity of the reaction product, the number of immunoreative processes and the length of immunoreactive processes in NM on the side of the brain ipsilateral to the cochlea removal. This initial change is first seen primarily at the periphery of the nucleus but is evident throughout NM by 6 hours after cochlea removal. After 12 hours the glia reaction is marked by intensely immunoreactive cell bodies and processes in patches throughout NM and bordering the nucleus. Degenerating terminals are not yet apparent. These results suggest that elimination of neuronal activity can activate glial expression of GFAP and elongation of astrocytic processes well before actual degeneration of axons or synaptic terminals. Supported by NINCDS grant #NS24522.

26.9 SYNAPSES IN EARLY NEUROGENESIS OF THE TANGENTIAL VESTIBULAR NUCLEUS OF THE CHICK EMBRYO. R. Petralia and K.D. Peusner. George Washington Univ. Sch. of Med., Washington, D.C. 20037.

The chick tangential nucleus (TN), in the lateral vestibular complex of the medulla, is composed essentially of two neuron types, principal (PC) and elongate cells (EC). Migration and differentiation of PC occur mainly between 6 and 13 days in the embryo (Golgi method; Peusner and Morest, 1977). The major input to the embryonic PC is the spoon endings (SE), formed by colossal vestibular fibers; small vestibular fibers also form terminals on the PC. Colossal fibers form synapses on PC around 8 days, with spoon ending formation between 10 and 13 days. In the absence of the vestibular nerve, the PC migrate and differentiate normally from 5-8 days, but thereafter degenerate and die by 13 days. Thus, completion of differentiation and survival may depend in part on spoon synapses. However, what influences cell migration and the outgrowth of axons and dendrites from 5-8 days is unknown and is the issue of the present ultrastructural investigation.

At the earliest age studied (5-6 days), the TN can be identified in light microscope, toluidine-blue stained sections, where fewer cells appear at progressively anterior levels of the TN, suggestive of a developmental gradient. The precursors of PC, the primitive epithelial cells (PEC), enter the TN from a medial-to-lateral direction, while at an earlier age the precursors of the EC proceed from a dorsal-to-ventral direction. At 5 days, the presumptive TN contains 3 types of processes identified by ultrastructure: vestibular fibers, external processes of PEC, and longitudinal fibers (presumptive dorsal spinocerebellar fibers). Between 5-6 days, the longitudinal fibers or their short branches form synapses on the lateral processes of the mediolateral PEC. At 6-6.5 days, the longitudinal fibers form synapses on the lateral processes of the postmigratory PC, while at 6.5-7 days the longitudinal fibers form synapses on the somata of the PC. These synapses are immature with few, round synaptic vesicles apposed to asymmetric membrane densities.

In conclusion, although the TN is an interstitial nucleus of the vestibular nerve, the very first synapses are formed by non-vestibular inputs. Conceivably migration and early differentiation of PC between 5-8 days may depend on contact by these first synapses. Moreover, these first synapses may influence axon outgrowth and connection to cervical spinal cord (Gross and Oppenheim, 1985; Cox and Peusner, 1987). Supported by NIH grant R01 NS18108.

26.10 RESPONSE OF THE RAT FETUS TO OLFACTORY STIMULATION PRESENTED IN UTERO. W.P. Smotherman. Laboratory for Psychobiological Research, Departments of Psychology and Zoology, Oregon State University, Corvallis, OR 97331.

Techniques for manipulating and directly observing the rat fetus in utero are providing information about mammalian behavioral development before birth [Smotherman & Robinson, Animal Behavior 34: 1859-1873, 1986]. Late in gestation rat fetuses are responsive to changes in their intrauterine environment and exhibit behavioral responsiveness to chemosensory stimulation [Smotherman & Robinson, Behavioral Neuroscience, in press].

Nineteen and 20-day-old rat fetuses exhibit a pattern of behavioral activation following intraoral infusion of a solution containing a novel olfactory stimulus. Fetuses that receive a 20 ul pulse of a lemon solution through an intraoral cannulae show a spike in behavioral activity coincident with the moment of infusion. The importance of olfaction in mediating this response is underscored by the virtually identical pattern of behavioral activation exhibited by fetuses that receive an intraoral infusion of citral -- a tasteless lemon odor. This conclusion is further supported by the results of experiments involving surgical transection of the olfactory bulbs. Following olfactory bulb transection, 19 and 20-day-old fetuses exhibit no change in spontaneous activity or in response to tactile stimulation, but fail to show behavioral activation in response to lemon or citral infusion. The effect of olfactory bulb transection is greater on day 19 than on day 20 of gestation suggesting that the behavioral responsiveness may be mediated by multiple sensory modalities in the older fetus.

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26.11 OLFACTORY DENERVATION IN THE NEWBORN (BUT NOT ADULT) RATS REDUCES THE NUMBER OF MITRAL AND TUFTED CELLS AND OF GLOMERULI IN THE OLFFACTORY BULB. E. Meisami and S. Emamian. Dept. of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.

Newborn male albino rats were denervated unilaterally by cutting the olfactory nerve in front and underneath the olfactory bulb (OB). At 30-days the operated and control OBs were removed, fixed, sectioned frontally and serially at 10um, and Nissl(thionin) stained. Similar procedures were carried out in normal 30-day rats and in rats operated at 30-days and examined at 60-days. All bulbs were subjected to morphometric and numerical histoarchitectural analysis. The results showed that in the normal rat: 1) no significant difference existed between the left and right OBs of normal rats; 2) mean number of glomeruli (based on counts of spherical or elipsoid periglomerular cell-assemblies) was about 3100 per OB, and mean glomerular diameter 71 um; mean values for total number of mitral and tufted cells were about 63000 and 130,000 per OB, respectively.

In the operated 30 day-old rats: 1) the denervated bulbs were markedly smaller(30%). 2) of the OB layers, only the olfactory nerve layer was completely absent, indicating lack of regeneration of the olfactory nerve; other OB layers (glomerular, external and internal plexiform and the internal granular) were, however, markedly (30-40%) reduced in thickness; glomerular layer appeared particularly disorganized; 3) While the total numbers of glomeruli, mitral and tufted cells in the control OBs of the operated animals did not differ significantly from those in the normal animals, these numbers were markedly reduced in the denervated OBs: glomerular diameter, 34%; glomerular number, 25%; mitral cell number, 31%; tufted cell number 64%. Comparison of distribution profiles of these bulbar elements in the control and operated bulbs revealed that the reductions occured along the entire OB length. OBs operated at 30-days and examined at 60-days were smaller and had slightly reduced numbers of the bulbar elements but the differences were not statistically significant. The results indicate that during the postnatal development (sukling period) the proliferating and growing fibers of the olfactory neurons exert profound transneuronal effects on the development and structural organization of the olfactory bulb.

26.12 PROLIFERATION PATTERNS DURING POSTNATAL DEVELOPMENT IN THE OLFACTORY BULB OF NORMAL AND UNILATERALLY DEPRIVED RATS. L.L. Frazier and P.C. Brunjes. Dept. of Psychol, Univ. of Virginia, Charlottesville, VA. 22903.

Unilateral external naris closure on postnatal Day 1 results in a 25% reduction in the volume of the olfactory bulb 30 days later (Brain.Res.Rev,11, 1). The small size of deprived bulbs has been shown to be due at least in part to dramatic decreases in cellular densities and numbers of the latest arising-relay (external tufted cells) and interneuronal (granule cells) cell populations and the latter groups' associated glia (Soc.Neurosci.Abst.,12, 125). Changes in cell number may result from alterations in cell proliferation, cell death, or both. Several lines of evidence suggest a key role for cell death, including observations that granule cell number in adult rats appears to be regulated by cell death (J.Comp.Neurol.,239, 117), and the finding that unilateral deprivation has little effect on laminar volume, cell density or number in the proliferative zone of the bulb, the subependymal layer (SUB). Nevertheless, evidence of a consistent developmental lag in the number of granule to relay cells has been reported, suggesting changes in proliferation rates should not be excluded. The current study assessed proliferation patterns in both normal and deprived bulbs. Occluded and sham-operated rat pups were injected with 3H-thymidine on postnatal Days 2, 5, 10, 20, or 30. Bulbs were removed 24 hr later, embedded in JB4, sectioned horizontally, and processed for autoradiography. Sections representing the middle of the SUB were selected and counts of the number of labelled vs. unlabelled cells made at 25, 50 and 75 % of the layer's rostral-caudal extent. A gradient of proliferation was found with higher numbers of labelled cells at 50 and 75% of the layer's extent at Days 2 and 5, and more at 75% at Day 10. Decreased numbers of labelled cells were found by Day 20, and only in the caudal SUB. The findings suggest a rostral to caudal proliferation pattern in early life. No differences in numbers or patterns of labelled cells were encountered between normal and deprived subjects at Days 2, 5 or 10, indicating the procedure does not affect early cellular proliferation. Supported by the ONR, Whitehall Foundation and NIH (NS23154)

26.13 ROLE OF HOST PLANT AND SENSORY ENVIRONMENT IN MATURATION OF OLFACTORY RECEPTOR SYSTEM OF THE BOLL WEEVIL, ANTHONOMUS GRANDIS. J. C. Dickens* and E. E. Moorman* (SPON: D. Stork.) USDA-ARS Boll Weevil Research Unit, Mississippi State, MS 39762.

We investigated maturation of olfactory receptors in boll weevils fed either their host plant, cotton, or an artificial diet from time of emergence. We recorded summated olfactory receptor potentials (electroantennograms=EAGs) from different age groups of weevils in response to serial dilutions of a non-specific host plant odorant (1-hexanol, a component of "green" odor), a specific host plant odor (beta-bisabolol, a sesquiterpene reported only from the host plant and its close relatives), and the specific aggregation pheromone of the weevil (grandlure, a mixture of four specific insect-produced odorants). In order to facilitate comparisons among dosage response curves, the following nonlinear model was used

$$V = \frac{Vmax}{1 + e^{-k * Lrate}}$$

where V is the response variable, Vmax is the maximum receptor potential and Lrate is the dosage of the odorant. No differences were found in either Vmax or k for insects of the same age and sex fed on their natural host plant containing both 1-hexanol and beta-bisabolol or the artificial diet which was devoid of these odorants. Vmax increased from day of emergence through day 4 of adulthood in both groups. While k tended to increase through day 6, these differences in k were not significant. Overall k values were different for each chemical and thus were indicative of separate receptor populations. If Vmax is considered a measure of acceptors responsive to each odorant, then the number of responding acceptors increases from emergence through day 4 regardless of diet or concomitant sensory environment. Since k is measure of the dynamics of acceptor response and remains fairly constant, changes in responses (V) elicited by a particular odorant must be due to changes in the size of the population of responding acceptors rather than dynamics of individual acceptors. Similar to the boll weevil, studies of the olfactory receptor system of the workerbee indicated maturation to take place on day 4 (Masson, C. M. and Arnold, G. A., J. Insect Physiol. 30:7, 1984). However, considerable plasticity was noted in the olfactory receptor system of the workerbee since EAGs elicited by a particular odorant varied according to whether individuals were reared in isolation or groups. Maturation of the olfactory receptor system of the boll weevil appears to be more predetermined, at least with regard to diet and concomitant sensory environment. These results correlate well with ecology and behavior of both the boll weevil and the workerbee.

26.14 POSTNATAL DEVELOPMENT OF THE ROSTRAL SOLITARY NUCLEUS IN THE ALBINO RAT: ANALYSIS OF PLANAR DENDRITIC LENGTH, MITOCHONDRIAL ENZYME ACTIVITY, AND GABA-LIKE IMMUNOREACTIVITY. P.S. Lasiter and D.L. Kachele*. Dept. of Psychol., Florida Atlantic University, Boca Raton, FL 33431.

Taste responses develop in lower-order central gustatory relays during a prolonged period of rats' postnatal life. We have previously reported that planar dendritic growth and activity of the mitochondrial-linked enzymes cytochrome oxidase (CO) and succinic acid dehydrogenase (SDH) is well-correlated with the development of taste responsivity in the second-order central gustatory relay located in the medial parabrachial nucleus (Lasiter, P.S. et al., Abst. Assoc. Chem. Soc., 9:85, 1987). The present studies examined development of neurons in the first-order central gustatory relay located in the rostral pole of the nucleus of the solitary tract (NST). Three distinct methodologies were employed; Golgi impregnations, histochemistry for CO and SDH, and staining for GABA-like immunoreactivity (GABA-LI). The rostral NST contains neurons with five distinct somatic morphologies. These neurons can be classified as small (F1) or large (F2) fusiform types, with mean aspect ratios (AR; minimum central cross-sectional diameter/maximum central cross-sectional diameter) of 0.274 and 0.372, respectively, small (M1) or large (M2) multipolar types with mean ARs of 0.459 and 0.461, respectively, and an oviform type (Ov) with a mean AR of 0.875. Morphometric analyses performed according to somatic type indicated that mean lengths of first- and second-order dendrites do not reliably increase between 11 days and 65 days. Mean first-order dendritic lengths were 34.56 μm for F1 types, 62.96 μm for F2 types, 31.98 μm for M1 types, 46.64 μm for M2 types, and 46.44 μm for Ov types. Histochemical studies indicated that activity of CO and SDH increases in the rostral NST from 10 days to 20 days, and that CO and SDH activity is asymptotic after approximately 25 days. GABA-LI immunoreactivity was confined to F1 and M1 neuron types in the rostral gustatory-responsive portion of the NST. Somatic GABA-LI of F1 and M1 neurons increased from 10 days to 20 days, and reached adult-like levels after approximately 22-25 days. These results indicate that the development of taste responsivity in the rostral NST during the first five weeks of life is probably not related to extensive radial dendritic growth. However, ontogeny of gustatory responsivity in the NST may be related to the maturation of peripheral receptor/fiber systems that provide input to NST neurons or maturation of neurochemical systems that promote synaptic transmission in the NST.

26.15 **THE PRENATAL "SENSITIVE PERIOD" OF ENVIRONMENTAL INFLUENCES ON THE DEVELOPING GUSTATORY SYSTEM.** **D.L. Hill and P.R. Przekop*. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.**

The development of taste in rat is a postnatal phenomenon in which sodium, initially a poor stimulus, becomes very effective. Development appears to involve the addition of an amiloride-sensitive sodium component to the receptor membrane which is absent in the immature rat. Since the largest developmental changes occur to salt stimuli, especially NaCl, the development of salt taste responses might be especially susceptible to environmental influences. Indeed, responses from the chorda tympani nerve to sodium salts are lower in rats fed a low-sodium (0.03%) diet beginning at 3 days gestation compared to controls. However, responses to non-sodium stimuli are unaffected. In adult rats, sodium deprivation has no effect upon multifiber chorda tympani responses. Therefore, a specific time period exists in which the taste system can be altered functionally by modifications in the diet. The present study examines this time period by instituting sodium deprivation during different periods of both pre- and postnatal development and comparing multifiber chorda tympani responses among deprivation groups. Concentration series (0.05-0.5M) of NaCl, NH_4Cl, KCl, and sodium acetate were flowed over the anterior tongue. The tongue was also stimulated with NaCl after lingual application of the epithelial sodium transport blocker, amiloride hydrochloride. Results suggest that the degree of response attenuation to sodium salts (NaCl & sodium acetate) is determined by the **prenatal** age at which sodium deprivation is imposed upon the animal. There appears to exist a continuum with given endpoints corresponding to periods of development when deprivation has a maximal effect through no effect. Importantly, these endpoints correspond to 8 days gestation (maximal effect) and 15 days gestation (no effect); deprivation imposed at 10 to 11 days gestation has an intermediate effect. Therefore, deprivation is not an all or none phenomenon in altering sodium responses. Moreover, the taste system remains plastic for a relatively short period of early development, with the degree of plasticity changing during this period. In summary, we have demonstrated there exists a "sensitive period" in which environmental factors have a permanent influence on the developing gustatory system. This period apparently occurs before the appearance of peripheral gustatory structures. Although these effects are reversible at the peripheral level, they may not be reversible in the central nervous system.

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27.1 TOLERANCE TO HALOPERIDOL-INDUCED "ANOREXIA" IS NOT CONTINGENT ON BEHAVIORAL EXPERIENCE. D. L. Wolgin and G. B. Thompson*. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

Two experiments were performed to determine whether tolerance to the "anorexigenic" effect of haloperidol is contingent on access to food in the drugged state. In the first experiment, groups of rats were given the drug (2.5 or 5 mg/kg) either before (Group Pre) or after (Group Post) a 30-min milk drinking session for 54 days. A control group (Group Sal) was given injections of saline. To control for cumulative deprivation during this phase of the experiment, the intakes of Group Post and Group Sal were yoked to that of Group Pre. On the test day (Day 55), all of the groups were given haloperidol prior to milk to assess the level of tolerance. During the chronic phase, rats receiving the drug prior to milk (Group Pre) recovered to only 45% (2.5 mg/kg) and 25% (5 mg/kg) of baseline levels of milk intake. On the test day, these rats showed less tolerance than rats given chronic injections of the drug after feeding (Group Post).

In the second experiment, dose-response curves were determined both before and after rats received chronic injections of either haloperidol (2.5 mg/kg) or saline for 14 days. During the chronic phase, haloperidol-treated rats were injected with the drug either before or after access to milk, as above. On the initial dose-response determination, haloperidol decreased milk intake at all dose levels (1.25, 2.5, and 5 mg/kg). Following chronic injections of the drug, there was a shift in the dose-response curves upward and to the right in both Group Pre and Group Post, but not in Group Sal. Comparisons of the ED50's before and after chronic exposure to the drug revealed a four-fold shift in both drugged groups (Group Pre = 3.97; Group Post = 4.66). These results suggest that tolerance to the "anorexigenic" effect of haloperidol is not contingent on access to food in the drugged state.

27.2 LONG-TERM EFFECTS OF MELPERONE ON ORAL MOVEMENTS IN RATS. P. Johansson*, L.M. Gunne*, and U. Bondesson*. (SPON:G. Ellison) Psychiatric Research Center, Ulleråker Hospital, S-750 17 Uppsala, Sweden.

Chronic administration of a variety of antipsychotic drugs, including haloperidol, fluphenazine, chlorpromazine, and thioridazine, has been found to elicit an increased rate of vacuous chewing movements (VCM) in rats (Gunne et al., Pharmacol. Biochem. & Behav. 25:897, 1986). On the other hand, the atypical neuroleptic, clozapine, has not been found to cause this effect over a wide dose range (Johansson et al., Psychopharmacol. Bull. 22:1017, 1986). This potential model for tardive dyskinesia (TD) has been applied to test a variety of antipsychotic drugs for their TD-inducing propensity.

Melperone is an effective antipsychotic drug (Bjerkenstedt et al., Arch. Psychiatr. Nervenkr. 226:157, 1978), with many characteristics similar to clozapine. There are few reports of TD connected with this drug.

In the present experiment rats were chronically given doses of melperone (25, 60, and 125 ug/ml) or haloperidol (2.0 and 5.0 ug/ml) in their drinking water for a period of 16 months. VCM rates were measured monthly. All groups had low starting levels of VCMs with no significant differences between groups. After three months the haloperidol groups started to show significant increases in VCMs compared to controls. This effect continued for the rest of the 16 months experiment. The melperone-treated groups did not differ from the controls. These doses of melperone do not appear to cause increased VCMs in this model of TD.

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27.3 NEUROLEPTIC-INDUCED ORAL DYSKINESIAS: METHODOLOGICAL ISSUES. A.D. Levy, R.E. See, E.D. Levin & G. Ellison. Dept. Psychology, UCLA, Los Angeles, CA 90024.

Animal models of neuroleptic-induced dyskinesias have been controversial; alterations in oral activity in laboratory animals have been described as a model of tardive dyskinesia (TD) or acute dystonia. Some researchers have observed early onset enhancement of oral movements resembling dystonia, others have observed a latent syndrome more characteristic of TD, while some have not observed alterations in oral activity.

In 3 separate experiments, rats were chronically administered haloperidol (HAL) in a variety of ways (chronic injections given twice weekly, subcutaneous implants, and chronic decanoate injections) and examined for oral movements in two testing environments -- in an open cage, and in a Plexiglas tube to facilitate observation of the oral region. In the open cage, oral activity was enhanced during neuroleptic exposure and subsided rapidly following drug withdrawal. In the tube, oral activity was low during drug treatments, and elevated upon drug withdrawal. The final study was designed to explain these trends. Rats were examined in each testing apparatus on each of 4 days of the chronic HAL injection cycle. Immediately following HAL injections oral activity was elevated and motor activity nearly eliminated in the observation cages, while oral movements in the tube were not altered. On subsequent days, motor activity scores began to rise, and oral movements returned to control levels.

The data suggest that the method of data collection has a substantial impact on interpretations. The negative correlation between oral and motor activity scores in the observations cages suggests that an artifact related to motor activity influences oral activity scores. Active rats show few oral movements in the cages, while most oral activity is observed when rats are otherwise immobile. The data have implications for TD research. Open cage environments may detect early onset neuroleptic-induced oral activity, while the tube may be better suited for observing latent neuroleptic-induced alterations in oral movements. Clearly animal models of TD and dystonia must be reevaluated to account for the differences obtained in each testing environment.

(Supported by MH39961).

27.4 BEHAVIORAL AND RECEPTOR CHANGES IN RATS FOLLOWING CHRONIC TREATMENT WITH HALOPERIDOL DECANOATE AND FLUPHENAZINE DECANOATE. R.E. See, J. Williams, & G. Ellison Dept. of Psychology, UCLA, Los Angeles, CA, 90024.

We have previously found that chronic neuroleptic administration in rats can produce a syndrome of late-onset increases in oral activity, particularly of small amplitude oral movements as recorded by a computerized movement detection circuit. In the present study, rats were treated for 10 months with either haloperidol decanoate, fluphenazine decanoate, or vehicle. Extensive behavioral observation utilizing this computerized system as well as a human observer showed the development of a complex syndrome of oral activity changes in the drug-treated animals, which persisted upon withdrawal.

In order to examine some of the possible changes in receptor populations that may underlie these behavioral changes, autoradiographic analysis was conducted on the brains of these neuroleptic-treated rats. Rats were killed by decapitation 50 days after drug withdrawal and the brains removed and stored at -80 C. Twenty micron coronal sections were thaw mounted onto subbed microscope slides. Both D1 and D2 receptors were localized as well as GABA receptors. For D1 binding, serial sections were incubated in Tris-HCl buffer (50 mM, pH 7.4) containing [^{3}H]SCH 23390 (77.7 Ci/mmole) at a concentration of 1.0 nM. Adjacent sections were incubated in buffer that included unlabeled SCH 23390 (1 uM) for nonspecific binding. For D2 binding, sections were incubated in buffer containing [^{3}H]spiperone (21.0 Ci/mmole) at a concentration of 1.4 nM, along with mianserin (1 uM), a 5-HT inhibitor. Nonspecific binding was represented by the addition of sulpiride (10 uM). For examining GABA receptors, sections were incubated in a Tris-citrate buffer (0.31 M, pH 7.1) containing [^{3}H]muscimol (39.4 Ci/mmole) at a concentration of 10 nM. GABA (0.2 mM) was used for nonspecific binding. Specific binding of each [^{3}H] ligand was as follows: [^{3}H]SCH 23390, 90-95%; [^{3}H]spiperone, 50%; and [^{3}H]muscimol, 75-80%.

Slide-mounted tissue sections were apposed to LKB Ultrofilm in X-ray cassettes for 3 to 6 weeks. Films were developed and then examined using an IBAS 1+2 image analyzer connected to a video camera. Differences in regional binding between groups will be discussed as well as correlations with behavioral changes found in the drug-treated animals.

(Research supported by MH 39961.)

27.5 CHRONIC NALTREXONE ALTERS MORPHINE-INDUCED DOPAMINE SYNTHESIS IN MESOLIMBIC AND NIGROSTRIATAL SYSTEMS. M. T. Bardo, J. L. Neisewander and R. B. Ennis*. Department of Psychology, University of Kentucky, Lexington, KY 40506.

Adult male rats were implanted with a slow-release pellet of naltrexone or they were given sham surgery. Ten days later, the naltrexone pellet was removed or a second sham surgery was performed. At one of various different intervals during or after pellet implantation, dopamine (DA) synthesis was assessed in the mesolimbic and nigrostriatal systems. Each animal was administered interperitoneally 100 mg/kg M-hydroxybenzylhydrate dihydrochloride, an inhibitor of the enzyme dihydroxyphenylalanine (DOPA) decarboxylase. Thirty min after injection, each animal was sacrificed by rapid decapitation and the olfactory tubercle, nucleus accumbens, anterior striatum and midbrain A9/A10 region were dissected for analysis of DOPA levels, an index of tyrosine hydroxylase activity. In order to assess the effect of morphine, in some experiments animals received either morphine (15 mg/kg, s.c.) or saline 30 min prior to M-hydroxybenzylhydrate.

The results indicated no significant change in DA synthesis either 1 or 10 days after the start of pellet implantation. As expected however, morphine increased DA synthesis in both the mesolimbic and nigrostriatal systems in sham control animals. More important, one day after naltrexone pellet removal, when mu-type opiate receptors are increased, there was an increased response to the DA-stimulating effect of morphine in olfactory tubercle. At 3 and 10 days after cessation of naltrexone exposure however, a decreased response to morphine was evident in olfactory tubercle, nucleus accumbens, striatum and A9/A10 region. The onset of subsensitivity to morphine occurred earlier in the nigrostriatal system than in the mesolimbic system. Thus, chronic naltrexone treatment induces a biphasic change in DA systems, with a period of supersensitivity followed by a rebound period of subsensitivity to opiates.

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27.6 **EFFECT OF NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE ON THE YAWNING BEHAVIOUR OF THE RAT.**

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Systemic administration of L-Monosodium Glutamate (MSG) to neonatal rodents produces lesions in the retinae, the circumventricular organs (e.g. the subfornical organ, area postrema) and several hypothalamic nuclei, resulting in a number of behavioural, metabolic and endocrinological abnormalities. The MSG treatment produces a loss of 80-90 % of the cell bodies of dopaminergic neurons whitin the arcuate nucleus and also of the pro-opiomelanocortin system, with an alteration of intra-hypothalamic neurotransmitter levels. The present study was carried out to investigate the effect of these lesions on the regulation of yawning behaviour. 27 male Sprague-Dawley rats were injected intraperitonelly with a solution of MSG (55.5 %) or equivolumetric doses of 13% NaCl (osmotic control) in an ascending dose schedule from 2.0 to 4.0 g/Kg on postnatal days 1-5. Histological analysis demonstrates a loss of neuronal bodies in the arcuate; and a diminution of 30 % of the hypophysis weigth ($p < 0.005$ Student t test). The behavioural observations were performed between 16-17 hrs. when the rats reached 30 and 60 days old. Yawns and penile erections were monitored and clocked by two observers, under standard conditions. Early postnatal administration of MSG resulted in a decrease of 50 % in spontaneus yawning frequency ($p < 0.005$ Mann-Whitney U test) and 36 % of apomorphine induced yawning (0.05 mg/Kg) ($p < 0.01$ Mann-Whitney U test) in 60 days old rats, but not at 30 days. The results suggest that direct damage of hypothalamic dopaminergic and pro-opiomelanocortin neurons, or its indirect effects on hypophyseal function (secretion of ACTH and MSH), may produce the above described significant decrease of yawning frequency.

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27.7 NEUROLEPTIC MALIGNENT SYNDROME IS ASSOCIATED WITH LOW LEVELS OF SERUM IRON. Patricia I. Rosebush*, (SPON: Michael F. Mazurek). Dept. of Psychiatry, Univ. of Toronto and Harvard Medical School.

Neuroleptic malignant syndrome (NMS) is a clinically-defined disorder characterized by fever, rigidity, and obtundation. Serum levels of creatine phosphokinase (CPK) are elevated in virtually all cases. While the aetiology of NMS is unknown, its pathogenesis is believed to involve an increased blockade of dopaminergic transmission.

We have studied 23 consecutive cases of NMS in 19 individual patients (4 patients developed NMS on 2 separate occasions). 21 of the 23 episodes (18 of 19 patients) were associated with subnormal levels of serum iron, with values falling 70% or more below the lower limit of normal in 7 patients. In each case for which data are available the serum iron returned to normal levels upon resolution of the NMS. Where multiple data points were available, there was a strong negative correlation between levels of serum iron and concentrations of serum CPK. There was no correlation between serum iron and body temperature.

These results raise the question whether the decrease of serum iron levels might play a role in the pathophysiology of NMS. Iron is known to be important for dopamine receptor function, low iron resulting in decreased dopamine receptor binding through a loss of dopamine receptors, while the affinity of the receptor remains unchanged. It is especially interesting in this regard that iron deficient patients are known to suffer from akathesia, a movement disorder generally believed to arise from dopaminergic blockade in the nigrostriatal pathway.

27.8 PLASMA PROLACTIN RESPONSE TO COCAINE AFTER HALOPERIDOL PRETREATMENT. D. B. Lozovsky, M.A. Sherer*, K.M. Kumor* and J.H. Jaffe*. Addiction Research Center, NIDA, Baltimore, MD 21224.

Cocaine inhibits neuronal uptake of norepinephrine, dopamine and serotonin (Carmichael, F.J. and Israel, Y., J. Pharmac. Exp. Ther. 186:253,1973; Ross, S.B. and Renui, A.L., Eur. J. Pharm. 2:181,1967; Snyder, S.H. and Coyle, J.T., J. Pharmac. Exp. Ther. 165:78, 1969; Ross, S.B. and Renuci, A.L., Eur. J. Pharm. 7:270,1969). Since these transmitters regulate neurohormones differently, neuroendocrine changes induced by cocaine might vary depending on the time course and dose responsiveness to cocaine and the levels of activity of the neurotransmitter systems involved. This variability might be responsible for the controversial results reported on the effects of cocaine on plasma prolactin (PRL) levels in drug abusers (Dakis, C.A. and Gold, M.S., Neurosci. & Behav. Rev. 9:469,1985, Gawin, F.H. and Kleber, H.D., Brit. J. Psychiat.147: 569,1985). The purpose of this study was to investigate whether cocaine can affect PRL secretion via non-dopaminergic mechanisms. Accordingly, we measured plasma PRL after cocaine administration to subjects pretreated with the dopamine receptor blocker, haloperidol. The subjects were five inpatient male cocaine users, aged 22 to 34 yrs. Four conditions (placebo/placebo, placebo/cocaine, haloperidol/placebo, and haloperidol/cocaine) were studied in a double-blind randomized design. Haloperidol (8 mg, IM) or placebo were given 20 min prior to cocaine (40 mg, IV) or placebo. Blood samples were taken 10 min before and 10 min after administration of haloperidol or placebo and 15,30, 45,60,90,120 and 180 min after administration of cocaine or placebo. Plasma PRL was measured by a double-antibody RIA. While there was a trend toward a slight decrease in plasma PRL after the placebo/cocaine as compared to the placebo/placebo condition, haloperidol induced the expected sustained rise in PRL. Infusion of cocaine after haloperidol resulted in additional increases in plasma PRL which were significant at 45 min time point (37% higher than after haloperidol/placebo). Similar trend was also noted for the 30 and 60 min time points. This PRL-elevating effect of cocaine in subjects with blocked dopamine receptors might result from the drug-induced inhibition of serotonin uptake.

27.9 COCAINE EFFECTS ON PLASMA VASOPRESSIN, CORTICOSTERONE, AND PROLACTIN IN THE RAT: TIME COURSE FOLLOWING ACUTE INJECTION. E.H. Ellinwood, Jr., J.K. Nishita, C.M. Kuhn, J.C. Ritchie, and L. Bero*. Dept. of Psychiatry and Pharmacology, Duke Univ. Med. Cntr., Durham, NC 27710.

The possibility that endogenous release of arginine vasopressin (AVP) in the brain interacts with drugs of abuse as epiphenomena has provided a potential basis for clinical utility. For example, in rats AVP can markedly alter the development and maintenance of tolerance to the physiological and behavioral effects of repeated exposure to ethanol. However, plasma AVP levels in rats are not merely reduced by ethanol, but follow a biphasic time course (elevated 5 minutes after i.p. injection and inhibited 30-60 minutes after injection). In the present study we examined the short-term time course of AVP release and that of two stress-related hormones, corticosterone (CORT) and prolactin (PRL), following the acute injection of cocaine.

Pairs of male Sprague-Dawley rats (250-310 g) were randomly injected i.p. with one of three doses of cocaine (10, 20, or 40 mg/kg) or saline. All rats were tested during hours 2-4 at the beginning of the colony light phase (12:12 LD-cycle). A separate control group received no injection. Rats were decapitated at 5, 10, and 20 minutes following the injection. Trunk blood was collected in chilled heparin-coated tubes and the whole brains were rapidly extracted and frozen on dry ice. The entire sacrifice and specimen collection procedure was completed within 2-3 minutes. Plasma AVP, CORT, and PRL were assayed by RIA along with osmolality (by freezing point depression) and electrolytes, sodium and potassium (NOVA 11 Analyzer).

Plasma AVP levels were not affected by the 10 mg/kg dose of cocaine and no significant change in AVP was found for any of the dosages at 5 minutes after their injection. Ten minutes after the 20 mg/kg cocaine injection, AVP levels were significantly elevated (10.5 $\pm$ 1.7 pg/ml) compared to saline-injected controls (6.1 $\pm$ 0.7 pg/ml; $p<.05$). This effect was no longer apparent at 20 minutes after injection. The 40 mg/kg cocaine injection produced similar but attenuated increases in AVP levels at 10 and 20 minutes after injection.

CORT levels were elevated by the cocaine injections and were not significantly different across the three time points. PRL, osmolality, and electrolyte levels were not affected by any of the cocaine regimens. Assays of specific brain tissue areas remain to be completed.

Rapidly changing AVP levels in response to acute cocaine injection may interact with some of the effects of cocaine.

27.10 EFFECTS OF CHRONIC COCAINE ON REGIONAL BRAIN METABOLISM, DOPAMINE AND OPIATE RECEPTOR BINDING. R. P. Hammer, Jr., D. Yoshishige* and S. A. Jacobson*. Dept. of Anatomy & Reprod. Biol., Univ. Hawaii School of Medicine, Honolulu, HI 96822.

Chronic exposure to cocaine is thought to affect dopamine systems in brain reward centers which produce reinforcing behavior. We used a combined autoradiographic deoxyglucose (DG)/receptor binding technique to study the influence of cocaine on brain metabolism and neurochemical systems. Osmotic minipumps which infused either 1 mg/kg/day or 10 mg/kg/day cocaine HCl, or saline were subcutaneously implanted into adult male Sprague-Dawley rats. After 2 weeks, animals were given 0.5 mCi/kg [^{3}H]2DG intravenously. Forty-five min later, brains were removed, frozen, and sectioned into adjacent series for 2DG autoradiography and receptor binding. 2DG series were immediately exposed to ^{3}H-sensitive film in X-ray cassettes. Receptor binding series were preincubated in buffer at 0°C (6 X 2 min) to remove ^{3}H label, and incubated in either 2.5 nM [^{3}H]naloxone in 50 mM Tris HCl (pH 7.4) and 100 mM NaCl at 0°C for 1 hr to label mu opiate receptors or 10 nM [^{3}H]sulpiride in 50 mM Tris HCl (pH 7.4) and 120 mM NaCl at 22°C for 1 hr to label D_2 receptors. After rinsing and drying, sections were exposed to ^{3}H-sensitive film. Regional autoradiographic densitometry was performed using a Quantitative Densitometry System. Optical density values were converted to absolute values using calibrated tissue standards. The 10 mg/kg/day dose caused significant ($p<0.05$) decrease of 2DG uptake in nucleus accumbens (NAc), substantia nigra pars compacta (SNc) and reticulata (SNr), but no change in striatum, globus pallidus, or locus ceruleus. Sulpiride binding was not affected by either dose in the NAc, SNr, or in any portion of the striatum. Dose-dependent alteration of naloxone binding, however, was observed. Opiate receptor density significantly ($p<0.05$) decreased in the basolateral nucleus of the amygdala, SNc, and dorsal raphe nuclei, and increased in the lateral hypothalamus, medial geniculate, and inferior colliculus compared to the same regions in saline-treated brains. Additional trends for altered binding were noted in the NAc, central nucleus of the amygdala, and ventral tegmental area. No other brain region of the 90 analyzed showed significant effects. While dopamine reuptake is known to be affected by cocaine, D_2 receptor binding was unaffected in this paradigm. Chronic cocaine exposure altered metabolism in several dopamine-related brain reward regions. Moreover, opiate binding was specifically affected in reward centers and not in other regions (with the exception of several auditory-related regions). These data suggest that the reinforcing action of cocaine may be mediated by opiate systems in brain reward centers, which are altered by chronic cocaine exposure. (Supported by USPHS Awards HD19951, DA04081 and NS01161 to R.P.H.)

27.11 PERSISTENT EFFECTS OF COCAINE ON RAT BRAIN TYROSINE HYDROXYLASE. Charles A. Harrington[1], Peter B. Silverman[1], Z.-Q. Zhu*[2]. [1]Department of Psychiatry, Mental Sciences Institute, University of Texas Health Science Center-Houston. [2]Department of Neurosurgery, Baylor College of Medicine-Houston.

A number of neurochemical and behavioral changes are associated with the acute administration of the central nervous system stimulant, cocaine. Cocaine increases striatal dopamine concentration after a single administration but animals given repeated administrations exhibited decreased levels of dopamine in the striatum. Levels of HVA are increased. In addition, animals treated multiple times with cocaine exhibit supersensitive motor activity response to subsequent cocaine challenges.

The activity of tyrosine hydroxylase (TH) has been found to be increased in animals given acute and chronic doses of cocaine. To further clarify the effect of cocaine on TH, we investigated TH with immunocytochemical methods in rat cortex and hippocampus at various times after subcutaneous administration of cocaine. In addition, the concentration of norepinephrine and dopamine were measured in cortex and hippocampus. Total biopterin co-factor levels were also quantified in hippocampus of animals at time periods in which increased TH was observed.

Male Sprague-Dawley rats were administered 100 mg/kg cocaine hydrochloride or vehicle subcutaneously of each of three successive days. One, three and seven days after the last dose of cocaine animals were sacrificed by decapitation. Tissue was processed by $HClO_4$ extraction for neurotransmitter measurement, by acid I_2 oxidation for total pterin analysis, or by glutaldehyde fixation for immunocytochemical analysis. Neurotransmitter and pterin levels were comparable between vehicle or cocaine treated animals at 1, 3 and 7 days after treatment. However TH levels were substantially increased, as demonstrated immunocytochemically, 7 days after cocaine treatment (although not at 1, or 3 days post treatment). We conclude that chronic or intermittent high frequency cocaine use may cause long term changes in the level of key enzymes, such as TH, in catecholamine metabolism in the central nervous system.

27.12 BENZOYLTROPINE AND TROPACOCAINE: EPIDEMIOLOGICAL AND BIOCHEMICAL EVIDENCE FOR CHOLINERGIC ACTIVITY IN THESE COCAINE-IMPURITIES. E.M. Meyer, A.J. Ruttenber*, L. Hearn*, J. Judkins*, M.-T. T. Nguyen*, R. Miller*, and C.L. Devane* (SPON: S.P. Baker). Univ. of Florida Schools of Medicine and Pharmacy, Gainesville, FL, Center for Disease Control, Atlanta, GA, and Office of the Medical Examiner for Dade County, FL.

Epidemiological evidence implicates the alkaloids benzoyltropine and tropacocaine in at least some of the toxic effects occassionally associated with street cocaine preparations. In 9 samples of street preparations obtained from Dec. 1983 through Nov. 1986, 7 contained tropacocaine (0.07-2.7 mg/g cocaine) and 2 others contained benzoyltropine (52 and 175 mg/g cocaine). At least 4 of the former and 1 of the latter group reported symptoms of atropine-toxicity, either central or peripheral.

We therefore investigated the possible actions of these two compounds on several cholinergic systems. Preliminary studies suggest that both compounds inhibit high affinity choline transport and coupled acetylcholine synthesis. ACh synthesis coupled to high affinity choline uptake was inhibited maximally by 85% and 92% by tropacocaine and benzoyltropine, respectively, at 100 uM concentrations. However, both drugs inhibited synthesis over 50% at 3-10 uM concentrations. Total choline uptake by these rat cerebral cortical synaptosomes was similarly inhibited. Interestingly, both compounds significantly inhibited the release of newly synthesized ACh from rat cerebral cortical synaptosomes. At 100 uM, these release-inhibition effects were not further enhanced by adding the muscarinic agonist oxotremorine. These results suggest that both compounds may be active at presynaptic M2 muscarinic receptors in this tissue; however, the alternative hypothesis of sodium channel inactivation is also being investigated.

In order to study the pharmacokinetic properties of these drugs, we also developed an HPLC separation procedure using a 5 micron trimethylsilane column with acetonitrile:phosphate buffer (20:80) containing triethylamine. This procedure separates benzoyltropine and tropacocaine from cocaine, nor-cocaine, and benzoyleecgonine, and allows us to correlate the disposition of the drugs with their behavioral and cardiovascular actions.

27.13 AUDITORY EVOKED POTENTIALS STUDIES OF THE CEREBRAL CORTEX, AMYGDALA, AND NUCLEUS ACCUMBENS DURING AMPHETAMINE ALTERED STATES. C.C. Turbes and G.T. Schneider*. Dept. of Anatomy, Creighton University Sch. of Med. Omaha, NE 68178.

These studies are made on cats and are concerned with changes in auditory evoked potentials related to the action of (d) and (l) amphetamine. Recordings in the cerebral cortex, nucleus accumbens and certain amygdala nuclei. The analog data is stored on FM tape for processing with a minicomputer. The data is processed in the time and frequency domains. Autospectral, coherence spectral and cross correlation estimates are performed on pre, during and post-amphetamine recordings. Comparisons are made with the pre and post-amphetamine recordings. Comparisons are made with the pre and post background electrical activity.

Changes in the analog and processed data show changes that relate to the action of d and l isomers and are different from each other. There are changes that relate to the amphetamine dosage level, the number of repeated doses and the region of the brain. The physical parameters of the stimuli are very important and must be controlled. There are changes in the analog and processed data that relate to the action of amphetamine.

These are evident in latency, amplitude, duration of the P and N peaks of the evoked potentials. There are changes in the frequencies and power of the auotspectral data as well as coherence spectra that relate to the actions of amphetamine. Time domain data shows changes in the cross correlation plots that relates to changes in the interaction between the brain regions studied. Comparisons studies are made with the cross correlation data and the coherence spectral data.

27.14 EFFECT OF A LOW DOSE REGIMEN OF 3,4,METHYLENEDIOXYMETHAMPHETAMINE UPON REGIONAL BRAIN NEUROTRANSMITTERS. R.E. Maloney*, M.D. Schechter, and B.K. Yamamoto. Dept. of Pharmacology, Northeastern Ohio Univs. Coll. of Medicine, Rootstown, Ohio 44272.

Previous studies have shown that the Schedule I drug MDMA ("Ecstasy", "ADAM") decreases 5HT content, synthesis and metabolism. These experiments have employed acute or subchronic dosing regimens using doses ranging from 5-30 mg/kg. To assess the effect of lower doses upon 5HT and other neurotransmitter systems, MDMA was administered to rats according to a pseudo-random dosing paradigm formally used to train rats to discriminate its stimulus properties (Schechter, Pharm. Biochem. and Behav. 26, 1987). Male Sprague Dawley rats were injected i.p. with 1.5 mg/kg ±MDMA 2-3 times /week for 6 weeks. Saline was administered on non-drug days. Control rats received daily injections of saline. Subsequently, all rats were sacrificed and the caudate, nucleus accumbens, hippocampus, hypothalamus, lateral septum, amygdala and prefrontal cortex were punch-dissected and assayed for norepinephrine, dopamine, serotonin and their metabolites by HPLC with electrochemical detection. Caudate, hypothalamus, amygdala and lateral septum all exhibited increases in 5HT and/or 5HIAA. These ranged from a 37% increase in amygdala 5HIAA to a 112% and 119% increase in caudate and amygdala 5HT, respectively. Hippocampus 5HIAA was decreased but 5HT was unchanged. Norepinephrine levels were not altered in any of the areas examined. Dopamine content was increased in caudate (32%), amygdala (80%), and lateral septum (58%). No changes were seen in DOPAC in any of these areas. No neurotransmitter changes were seen in nucleus accumbens and prefrontal cortex. Body weight increases did not differ between control- and MDMA-treated animals over the course of the experiment. In conclusion, a low dose of MDMA administered on an intermittent schedule for 6 weeks produced selective increases in regional neurotransmitter content. The most consistent and dramatic changes were upon the serotonergic system with the dopaminergic system affected to a lesser extent.

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27.15 BIPHASIC BEHAVIORAL AND NEUROCHEMICAL EFFECTS FOLLOWING N-ETHYL-METHYLENEDIOXYAMPHETAMINE (MDE) ADMINISTRATION J.W. BOJA, B.K. YAMAMOTO AND M.D. SCHECHTER. Department of Pharmacology, Northeastern Ohio Univ. College of Medicine, Rootstown, Ohio 44272

The psychoactive drug methylenedioxymethamphetamine (MDMA) has the ability to release both dopamine (DA) and serotonin (5-HT) [Schmidt, et al., Biochem. Pharmcol. 36:747-755, 1987] in the rat brain and its N-ethyl analog MDE also releases DA [Boja, et al., Soc. Neurosci. Abstr., 12:608, 1986]. In order to determine the behavioral effects of MDE, male rats trained to discriminate 0.8 mg/kg amphetamine (AMPH) from 1.4 mg/kg norfenfluramine (NF), using a two-lever operant discrimination task, were administered 2.0 mg/kg MDE and tested at 20 min intervals for 180 min. NF-like responding was prevalent at 20 and 40 min following MDE administration, whereas AMPH-like responding was prevalent at 100-160 min. Vehicle-like responding was seen at 180 min post-MDE administration.

In order to investigate the neurochemical effects of MDE in awake behaving animals, naive male rats were chronically implanted with stearate-modified carbon paste electrodes in the caudate nucleus and allowed to recover 72 hrs prior to testing. In vivo voltammetric recordings, using linear sweep scans from -0.2 to +0.4V and semidifferential signal processing, were made at five min intervals. Peak DA current was measured at +0.15V. MDE (15mg/kg) was injected after a stable baseline was established. Data are expressed as percent of the baseline electrochemical signal (± SEM).

MIN	DA	MIN	DA
0	100.0 (1.1)	100	123.0 (6.9)
20	87.9 (7.4)	120	113.8 (10.9)
40	98.5 (3.0)	140	109.9 (15.7)
60	117.0 (4.9)	160	108.9 (5.5)
80	125.2 (7.4)*	180	97.3 (12.9)

*Significantly different from baseline, $p<0.05$.

These results indicate that MDE initially inhibits DA efflux and precludes AMPH-like discrimination, thus resulting in NF-like responding. After 60 min following MDE administration, DA efflux increases and is maximal by 80 min. At this time AMPH-like discrimination parallels DA efflux which occurs between 60-160 min following MDE administration.

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27.16 THE ACUTE EFFECTS OF 3,4,METHYLENEDIOXYMETHAMPHETAMINE ON TRANSMITTER CONTENT IN LIMBIC BRAIN AREAS. L.J. Spanos and B.K. Yamamoto. Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

3,4-Methylenedioxymethamphetamine ±(MDMA), an amphetamine analog, has recently been classified as a Schedule I drug due to its abuse potential. Previous studies have shown that at high doses, MDMA is neurotoxic to serotonergic neurons. However, the drug has been reported to have therapeutic value because it enhances communication and sensory awareness. This suggests a limbic component to its mechanism of action. To test this hypothesis, four limbic brain regions were examined to determine the possible differential effect on neurotransmitter metabolism in response to this drug. Male Sprague-Dawley rats were injected i.p. with 2.5, 5 and 10 mg/kg of ±MDMA. Control rats were injected with saline vehicle. All animals were sacrificed at two different time points (60 and 120 minutes). The nucleus accumbens, amygdala, lateral septum, and medial prefrontal cortex were punch-dissected and assayed for norepinephrine, dopamine, serotonin and their metabolites using HPLC with electrochemical detection. No changes were observed at the lowest dose in any of the brain areas examined. At the 5 and 10 mg/kg dose however, consistent and significant decreases (27-82%) in serotonin occurred in all regions. Nucleus accumbens and lateral septum 5HIAA content also decreased by approximately 25% while medial prefrontal cortex showed a 62% increase in this metabolite at both time points. Dopamine content increased by 35% in nucleus accumbens while DOPAC decreased by the same amount. DOPAC also was decreased by 50% in amygdala but was not altered in any other brain region. No other alterations in neurotransmitter metabolism were observed. In conclusion, the acute administration of ±MDMA produced selective and dramatic decreases in the serotonin content of limbic brain regions while transient and subtle changes were observed in dopamine and norepinephrine.

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27.17 STUDIES ON THE ROUTE OF ADMINISTRATION AND DURATION OF EFFECT OF (±)METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN RATS. K.T. Finnegan*, G.A. Ricaurte*, S.J. Peroutka, I. Irwin* and J.W. Langston. Inst. for Med. Res., San Jose, CA 95128; Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Recreational use of MDMA has become increasingly widespread. Research in experimental animals has raised concern that this drug may be neurotoxic. Specifically, subcutaneous administration of MDMA has been found to produce a long-lasting depletion of serotonergic nerve terminal markers in rats and monkeys. However, the relevance of these findings to humans, who typically self-administer the drug orally, is unclear. Another unanswered question relates to whether or not serotonin (5HT) levels recover with time after MDMA administration. In this study, both of these issues were addressed.

Groups of rats (n=4) were administered MDMA orally (PO) (7.5, 15.0, 30.0 mg/kg) twice daily for 4 days. Two weeks after drug administration, rats were killed, the hippocampi dissected free, and the concentration of 5HT determined. To probe for possible recovery of 5HT with time, groups of rats (n=4) were injected subcutaneously (SC) with 20 mg/kg MDMA twice daily for 4 days. Individual groups of rats were then killed 2, 7, 10, 13 and 20 weeks after drug administration for the determination of hippocampal 5HT.

Orally administered MDMA produced a long-lasting, dose-related depletion of 5HT in the hippocampus (control 0.37 ± 0.04; 7.5 mg/kg 0.18 ± 0.01; 15 mg/kg 0.15 ± 0.01; 30 mg/kg 0.09 ± 0.01). These results are comparable to those previously reported after SC administration of MDMA. Preliminary results from our laboratory indicate that the PO and SC routes may indeed produce similar effects. For example, 15 mg/kg of MDMA given SC produced a 62.5% depletion of 5HT; this same dose given PO produced a 59% depletion.

In the second experiment, 5HT determinations at various times after MDMA treatment revealed partial recovery. Two weeks after MDMA treatment, hippocampal 5HT was reduced by 64% (control 0.41 ± 0.01; experimental 0.15 ± 0.02). However, 5HT concentrations had recovered to 62% of control values by 20 weeks (control 0.372 ± 0.01; experimental 0.23 ± 0.017).

These results suggest that orally administered MDMA may be no less toxic than when the drug is administered parenterally. Further, these findings indicate that there is partial recovery of 5HT levels with time. This recovery may reflect regenerative sprouting of 5HT nerve fibers. Whether actual functional reinnervation occurs, however, remains to be determined.

27.18 CEREBROSPINAL FLUID MONOAMINE METABOLITES IN CHRONIC HUMAN USERS OF 3,4 - METHYLENEDIOXYMETHAMPHETAMINE (MDMA; ECSTASY). S. J. Peroutka and K. F. Faull. Departments of Neurology, Pharmacology, Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) is a novel psychoactive agent which is used recreationally in the United States. Recently, this compound has been shown to destroy serotonergic nerve terminals in rats. The present study attempted to determine if cerebrospinal fluid metabolites of serotonin, dopamine, and/or norepinephrine were altered in chronic human users of MDMA. The initial analysis involved four people who averaged 20-25 doses of approximately 150 mg MDMA over a six-month time period. The subjects remained free of psychotropic medications for at least two weeks prior to a lumbar puncture. The lumbar puncture was performed between 7:00 and 9:00 a.m. The patients fasted since the evening prior to the study. 15 cc of cerebrospinal fluid was collected, 4 cc of which was placed in an ascorbate containing test tube and immediately frozen on dry ice. 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by gas chromatography/mass spectrometry. Control values were obtained from neurological and psychiatric patients who had no known history of psychoactive drug abuse. In three of the four patients, all cerebrospinal fluid metabolites were within normal limits. In the fourth subject, the MHPG level was within normal limits. However, the levels of 5-HIAA, DOPAC, HVA were reduced to approximately 25 to 40% of control values in this single patient. This individual denied any long-term clinical effects from psychotropic drug use except for possible alterations in sleep patterns. The subject had taken a total of approximately 4 to 5 grams of MDMA in addition to numerous other psychoactive medications including d-LSD, psilocybin, mescaline, cocaine and marijuana. The implications of these findings for both the clinical and recreational use of drugs such as MDMA will be discussed.

27.19 EFFECTS OF MPTP ON CENTRAL DOPAMINERGIC SYSTEMS IN A LIZARD E. Font* (1), R.C. Switzer (2), N. Greenberg (1) and E.F. O'Connor (3) (SPON: J. LUBAR). (1) Life Sciences Grad. Prog. in Ethology, Univ. of Tennessee, Knoxville TN 37996. (2) Memorial Res. Ctr., Univ. of Tennessee, Knoxville TN 37920. (3) Dept. of Psychology, Univ. of Tennessee, Knoxville, TN 37996.

Systemic administration of MPTP causes destruction of dopaminergic neurons in the substantia nigra of humans, primates, dogs, cats and mice, mimicking idiopathic Parkinson's disease. With few exceptions, the neurotoxicity of MPTP in non-mammalian species remains unexplored. The present study was undertaken to investigate the neurotoxic effects of MPTP in the lizard, Anolis carolinensis (Sauria, Iguanidae).

Twenty-six male A. carolinensis were injected i.p. with MPTP in doses ranging from 4 to 100 mg/kg, producing a variety of behavioral abnormalities. These included pronounced color changes and nuchal crest erection, indicative of an acute stress response, as well as hypokinesia. Seven of the lizards receiving doses over 57 mg/kg showed clonic jerks and severe postural rigidity, and died within 48 hrs of injection. The remaining lizards recovered after a variable period of time. Following a survival time of 3 to 13 days they were sacrificed using sodium pentobarbital. The brains of all 26 animals were removed and prepared for histological examination using a modification of the cupric-silver technique. This technique has been shown by one of us (RCS) to distinctly reveal the neuropathological changes following MPTP administration in mice. In the lizards, axons in different stages of degeneration, corresponding to the different survival times, were observed ascending in the lateral forebrain bundle to a terminal station in the striatum. This appears to be homologous to the dopaminergic nigrostriatal projection of mammals. Degenerating cell bodies were observed in the substantia nigra and ventral tegmental area in animals that were sacrificed at 5 days.

Research now under progress will clarify the neurotoxic effects of MPTP in this animal model using histofluorescence and chromatographic analysis.

27.20 REGULATION OF DOPAMINE RECEPTOR SENSITIVITY: EFFECTS OF 1-METHYL-4-PHENYLPYRIDINIUM (MPP^+) ON PRIMING. B.E. Mileson*, M.H. Lewis, R.B Mailman. Curriculum in Toxicology, Depts. of Psychiatry and Pharmacology, Biol. Sci. Res. Ctr., Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

Male Sprague Dawley rats were anesthetized with ether, and received 80 ug of MPP^+ (intracisternally, IC) in 20 ul of vehicle. One to two weeks following IC injection, MPP^+ and vehicle treated control rats were challenged with apomorphine (0.3 mg/kg, sc). Apomorphine-induced behaviors were quantified using a computer supported observational method by observers unaware of animal treatments. MPP^+ treated animals displayed significantly more licking and gnawing in response to apomorphine ($p < 0.05$) than vehicle treated controls. While this observation could be explained by MPP^+-induced dopamine receptor supersensitivity, it could also be due to a "priming" effect of MPP^+. ["Priming" is the observation that initial exposure to a drug results in enhanced response to subsequent doses of that drug, a phenomenon seen with several classes of compounds, including dopamine receptor ligands. Thus, behavioral stereotypies induced by apomorphine (0.3 mg/kg, sc) are significantly less intense in drug naive rats than in rats pretreated two weeks earlier with this dose of apomorphine.] To test these alternate hypotheses, a second apomorphine challenge was performed four weeks later. In this second challenge, the MPP^+-treated animals showed no increases in licking or gnawing from the first challenge. Conversely, the control rats displayed an increased response to apomorphine challenge, as expected from the "priming" effect of the previous dose. There was no difference between MPP^+ and control rats in this second challenge. After a minimum of five days to permit elimination of apomorphine, the rats were decapitated, and striatal dopamine, DOPAC and HVA concentrations measured. No differences were seen between control and lesioned rats, clearly confirming that permanent generalized decreases in striatal dopamine concentrations did not elicit behavioral supersensitivity to apomorphine. In a separate study, dopamine and its metabolites were measured immediately after IC injection of MPP^+. After four hr (but not 1 hr), striatal DA and DOPAC concentrations were profoundly elevated. These data are consistent with the hypothesis that MPP^+ (administered IC) causes priming of the dopaminergic system, possibly due to transient increases in striatal DA triggered by MPP^+ insult.

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27 PO BROMOCRIPTINE INTERACTS WITH DESIPRAMINE IN COCAINE WITHDRAWAL. R.H. Loiselle, A.J. Giannini, M.S. King*, L.R. DiMarzio*, C.A. Armbrecht*. Northeast Ohio Med. Coll., P.O. Box 2169, Youngstown, OH 44504.

Thirty-six cocaine abusers were withdrawn from cocaine over a 90-day period. Twelve received placebo (group P), 12 received bromocriptine 2.5 mg. q.i.d. plus placebo (B-P), and 12 received bromocriptine 2.5 mg. q.i.d. plus desipramine 200 mg. q.d. (B-D). Both B-D and B-P were significantly more effective in controlling withdrawal symptoms than P. ($p<.001$ for both) throughout the duration of the study. After 30 days ($p<.05$) 60 days ($p<.05$) and 90 days ($p<.02$) B-D was more effective than B-P in controlling withdrawal symptoms. This study supports both the dopamine-depletion and dopamine-receptor supersensitivity models in cocaine withdrawal.

27PO COMPARISON OF BROMOCRIPTINE AND AMANTADINE IN THE TREATMENT OF ACUTE COCAINE WITHDRAWAL. A.J. Giannini, R.H. Loiselle, B.A. Sullivan* and M.C. Giannini*. Northeast Ohio Coll. Med., P.O. Box 2169, Youngstown, OH 44505.

Thirty age-matched white males in the 20's and thirties who had abused cocaine daily for at least six months agreed to be detoxified. One-third of this group were given either amantadine (A), bromocriptine (B), or placebo (P). Both amantadine and bromocriptine are central dopamine agonists. Amantadine was given at a dosage of 100 mg. q.6 hrs., bromocriptine 2.5 mg. q.6 hrs., and placebo cap 1 q.6 hrs. The response was measured daily for 10 days. By the end of day 2, both the A and B groups showed significant improvement over placebo ($p<.01$ for both groups). This improvement continued over the study, though by the end of the seventh day the difference between A and B groups and P group were only ($p<.05$). Throughout the study there were no significant differences between A and B groups. Since both amantadine and bromocriptine are dopamine agonists, this study supports the dopamine-depletion hypothesis of cocaine abuse and withdrawal.

CHRONIC DRUGS AND NEUROTOXICITY II

28.1 EFFECT OF EXERCISE ON BENZODIAZEPINE TOLERANCE ASSESSED WITH AN OPERANT BEHAVIOURAL TASK IN RATS. A.J. Goudie and S. Kaney*. Dept of Psychology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

McMaster and Carney (1986) reported that chronic exercise produces tolerance to behavioural actions of muscarinic antagonists (Pharmacol. Biochem. & Behav. 24: 865). We report here that tolerance to the sedative (rate-supressant) action of the short-acting benzodiazepine midazolam is facilitated by previous (remote) exercise in a running wheel.

Rats responded for food on a Fixed Ratio 15 schedule (15 min sessions). After training, subjects were run in 4 weekly cycles in which midazolam tolerance was assessed on consecutive Fridays, i.e. all subjects received midazolam before these operant sessions. On Mondays, subjects in 4 groups (n=8) were treated according to a 2 x 2 factorial design:-

Group	Treatment	Exercise Condition
1	Midazolam	Forced exercise
2	Midazolam	No exercise
3	Saline	Forced exercise
4	Saline	No exercise

On Tuesdays through Thursdays all subjects responded (without any drug treatment) in operant sessions. The rate-suppressant actions of midazolam on Fridays were determined by expressing response rates as percentages of mean response rates on the preceding Wednesday and Thursday. Midazolam was administered at 1 mg/kg (s.c.). Forced exercise involved placing subjects for 80 mins in a wheel rotating at a constant speed of 6 r.p.m. The duration of exercise was chosen to cover the total duration of time for which midazolam is behaviourally active in rats at 1 mg/kg.

The response levels (%) obtained on consecutive Fridays were analysed with a 2 x 2 repeated measures ANOVA. There was a significant effect of repeated tolerance test days ($p<0.001$); i.e. tolerance developed rapidly to the rate-suppressant action of midazolam. There was a significant main effect of exercise ($p<0.05$), animals that received exercise on Mondays showed more tolerance than those that received no exercise. There was no exercise x treatment interaction ($F<1$); i.e. the facilitatory effect of exercise on tolerance was seen in animals that were exercised under drug and under saline. The effect of type of treatment administered on Mondays approached, but did not reach, significance ($F=3.99$, df=1, 28). These data show that exercise remote from drug treatment facilitates tolerance by a mechanism which is at present unknown, but which may involve stress effects (cf. Peris and Cunningham, Alcohol Drug Res. 7: 187, 1987).

28.2 **COMPARISON OF BICUCULLINE, FLURAZEPAM AND PICROTOXIN SEIZURE THRESHOLDS IN DIAZEPAM DEPENDENT-WITHDRAWN AND BARBITAL DEPENDENT-WITHDRAWN RATS.** W.M. Bourn and C.E. Reigel (SPON: J.W. Dailey). Sch. of Pharmacy, NE Louisiana Univ., Monroe, LA 71209 and Dept. of Basic Sci., Univ. of Illinois Col. of Med. at Peoria, Peoria, IL 61656.

Barbiturates and benzodiazepines (BZs) are capable of producing physical dependence in humans following sufficient chronic high dosage administration. Abrupt withdrawal of these agents in physically dependent individuals results in the CNS depressant withdrawal syndrome. This syndrome is characterized by excessive CNS excitation, typified by grand-mal type convulsions in severe instances. Agents in one class are capable of cross-substituting for agents of the other class and maintaining a physical dependence. Upon withdrawal of the second agent, symptoms of the CNS withdrawal syndrome appear.

BZs and barbiturates are generally considered to cross-substitute in animal models of physical dependence. However, we have previously reported incomplete cross-substitution from barbital to diazepam (DZ) physical dependence in the rat (Fed. Proc. 41: 1542, 1982). Using susceptibility to sound-induced seizures as the withdrawal measure (characteristic of barbiturate withdrawal), DZ did maintain the barbital physical dependence. However, upon withdrawal of DZ, susceptibility to sound-induced seizures was not present. These results suggested that barbiturate and BZ physical dependence are similar, but not identical in rats. Analogous to this, barbiturates and BZs both augment GABAergic activity, but do so through different receptors. BZ binding to BZ receptors enhances GABA binding whereas barbiturate binding to the barbiturate receptor prolongs the opening of the GABA/chloride ionophore initiated by GABA receptor activation.

It is possible that enhanced net GABAergic activity initiated at different receptors within the GABA/BZ/chloride ionophore receptor complex could maintain a physical dependence when a BZ is cross-substituted for a barbiturate. Adaptive changes due to chronic receptor occupation could dissipate following cross-substitution to an agent acting at another receptor within the GABA/BZ/chloride ionophore complex. This could account for the lost of sound-induced seizure susceptibility upon DZ withdrawal discribed above. To test this hypothesis, IV seizure thresholds were determined for convulsant agents that bind to the GABA receptor (bicuculline), BZ receptor (flurazepam) and barbiturate receptor (picrotoxin) in barbital and DZ dependent-withdrawn rats and non-dependent vehicle controls. Barbital withdrawn and DZ withdrawn rats exhibited lower flurazepam (FLZ) and picrotoxin seizure thresholds than controls. FLZ seizure threshold was lowered more in DZ withdrawn rats than in barbital withdrawn rats. Picrotoxin seizure threshold was lowered more in barbital withdrawn rats than in DZ withdrawn rats. Bicuculline seizure threshold was lowered only in barbital withdrawn rats.

28.3 PICROTOXIN EFFECTS ON FENVALERATE-INDUCED LOCOMOTOR ACTIVITY. K.M. Tolson, W.M. Bourn, and F.O. Risinger. School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209-0470.

The neurotoxicity of fenvalerate (FNV), a synthetic pyrethroid of the Type II class, seems to be associated with the mammalian GABAergic system. In vitro studies (Lawrence & Casida, Science, 221:1399, 1983 and Crofton et al., The Toxicologist, 6:197, 1986) have linked FNV and other Type II pyrethroids with the TBPS/picrotoxinin binding site(s). The present study examined the relationship between FNV and the GABA receptor-ionophore complex with locomotor activity as the dependent variable.

The time course of FNV toxicity was determined for both male and female Sprague-Dawley rats. Animals were dosed orally with FNV (100 mg/kg) or corn oil vehicle. Time to onset and activity levels were measured in an electronic activity monitoring system. Two activity levels were monitored continuously for four hours after dosing--head/limb movement and whole body movement. Onset of the toxicity syndrome, characterized by an increase in activity, was achieved within the first hour of testing in female rats ($p<0.01$). In male rats the onset did not occur until the second hour of testing ($p<0.01$). After the initial onset, activity levels remained high ($p<0.01$) as both sexes progressed into the toxicity syndrome of FNV.

If picrotoxin (PTX) and FNV share a common binding site, one would expect pretreatment with a subconvulsive dose (2 mg/kg) of PTX to alter the binding and subsequent toxicity syndrome of FNV. PTX was administered ip to female rats thirty minutes prior to FNV exposure (100 mg/kg). PTX alone produced a decrease in activity ($p<0.01$) within the first hour of testing. Rats receiving PTX prior to FNV also exhibited a decrease in activity ($p<0.01$) within the first hour, but by the fourth hour there was an increase in activity ($p<0.01$) associated with the onset of the FNV toxicity syndrome. Thus, pretreatment with PTX was found to delay the time to onset by three hours in female rats. These findings provide further (behavioral) evidence that links Type II pyrethroids with the picrotoxinin domain within the GABA receptor-ionophore complex.

28.4 FOOD AVERSION TO DEOXYNIVALENOL (VOMITOXIN) IN RATS AND THE ROLE OF THE AREA POSTREMA. K.-P. Ossenkopp, W. A. Rapley*, and M. Hirst. Depts. Psychology and Pharmacology & Toxicology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

The presence of vomitoxin in livestock feed is associated with food rejection and reduced weight gain. The present experiments used a rat model to investigate the nature and possible underlying mechanisms of the vomitoxin-induced food rejection response. In Experiment 1 male albino rats (Sprague Dawley) were maintained in an isolated operant conditioning chamber and obtained food pellets by pressing a lever on a continuous reinforcement (CRF) schedule. After a training period with normal Noyes pellets, the rats were presented with pellets containing various levels of vomitoxin (3, 6, or 12 ppm). Number of lever presses, number of pellets consumed, weight gain and water intake were monitored and compared to control rats over a 10 day period. Increasing levels of vomitoxin produced significant decrements in body weight gain and rapid dose-related rejections of the food pellets. In Experiment 2 male albino rats were given thermal cautery lesions of the area postrema (AP), a circumventricular organ known to be a chemoreceptor for blood-borne toxins, or sham lesions. Following a 10 day recovery period the rats were again maintained in the isolated operant conditioning chambers in the same manner as in the first experiment. When the rats were presented with food pellets containing 48 ppm of deoxynivalenol both the AP lesioned and sham lesioned rats exhibited significant decrements in weight gain. The sham lesioned animals exhibited high levels of pellet rejection whereas the AP lesioned rats showed much lower levels of rejection ($p < .01$). However, number of pellets consumed did not differ between the two groups. Thus, the AP seems to be necessary for high levels of food aversion displayed in response to food pellets containing the vomitoxin. However, integrity of the AP does not seem to be important for reductions in body weight gain in response to vomitoxin contaminated food.

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28.5 ANTAGONISTIC EFFECT OF ZINC ON MORPHINE-INDUCED ANALGESIA, TOLERANCE AND NALOXONE-PRECIPITATED WITHDRAWAL SYNDROME IN RATS. M. Baraldi. Dept. of Pharmaceutical Sciences, Section of Pharmacology and Pharmacognosy, Modena University,41100 Modena, Italy.

There are several evidence suggesting that endogenous opioids and zinc may interact to regulate neuronal functions in brain areas where they are co-localized. Zinc has been shown to inhibit in vitro opioid binding by reducing in a dose related fashion both the affinity and the number of binding sites. This effect could be related to the high affinity of zinc for the SH- groups present in opioid receptors, since thiol-reducing agents can restore the binding properties of opioid receptors altered by the exposure to the oxidative effect of the metal. Furthermore it is noteworthy that the Enkephalin-degrading enzymes are zinc-metal enzymes and that thiorphan inhibits the enkephalinase activity presumably by binding zinc which resides in the active sites of the enzyme. These data prompted us to test the behavioral and biochemical effects induced in normal rats and in morphine-tolerant rats by the supplementation of pharmacological doses of zinc which we demonstrated to increase the release of ACTH from anterior pituitary and to induce hyperalgesia. Pellets of morphine (75 mg) or placebo were implanted subcutaneously to rats which received at the same time a subcutaneous depot injection of ZnO2 (110 mg/Kg) or the vehicle alone. Here we report that the administration of zinc reduced the analgesic effect of morphine and the Naloxone-precipitated withdrawal syndrome. Interestingly the treatment with morphine alone induced a reduction of zinc in plasma and in brain tissue and a decreased presence of ACTH in plasma, that is exatly the opposite effect induced by the supplementation of zinc alone. A further indication of the antagonistic effect of zinc and morphine is represented by the ability of morphine to prevent the oxidative effect of zinc on opioid receptors. These observations could be of value in order to test the effect of zinc supplementation in opiate addiction.

28.6 EFFECT OF CHRONIC LEAD INTOXICATION ON DOPAMINE METABOLISM AND TRANSMISSION IN THE RAT NEOSTRIATUM. D. Martínez-Fong*, M. E. Gutiérrez* and J. Aceves* (SPON. J. Alanís). Dept. of Physiol. Biophys. and Neurosci. Centro de Investigación y de Estudios Avanzados del IPN. 07000, México, D. F.

Rats intoxicated with low lead levels show hyperactivity and stereotyped behavior. Increase in dopamine (DA) metabolism and transmission in neostriatum and nucleus accumbens septi elicits this same behavior. Then, increase in DA metabolism and neurotransmission would be expected in these brain areas of lead intoxicated animals. Here we have studied this possibility. We studied the effect of lead intoxication on the activity of some enzymes involved in DA metabolism; the effect on DA transmission was judged observing modifications in rotational behavior induced by amphetamine in animals with 6-hydroxydopamine lesion. Female rats were exposed to 1400 ppm of Pb acetate via drinking water two months before and during pregnancy, and also during lactation until 21 days postpartum. At this time, the same Pb solution was given to male offsprings until their sacrifice at 3 months of age. Animals of the same age were used as controls. In Pb-intoxicated animals, the quinonoide dihydrobiopterin reductase (QDBR) activity and the levels of tetrahydrobiopterin (BH4) increased 33 and 40% respectively, but cyclohydrolase activity remained normal, suggesting that the increase in TH4 (tyrosine hydroxylase cofactor) was due to increase in QDBR activity. Tyrosine hydroxylase activity, as judged by the increase in DOPAC (measured voltametrically in vivo) after the blockade of the DA presynaptic autoreceptors with haloperidol, was also found increased in intoxicated animals. Paradoxically, rotational behavior (turns/min) was significantly reduced in intoxicated animals. Pb concentrations were significantly increased in striatal tissues and blood. The results suggest that Pb intoxication increases, as expected, DA metabolism in neostriatum. Pb intoxicated animals also showed the paradoxical response to amphetamine, which is a common feature of plumbism in mammals.

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28.7 ACUTE LEAD EXPOSURE REDUCES GLUCOSE UTILIZATION IN THE RAT BRAIN. John M. Bertoni and Pamela M. Sprenkle *, Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107

The 2-deoxyglucose autoradiographic method was used to assess the effects of acute lead exposure on regional brain glucose metabolism in the rat. Eight male Long-Evans hooded rats weighing between 317 and 364g were cannulated for access to the femoral artery and vein, then given an IV bolus of lead acetate (50mg PbAc/kg or 27mg Pb/kg) or saline and observed for at least 7 hours prior to the start of the 2-deoxyglucose experiment. Under controlled conditions of light and sound, the rats were injected with 14C-2-deoxyglucose, then sacrificed after 45 min. The brains were frozen in cooled isopentane at -40 ° C and 20 u sections were made. Every third slice was dried on glass slides and apposed to Kodak SB5 X-ray film for 5 days. Local cerebral metabolic rates for glucose (lCMRglu) were measured by densitometry with a computerized image analyzer. Four other rats were similarly given lead but without 2-deoxyglucose, and blood samples were taken at -1, 15, and 30 min and at 1, 2, and 6 hours, with corresponding mean (± SE) lead levels in the blood of 1.8 ± 1.4, 156 ± 14.6, 182 ± 20.4, 155 ± 17.4, 134 ± 12.5 and 36 ± 2.3 ppm. Whole brain lead was 0.45 ± 0.22 and 1.20 ± 0.15 ppm in the control and lead-treated groups, respectively ($\bar{p}<0.025$). The greatest reductions in lCMRglu occurred in the following structures: medial geniculate bodies (15.6 ± 2.5%), anterior commissure (11.8 ± 6.2%), and auditory cortex (10.4 ± 4.1%). The auditory centers are known to be among the most metabolically active in the mammalian brain. These data support the hypothesis that brain areas with the highest glucose metabolism, highest Na,K-ATPase activity and greatest synaptic densities are most vulnerable to the effects of lead.

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28.8 HEMODYNAMIC EFFECTS OF MERCURY CHLORIDE IN INTACT AND IN ISOLATED PERFUSED RABBIT HEARTS. H.M. Rhee and B.H. Choi. Department of Pharmacology, Oral Roberts University School of Medicine, Tulsa, OK 74137 and Department of Pathology, University of California Irvine, Irvine, CA 92717.

Although it is well known that both organic and inorganic mercurial compounds are capable of producing severe toxic damage to the central nervous system the precise mechanisms of cellular damage in the brain are poorly understood. Since inorganic mercurial compounds do not readily cross the blood-brain-barrier and are also known to cause acute cardiovascular effects in intact animals the pathogenesis of neurotoxic damage in inorganic mercury intoxication may be different from that of organic mercury in that the CNS damage may be greatly influenced by direct action of these compounds to peripheral organs. In order to characterize the acute effect of mercuric chloride New Zealand white rabbit (1.5-2.5 kg) were anesthetized (pentobarbital 30 mg/kg) and instrumented as reported previously (J Pharmacol Exp Ther 234: 534, 1985). At the same time the left renal sympathetic nerve activity was monitored during the injection of mercury to test whether or not mercury alters nerve activity which might produce hemodynamic alteration secondarily. In intact anesthetized rabbits an intravenous injection with small doses of mercuric chloride (100 µg/kg or less) did not produce significant changes in systemic blood pressure, heart rate or renal nerve activity. Large doses (500 µg/kg or more), on the other hand, depressed these parameters significantly. For example, a fast bolus injection of mercuric chloride (2 mg/kg) produced a state of shock with transient increase in the nerve activity. Since this cardiovascular action of mercuric chloride suggested a direct action of mercury to the heart, the action of mercuric chloride was tested in isolated perfused rabbit hearts. The isolated hearts were retrogradely perfused with Krebs-Henseleit solution as reported previously (Oxygen Transport to Tissue, Lubbers et al (eds), Vol V, Plenum Press 1984, p. 389) with different concentrations of mercury at 37°C. One mg% of mercuric chloride solution reduced the contractile force by 30% in 10 sec and the first derivative of intraventricular pressure (dP/dt) was also reduced proportionately. These results suggest that hemodynamic action of mercuric chloride was indeed due to its primary action on the heart and that these effects must be taken into account when analyzing the vulnerability and damaging effects of mercurial compounds to the central nervous system.

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28.9 EFFECTS OF INHALED p-XYLENE ON LEARNING AND MEMORY IN RATS. P.J. Bushnell. Neurotoxicology Division, US EPA, Res. Tri. Park, NC 27711.

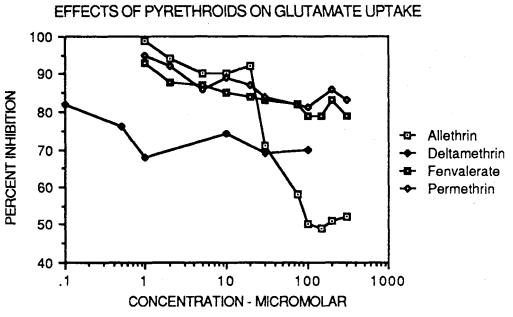
p-Xylene is a prevalent solvent and chemical precursor used in both industry and household products. While the population at risk for exposure is thus quite large, little is known about its neurobehavioral effects. Young adult male Long-Evans rats inhaled p-xylene at concentrations of 0 to 1600 ppm, 4 hr per day for 1 to 5 days, and were evaluated immediately following exposure on several learning and memory tasks. Autoshaping was carried out across 5 successive days with p-xylene exposure in the morning followed by testing in the afternoon. For this test, the retraction of a single lever on a variable-time 50" schedule predicted delivery of a food pellet. When the force required to depress the lever was <0.10 N, acquisition of the barpress response was faster in animals having inhaled 1600 ppm p-xylene than in air-exposed controls. When the force was increased to 0.20 N, however, p-xylene exposed rats acquired the response no faster than controls. Learning was also assessed in the 8-arm radial maze (8-ARM) by baiting a different set of 4 arms daily and measuring decline in number of errors (entries into unbaited arms) required to obtain the rewards across 10 trials/day. Rats having inhaled 1600 ppm p-xylene made more errors in this repeated acquisition paradigm. To asses working memory, rats were trained in an operant chamber on a discrete-trial conditional delayed response paradigm. These rats received pellets for pressing one of two levers in the choice phase of a trial if that lever had been presented alone in the prior sample phase of that trial. On half the trials, a cue light indicated the correct response, thus providing a discrimination control condition within the task. Performance on both uncued (memory) and cued (discrimination) trials was unaffected by prior inhalation of 1600 ppm p-xylene. It thus appears that acute p-xylene inhalation at 1600 ppm (16 times the TLV) does not affect memory in rats. The increase in rate of barpress acquisition may be explained on the basis of decreased motor control induced by p-xylene. The increase in errors in the repeated acquisition test may reflect impaired ability to process spatial information after p-xylene exposure.

28.10 EFFECTS OF CHRONIC EXPOSURE TO TRICRESYL PHOSPHATE IN F344 RATS AND B6C3F$_1$ MICE: BEHAVIORAL AND BIOCHEMICAL CORRELATES. R. Irwin*, G. B. Freeman, R. Trejo*, M. Hejtmancik* and A. Peters* (SPON: S. Tjioe). Battelle, Columbus, OH and National Toxicology Program, RTP, NC.

Subchronic exposure to tricresyl phosphate (TCP) (0-13000 ppm TCP in rats; 0-4200 ppm in mice), an organophosphate of known neurotoxicity, decreases forelimb grip strength in male rats and both forelimb and hindlimb grip strength in male and female mice. It was suggested that a TCP-induced depression of body weight accounted for the observed grip strength impairment in rats, but not in mice (Haggerty et al., The Toxicologist, 6: 218, 1986). In an ongoing chronic dosed feed study (2 years), F344 rats are exposed to 0, 75, 150, 300 or 600 ppm, while B6C3F$_1$ mice receive 0, 60, 125, or 250 ppm. After 13 weeks of dosing, forelimb gripstrength was unaffected by TCP in both mice and rats. Hindlimb grip strength decreased in male rats (300 and 600 ppm), but not in female rats. In mice, decrements in hinglimb grip strength were observed in males (250 ppm) and females (125, 250 ppm). TCP had no effect on food consumption in either species. In addition, all dose groups of both sexes exhibited group mean body weight values that were similar to those of respective controls. As a biochemical index of potential toxicity, serum cholinesterase levels showed a dose-dependent reduction in both rats and mice. In rats, serum cholinesterase declined significantly relative to control in the 300 (-18%) and 600 (-27%) ppm male dose groups and the 150 (-23%), 300 (-35%) and 600 (-49%) ppm female dose groups. Serum cholinesterase values in all dose groups of mice were significantly lower than control [males: 60 ppm (-31%), 125 (-53%), 250 (-72%); females: 60 ppm (-28%), 125 (-56%), 250 (-77%)]. These results suggest that grip strength is sufficiently sensitive to detect sex and species differences in the behaviorally neurotoxic effects of TCP.

28.11 EFFECTS OF PYRETHROID INSECTICIDES ON THE ACTIVE UPTAKE OF GLUTAMATE INTO RAT BRAIN SYNAPTOSOMES. A. S. Bloom and A. T. Accardi. Dept. of Pharmacology and Toxicology, Med. Col. of Wisconsin, Milwaukee, WI 53226.

The Na^+-dependent binding site for glutamate in brain membranes is thought to be associated with the high affinity uptake of that putative neurotransmitter into neurons. We have previously demonstrated that pyrethroid insecticides inhibit the Na^+-dependent binding of glutamate to rat brain membranes (Neurosci. Abs., 11 :632, 1985). We have now investigated the effects of several pyrethroids on glutamate uptake. The uptake of ^3H-glutamate was measured in a crude mitochondrial preparation of rat forebrain at 37° C. Pyrethroids were added using DMSO as the vehicle. Allethrin (ALL), permethrin (PER), deltamethrin (DEL) and fenvalerate (FEN) were examined. All of the pyrethroids



tested inhibited the uptake of glutamate. ALL had the most effect, inhibiting 50% at a concentration of 100μM. DEL was the most potent, producing significant inhibition at concentrations as low as 0.1 μM. However the maximum inhibition observed with DEL was only 30%. PER and FEN were less active. Kinetic studies indicated that the inhibition was noncompetative in nature. The above data demonstrate that the pyrethroid insecticides can inhibit the high affinity glutamate uptake system. It is possible that this action is due in part to an effect on glutamate binding to the carrier in this system. (Supported by USPHS grant ES 03102).

28.12 SODIUM BENZOATE INCREASES FREE TRYPTOPHAN (TRP) IN BLOOD AND SEROTONIN (5-HT) FLUX IN CORTEX OF HYPERAMMONEMIC SPF MICE. M.L. Batshaw, S.L. Hyman, J.C. Coyle, and I. Qureshi (spon. James C. Harris). Depts. Peds., Psych. and Kennedy Inst. Johns Hopkins Meds Inst., Balto. and Hopital Ste. Justine, Montreal

Sodium benzoate has proven effective treatment of hyperammonemia in children with inborn errors of urea synthesis. It provides an alternative pathway for waste nitrogen excretion as hippurate. Normal dose is 1.75 mmol/kg/d. Toxicity associated with high dosage benzoate (>4 mmol/kg/d) simulates ammonia toxicity with anorexia and lethargy. The mechanism of these behavioral abnormalities is unclear but may involve altered serotonin metabolism. We have previously shown that hyperammonemia leads to increased serotonin flux in cortex of rats who received infusions of urease (Pediat Res 20:1310-15,1986). We now report that benzoate also increases cortical serotonin flux. We injected i.p. 1 or 5 mmol/kg of sodium benzoate or sodium acetate (control) in congenitally hyperammonemic Spf/y and in normoammonemic CD-1 mice with sacrifice 1 hr. later (n=10 each group). Plasma benzoate levels were similar in both animals (mean) 35 uM and 6362 uM for 1 and 5 mmol/kg injections respectively. These are comparable to levels found in children receiving similar dosage. Benzoate also accumulated in cortex, 29 pmol/mg tissue and 2239 pmol/mg respectively. Compared to CD-1 mice, Spf mice (all conditions) had higher cortical levels of HIAA ($p<.001$), 5-HT and Trp ($p<.005$). Benzoate treatment resulted in a significant increase in HIAA, $p<.001$, and 5-HT, $p<.05$ in CD-1 mice and an increase in HIAA, $p<.001$ in Spf mice. There was no treatment effect on cortical levels of norepinephrine, GABA or amino acids. Benzoate treatment resulted in an increase in free serum Trp levels in CD-1, $p<.001$, and Spf mice, $p<.09$. Sodium acetate had no effect on free Trp or cortical serotonin flux. The effect of benzoate on free Trp and on serotonin flux was most evident with the 5 mmol/kg dose. These results suggest that the effect of hyperammonemia and benzoate may be synergistic in increasing serotonin flux. The mechanism of the benzoate induced changes appears to involve competition of benzoate with Trp for albumin binding sites in blood. This study indicates that benzoate toxicity may be linked to altered serotonin metabolism and that the treatment margin of safety is rather narrow.

ACTION POTENTIALS AND ION CHANNELS I

29.1 **MORE THAN ONE RNA SPECIES IS INVOLVED IN CONTROLLING INACTIVATION OF RAT BRAIN Na CHANNELS EXPRESSED IN XENOPUS OOCYTES.** D.S. Krafte*, T.P. Snutch*, J.P. Leonard, N. Davidson*, & H.A. Lester. Divs. of Biology and Chemistry, Caltech, Pasadena, CA 91125.

We have injected RNA from the brains of 18 day old rats into Xenopus oocytes in order to study the properties of voltage-dependent Na channels from this tissue. Oocytes were injected either with unfractionated poly(A+) (RNA_u) or poly(A+) RNA that was size fractionated over a sucrose gradient. We identified a fraction (RNA_α) that contained the 9 kb mRNA encoding the α subunit of the Na channel both by Northern blot analysis and by electrophysiological recording. Currents elicited by this fraction exhibited a 3 fold or greater slowing of the macroscopic inactivation rate as compared to currents following injection of RNA_u. In addition, the steady-state voltage dependence of inactivation was shifted in the depolarizing direction (V1/2 = -45 mV for RNA_u vs. -34 mV for RNA_α). To eliminate the possibility that these effects were an artifact of our manipulations we pooled all the fractions from the gradient and injected the pooled RNA into oocytes. Macroscopic inactivation of Na currents elicited by pooled RNA was indistinguishable from that following injection of RNA_u. The mechanism behind the slowing of the inactivation kinetics was investigated at the single channel level using both cell-attached and excised outside-out patches. In both cases a greater tendency for the channels to reopen appears to underlie the macroscopic observations. This gating behavior is illustrated in the current traces below in response to a voltage step from -100 mV to -20 mV in a cell-attached patch (left-RNA_u, right-RNA_α, calibration bars = 2 pA and 4 ms). The single channel conductance was approximately 13 pS for channels induced by both RNA_u and RNA_α injections. Supported by NS-11756 and by fellowships from NIH and AHA.

29.2 TTX-sensitive Na channels expressed in Xenopus oocytes injected with rabbit heart RNA. Fedora Sutton*, Kiyonori Yoshii*, Norman Davidson* and Henry A. Lester. Division of Biology 156-29, Caltech Pasadena CA 91125.

We are characterizing the types of sodium channels expressed in cardiac tissue. RNA was isolated from atrium, ventricle, or whole rabbit or rat heart (ages 8 to 25 d). Based on Northern blot analysis with the neuronal specific probe pSCG-10, there was no detectable contamination with neuronal RNA. Two hundred ng of RNA was injected into oocytes in 50 nl of water. After 2 d incubation at 20°C, voltage-dependent Na currents (100-500 nA peak) were observed with a standard two-electrode voltage-clamp circuit. With either ventricular or atrial RNA, these currents were blocked by TTX; the dose response relation suggests that at least 90% of the channels were blocked by the binding of a single TTX molecule with a dissociation constant of 8 ± 2 nm. This affinity equals that for blockade of the 5-10 fold larger Na currents induced by rat or rabbit brain RNA. This sensitivity to TTX contrasts with the dissociation constant of at least 1 μM observed with voltage-clamp analysis of ventricular muscle. Perhaps TTX insensitivity requires a post-translational modification that oocytes cannot perform. Supported by HL-35782 and by NIH fellowship to F.S.

29.3 **Evidence for alpha-2,8-linked polysialic acid in the glycopeptide of the voltage-sensitive Na channel from *E. electricus*.**

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All of the known homologous voltage-sensitive Na channels from vertebrates are glycoproteins. The protein from eel electroplax is formed of a single peptide of 208 kDa, which when normally glycosylated runs on SDS-gels as a diffuse band of ~290 kDa. Compositional analysis indicates 29% of the glycopeptide is carbohydrate, enriched in sialic acid and N-acetylhexosamines (Miller, Agnew & Levinson, 1983, Biochem.22,462). We have investigated susceptibility of the carbohydrate to exo and endoglycosidases and here report the effects of treatment with two neuraminidases. Denatured glycopeptide was prepared with by our previously reported DEAE ion-exchange and Sepharose gel filtration protocols. Electrophoretic subfractions were prepared as well resolved substrate by preparative SDS-gel electrophoresis.

When treated with the exoneuraminidase from *C. perfringens* (37 C, 0.8 U/ml, 3 h), SDS-PAGE showed the average apparent size on 5.5% gels was decreased from 290 kDa to 220 kDa. This was prevented by the inhibitor 2-deoxy-3-dehydro-N-acetylneuraminic acid. A second enzyme tested was endo-N-acetylneuraminidaase (Endo-N) from K1F bacteriophage induced *E.coli* (Hallenbeck, et al, 1987, J.Biol.Chem.,262,3553). Endo-N is specific for long unbranched polysialic acid homopolymers of minimal chain length 5; cleavage leaves the proximal 4 sialic acids unhydrolyzed. Treatment with this enzyme under similar conditions produced a similar increment in electrophoretic mobility, indicating apparent loss of of 70 kDa (25%). Thus, a sizeable fraction of the sialic acid appears to be linked in unbranched homopolymers of polysialic acid. It can be estimated that removal of all ~113 sialic acid residues would cause minor losses of both mass (12%) and charge (<2% of SDS-denatured peptide). Thus the sialic acid-bearing moiety may form an extended structure that contributes to a large hydrodynamic resulting in substantial retardation on acrylamide gels.

To our knowledge the electroplax Na channel is only the second protein from excitable tissues reported to exhibit polysialic acid, the other being the embryonic neural cell adhesion molecules (NCAM) involved in cell-cell interactions during development. Experiments are in progress with native glycopeptide to test whether the polysialylation plays a role in channel function.

29.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF SODIUM CHANNELS IN HIGH FREQUENCY PACEMAKING NEURONS OF ELECTRIC FISH. L. Maler, M. Ellisman, and S.R. Levinson. Dept. of Anatomy, University of Ottawa, Ottawa, Ont. K1H 8M5, Dept. of Neuroscience, UCSD, La Jolla, CA 92093, and Dept. of Physiology, University of Colorado, Denver CO 80262.

The electrical behaviour of neurons is dependent on voltage sensitive ion channels; although the differential distribution of ion channels over the somatic, dendritic, and axonal membranes contributes to the integrative properties of the neuron it has not been possible to directly visualize this distribution. Sodium channels are located on unmyelinated axons and are densely clustered at axonal nodes and the initial segment. Recently an antibody has been developed against the sodium channels isolated from the electric organ of Electrophorus electricus, a gymnotiform fish. It was also verified that this antibody produced reliable localization of sodium channels on electrocytes and at nodes of Ranvier of the eel (Ellisman & Levinson, 1982). We have used immunocytochemistry to study the distribution of sodium channels on functionally defined neuronal populations of another gymnotiform fish, Apteronotus leptorhynchus. This species has an electric organ discharge (EOD) which ranges from 600-1000Hz. The EOD frequency is set by a medullary pacemaker nucleus which contains pacemaker cells with intrinsic axons and relay cells whose axons innervate spinal electromotor neurons. The pacemaker nucleus is rich in saxitoxin binding sites and the pacemaker potential is a TTX sensitive sodium current (Reed, Stys & Maler, unpubl. obs.). A subset of the electrosensory afferent system discharges in a phase-locked one-to-one fashion to the EOD (i.e. 600-1000Hz) - the phase coder or T system. Phase coders project to a specific population of neurons in the medulla (spherical cells) and these in turn project to adendritic giant cells in the midbrain (Carr et al., 1986); both spherical and giant cells maintain a one to one phase-locked discharge to the EOD. In the pacemaker nucleus sodium channels are located in the plasmalemma at nodes and the initial segment of pacemaker and relay cells. The somatic plasmalemma of both pacemaker and relay cells is densely labelled by the sodium channel antibody and this labelling extends onto the proximal dendrites of the relay cells. The plasmalemma of the large club endings generated by pacemaker cells are also strongly immunoreactive for the sodium channel protein. In contrast, the spherical and giant cells which fire at the same rate as the pacemaker cells, do not have sodium channel immunoreactivity associated with their plasmalemma; nodes of Ranvier on the axons of these cells are labelled by the sodium channel antibody. These results demonstrate that a high density of somatic sodium channels is important for cells which generate the pacemaker discharge, but not for cells which merely passively follow the discharge.

29.5 EFFECTS OF NEURAMINIDASE TREATMENT OF BATRACHOTOXIN-MODIFIED EEL PURIFIED SODIUM CHANNELS IN PLANAR LIPID BILAYERS. E. Recio-Pinto, W.B. Thornhill, D.S. Duch, S.R. Levinson, and B.W. Urban*. Depts. Anesthesiology and Physiology. Cornell U. Med. Coll., New York, N.Y. 10021. Dept of Physiology, Univ of Colorado, School of Medicine, Denver, CO 80262.

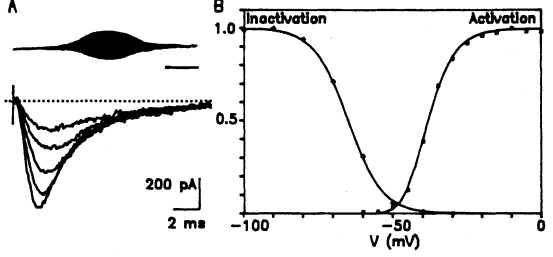
Treatment with neuraminidase, which removes sialic acids, resulted in a decrease in the apparent molecular weight of the purified eel sodium channel from 290 to 210 kDa as detected on polyacrylamide SDS gels. Neuraminidase treated (5 hrs) and untreated purified, reconstituted sodium channels were fused with neutral planar bilayers in the presence of batrachotoxin as previously described (Recio-Pinto, E., Duch, D.S., Levinson, S.R. and Urban, B.W., 1987; J. Gen. Physiol., in press). In symmetrical 0.1 M NaCl the mid-point potential of the steady state activation curve was lower in the neuraminidase treated (-46 mV) than in untreated (-76 mV) channels. Neuraminidase treated channels frequently showed subconductance-like states (two major peaks around 16 pS and 8 pS), which was the predominant one in untreated channels. Some neuraminidase treated channels also showed a non-linear current-voltage relationship. In other experiments untreated reconstituted sodium channels were first fused with planar bilayers and then treated with neuraminidase (symmetrical 0.1 M NaCl). Neuraminidase treatment produced a shift in the mid-point potential to more depolarized potentials of about 20 mV. In addition, neuraminidase treatment increased the frequency of subconductance-like states. We conclude that removal of sialic acids with neuraminidase accounts for these changes in channel behavior, by either decreasing the negative charges in the extracellular surface of the channel and/or allowing conformational changes of the channel.

29.6 **VOLTAGE-CLAMP ANALYSIS OF Na^+ CURRENTS IN ISOLATED NEURONS FROM THE GUINEA PIG CNS**

Alan R. Kay * & Robert K.S. Wong. (SPON : K.-W. Yau) Dept. Neurology, Columbia University CPS, New York, NY 10032.

Macroscopic Na^+ currents were measured in the whole-cell mode of patch-clamping, from neurons isolated (method of Kay & Wong, J. Neurosci Methods, 16, 227,'86) from the hippocampus, neocortex and thalamus of mature guinea pig brain. K^+ currents were blocked by external TEA, Cs & 4AP, and internally by Tris. Ca^{2+} currents were blocked by 200 μM Cd^{2+}.The Na^+ current could be controlled throughout its range of activation when the external Na^+ was reduced to 30 mM (see fig. A ; hippocampal CA1 neuron (scale 10 μm) depicted above current traces; reponses to steps to -42, -40, -38, -36 & -34 from -90 mV). Adequate voltage-clamp was also ensured by the electrotonic compactness of the cells (length of processes $< 0.1\lambda$), low access resistance of electrodes (2 -5 MΩ), and small capacitance of the cells (2 -15 pF). In some instances unclamped Na^+ currents were observed together with controlled Na^+ currents; such recordings were always associated with the presence of fine branches.

The steady-state activation and inactivation curves (see Fig. B; hippocampal CA1 neuron) of the Na^+ current overlapped to a small extent, consistent with the small 'window-current' observed. Moreover, in none of the areas from which Na^+ currents were recorded, was a separate persistent Na^+ current evident. We attempted, without success, to record this putative current in the presence of a number of different Ca^{2+} blockers (La^{3+} Co^{2+} Mn^{2+} & Ni^{2+}), as Cd^{2+} has been reported to block the persistent Na^+ in cardiac Purkinje fibres (Carmeliet, 1987). In addition to the fast inactivation of the Na^+ current which occurs with time constants in the range 2 - 20 ms, a slow phase of recovery from inactivation (half-time 30 s) was observed. Complete recovery from this phase of inactivation doubled the Na conductance available for activation. (Supported by NIH grant NS 24682 & the Klingenstein Foundation).



29.7 SOMA-DENDRITIC LOCALIZATION OF Na^+ CHANNELS IN PYRAMIDAL NEURONS ACUTELY ISOLATED FROM RAT NEOCORTEX. J.R. Huguenard, O.P. Hamill*, and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Although the electroexcitability of dendrites has been studied directly in cerebellar Purkinje cells and hippocampal pyramidal neurons, much less is known about the properties of dendritic membranes in mammalian neocortical neurons. We have directly measured Na^+ current in dendritic membranes of pyramidal neurons from rat sensorimotor cortex, using the acute cell isolation procedure of Kay and Wong (J. Neurosci. Meth. 16: 227, 1986).

Isolated pyramidal neurons were easily identified on morphological criteria. We applied the patch clamp technique to such neurons from P3-P8 animals under conditions which isolated Na^+ currents. Two techniques were used to localize the current to dendritic vs. somatic membrane areas. The first involved differential perfusion of a cell during whole cell voltage clamping so that only a portion of the membrane was exposed to a Na^+-containing solution, whereas the rest of the neuron was bathed in 0 Na (choline substitution). Under these conditions we found that the amplitude of the Na^+ current (normalized to membrane area) was approximately equal over apical dendritic and somatic membranes (ca. 40 pS/μ^2). A second technique involved direct measurement of single channel currents with cell-attached patches from different membrane sites. At least one somatic site and 1-3 sites on the primary apical dendrite up to 100μ from the soma were sampled sequentially in each cell. This technique provided results similar to those of the differential perfusion experiments. The density of Na^+ channels per equivalent patch was uniform throughout the dendro-somatic membrane area. Furthermore, the kinetic properties of the channels were consistent with the idea of a uniform population distributed through the entire membrane. We conclude that there is a relatively uniform distribution of Na^+ channels over the somatic and primary apical dendritic membranes of cortical pyramidal neurons at these ages and that the regenerative Na^+ currents in the major dendritic processes are equivalent to those of the soma.

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29.8 DIAZEPAM (VALIUM) ACTIONS ON MOUSE AND GUINEA PIG AURICULAR MUSCLE. E. Gijón*, M.C. Márquez-Orozco*, A. Márquez-Orozco* and X. García*.(SPON: B. Ortega-Corona). Department of Physiology and Department of Embryology. School of Medicine. National Autonomous University of México. México, D.F. 04510. MEXICO.

It has been described that benzodiazepines reduce the action potential duration and cause a negative inotropic effect on heart muscle from dog, cat, guinea-pig and rabbit, and this has been explained as a calcium current diminution (Akutagawa, K., Makino, M. and Ishii, K., Japan. J. Pharmacol., 33:845-850, 1983). Bones from mouse treated with diazepam, fix greater quantities of calcium (Márquez-Orozco, M.C., Márquez-Orozco, A., Alvarez, G. and Sámano-Bishop, A., Bol. Soc. Mex. Cienc. Fisiol. 7(1): 10-15, 1984). Our studies on mouse auricular muscle showed that diazepam increases the action potential duration in a reversible form and causes positive inotropic effect (García, X., Márquez-Orozco,M.C., Márquez-Orozco, A. and Gijón, E., Res. C. Nacl. Farmac. and Reg. Reu. latinoam. Assoc. Pharmacol. 10: 101, 1986. Further studies on diazepam action on electrical and mechanical activity of left auricular muscle from CD-1 stump mouse and Hartley guinea-pig, mounted horizontally in a constant temperature bath (34º C) perfused with Krebs solution, Ca2+ 1.25 mM, bubbled with a mixture of 98% O2 and 2% CO2. Diazepam ampules (Valium 10) Roche, were used, with a final bath concentration of 1.75X10-4 M. It was found in preparations electrically driven, from mouse and guinea-pig, that diazepam induces an increase in action potential duration measured at 20% repolarization, an increase in amplitude and duration of reverse potential, a decrease in the rate of spike depolarization, and modifies the conduction velocity of the action potential. It produces in preparatios with spontaneous activity, positive chronotropic and inotropic effects, and inotropic effect in electrically driven preparations. The change in action potential duration on mouse preparations, is higher than on guinea-pig preparations, c.a. X3. These findings support that diazepam action may be due to an increase in the inward calcium current and a reduction in the inward sodium current.

29.9 PYRETHROID INSECTICIDES AND DDT MODIFY ALKALOID-DEPENDENT ACTIVATION OF THE VOLTAGE-SENSITIVE SODIUM CHANNEL. J. R. Bloomquist and D. M. Soderlund. Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456.

The actions of pyrethroid insecticides and DDT on alkaloid-dependent activation of the voltage-sensitive sodium channel were studied using measurements of ^{22}Na uptake into mouse brain synaptosomes. Deltamethrin and DDT at near-saturating concentrations enhanced maximal sodium uptake (E_{max}) stimulated by high concentrations of veratridine with little effect on potency measured as half-maximal uptake ($K_{0.5}$). Cismethrin, however, increased the potency of veratridine-dependent uptake 3-fold with no increase in E_{max}. All three insecticides also enhanced sodium uptake in the presence of 1 µm batrachotoxin. With DDT (100 µm), the $K_{0.5}$ for batrachotoxin activation was shifted to 2-fold lower concentrations with no change in E_{max}. In contrast, deltamethrin, cismethrin, and DDT inhibited activation of the sodium channel by aconitine. Cismethrin (10 µm) was a noncompetitive inhibitor of aconitine-dependent uptake, decreasing E_{max} 34% without affecting the potency of aconitine. The inhibition of aconitine-dependent uptake by 100 µm cismethrin was expressed in the presence of 1 µm sea anemone toxin II, where E_{max} was decreased 60%.

The inhibition of aconitine-dependent sodium uptake by these insecticides suggests a possible overlap or negative allosteric coupling between the binding sites for insecticides and aconitine that differs from the enhancement of uptake observed with these compounds and either veratridine or batrachotoxin. Thus, the actions of DDT and pyrethroids on the voltage-sensitive sodium channel reveal unique characteristics of the action of aconitine that are not shared by veratridine and batrachotoxin. This work was supported by NIH Grant ES02160. We thank Dr. John Daly for supplying a sample of batrachotoxin.

29.10 YOHIMBINE'S EFFECT ON THE BINDING OF BATRACHOTOXININ TO MOUSE BRAIN SODIUM CHANNELS. I. Zimanyi*, A. Lajtha, E.S. Vizi, and M.E.A. Reith. Center for Neurochemistry, N.S. Kline Institute, Ward's Island, New York, NY 10035, and Institute of Exp. Med. Hung. Acad. Sci., Budapest POB 67, H-1450, Hungary,

Local anesthetics, such as procaine and lidocaine inhibit the binding of [^{3}H]BTX-B to sodium channels of rat or mouse brain, involving a specific receptor, and although their inhibitory effect appears to be competitive, the mechanism is best described by the allosteric model for heterotropic cooperative interactions (Reith M.E.A. et al., J. Biol. Chem., 261:7300, 1986).

Although yohimbine is best known as an alpha$_2$-adrenoceptor antagonist, it also has a meaningful local anesthetic activity. Previous electrophysiological and biochemical studies indicate that yohimbine binds to the gating molecules of sodium channels and modifies their opening and closing rates. Revenko et al. (Neuroscience, 7:1377, 1982) suggested that yohimbine and local anesthetics share a common receptor in sodium channels.

In the present study [^{3}H]BTX-B binding to mouse brain cortex was used as a tool to investigate the interactions of yohimbine and other local anesthetics with the gating mechanism of voltage-dependent sodium channels.

Yohimbine inhibited the [^{3}H]BTX-B binding with an IC_{50} of 9.6 uM. The Hill coefficient was 1.07 $\pm$ 0.04 (n=4), suggesting that yohimbine interacts with single class of binding sites. Saturation analysis with 5-200 nM of BTX-B in the absence and presence of 10 uM yohimbine indicated a K_d of 27 nM and 61 nM, and a B_{max} of 3.5 and 4.2 pmol/mg of protein, respectively, in agreement with yohimbine beeing a competitive inhibitor of BTX-B binding.

Kinetic studies of the dissociation rate of the BTX-receptor complex indicated that yohimbine, unlike that procaine or lidocaine had no effect on the rate of aconitine-induced dissociation of [^{3}H]BTX-B. The values of the k_{-1} in the absence and in the presence of 10 uM yohimbine were 0.0061 and 0.0066 min^{-1}, respectively. Therefore the mechanism by which yohimbine inhibits BTX-B binding is different from that for local anesthetics. The latter drugs shift the sodium channel to an inactive form from which BTX-B dissociates with a high rate. The data taken together strengthen the idea that yohimbine competes with BTX for the BTX receptor sites themselves, while other local anesthetics act through their own binding sites. The effect of yohimbine shown here suggests that local anesthetic action of compounds can be achieved by mechanism other than those involving receptors in the channel distinct from the BTX site. Supported by NIDA grant DA 03025, and by a grant for Cocaine Research from N.Y. State.

29.11 IN VIVO ANTICONVULSANT ACTIVITY AND IN VITRO BLOCKADE OF REPETITIVE ACTION POTENTIALS BY TWO PUTATIVE ANTICONVULSANTS, RALITOLINE (CI-946) AND CI-953. C.P. Taylor, D.M. Rock, M.J. McLean, and R.L Macdonald. Dept. Pharmacol., Warner-Lambert/ Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105 and Dept. Neurol., Univ. Michigan Sch. Med., Ann Arbor, MI 48109.

Although anticonvulsants have been used for the clinical treatment of partial seizures for many years, there is a need for new agents with better efficacy and fewer side effects. Ralitoline ((Z)-N-(2-chloro-6-methylphenyl)-2-(3-methyl-4-oxo-2-thiazolidineylidene)acetamide) and CI-953 (N-(2-chloro-6-methylphenyl)-N'-4-pyridinyl urea, monohydrochloride) have related chemical structures that are different from those of known anticonvulsants. Both compounds were tested by the Antiepileptic Drug Discovery Program of NINCDS. Ralitoline and CI-953 prevented tonic extensor seizures from maximal electroshock in mice with ED_{50} values of 15 mg/kg and 12 mg/kg PO respectively. Ataxia was the first dose-related side effect in mice, with ED_{50} values of 205 mg/kg and 240 mg/kg respectively. Thus both compounds are expected to prevent tonic-clonic seizures. Neither compound completely protected mice from clonic seizures in mice produced by the convulsants pentylenetetrazol, bicuculline or picrotoxin and so neither compound is expected to prevent absence seizures.

Rats were kindled with electrical stimuli from chronically-implanted electrodes in the dorsal hippocampus until generalized (stage 5) seizures were reliably obtained (methods of Vartanian and Taylor, Neurosci. Abstr. 10:406,1984). After administration of ralitoline or CI-953, the electrical current necessary to produce a focal afterdischarge was increased in a dose-related manner, at doses that did not cause ataxia. Thus both compounds would be expected to prevent partial seizures.

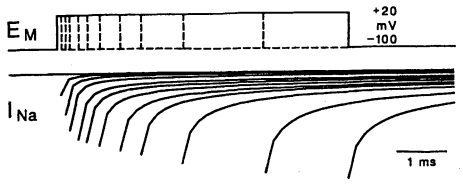
Spinal cord neurons were cultured from neonatal mice and drugs were added to the bathing physiological solution (McLean and Macdonald, J. Pharmacol. Exp. Ther. 227:779,1983). Intracellular recordings in vitro showed that ralitoline (0.1 to 1.0 µg/ml) or CI-953 (0.2 to 3 µg/ml) blocked sustained repetitive action potentials elicited by depolarizing current steps (450 msec) but did not block the initial spike of the train. Spontaneous action potentials (caused by spontaneous synaptic activity) were decreased with higher drug concentrations (3 µg/ml of ralitoline, 10 µg/ml of CI-953). Postsynaptic GABA and glutamate iontophoretic responses were not altered at 30 µg/ml (ralitoline) or 20 µg/ml (CI-953).

These results indicate that both experimental drugs may act specifically on voltage-sensitive sodium channels (Catterall, Trends Pharmacol. Sci. 8:57, 1987).

29.12 MODIFICATION OF SODIUM CHANNELS IN THE RESTING STATE BY FENFLUTHRIN M. Nishio* and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The pyrethroid insecticides are known to modify the gating kinetics of sodium channels. However, it has been a matter of controversy whether pyrethroids modify the sodium channel in the open state or in the resting state. Experiments were performed with squid giant axons using fenfluthrin, a new fast-acting pyrethroid. It is a type I pyrethroid devoid of a cyano group at the α position. Sodium channel currents were recorded with internally perfused squid giant axons under voltage clamp conditions. The external solution contained (mM): 110 NaCl, 345 TMA-Cl, 50 CaCl and 5 HEPES with the final pH adjusted to 8.0. The internal solution contained (mM): 250 Cs-glutamte, 20 NaF, 400 sucrose and 30 Na-phosphate with the final pH adjusted to 7.3.

The internal application of fenfluthrin inhibited the falling phase of the sodium current during the depolarizing pulse and prolonged the sodium tail current associated with step repolarization. If the pyrethroid modified the channel in its open state, the rate at which the channel arrived at the modified open state, which is represented by the time course of the development of tail current amplitude, would be dose-dependent. The membrane was depolarized to +20 mV (the sodium equilibrium potential) for various durations and the tail currents associated with repolarization to -100 mV were recorded. The time course of the development of tail current amplitude was expressed by two exponential functions. Each of the fast and slow time constants remained unchanged at various concentrations of fenfluthrin (1-30 µM), indicating that the rate of arrival of channels in the modified open state is independent of dose. The decay of tail current, which represents the closing kinetics of modified channels, was dual exponential. In the concentration range tested, the time constants of both fast and slow components were independent of dose. These results suggest that fenfluthrin modifies the sodium channel in its resting state. Supported by NIH grant NS14143.



29.13 KINETICS OF BLOCKING ACTION OF PSYCHOTROPIC DRUGS ON SODIUM CHANNELS IN SINGLE GUINEA PIG VENTRICULAR MYOCYTES. T. Narahashi, N. Ogata* and M. Nishimura* (SPON: C. Wu). Department of Pharmacology and Reingold ECG Center, Northwestern University Medical School, Chicago, IL 60611.

A major side effect of the psychotropic drugs such as neuroleptics or antidepressants is cardiac toxicity. The mechanism underlying this side action remains to be clarified. We have shown that chlorpromazine, a phenothiazine neuroleptic, directly affects voltage-gated sodium and calcium channels in mouse neuroblastoma cells (Ogata, N. and Narahashi, T., Biophys. J., 51:430a, 1987). Such a direct action of chlorpromazine on the ion channels might also play a pivotal role in the generation of the cardiac toxicity. We, therefore, studied actions of several representative psychotropic drugs on the sodium channel kinetics in guinea pig ventricular myocytes. The myocytes were enzymatically isolated and voltage-clamped using the whole cell patch clamp technique at 20-22°C. Chlorpromazine, imipramine (a tricyclic antidepressant) and haloperidol (a butyrophenone neuroleptic) produced concentration-dependent and fully reversible block of the sodium current evoked after a long resting period at negative holding potential of -120 mV. The order of potency for this tonic block was chlorpromazine (KD, 3×10^{-6}M) > imipramine (10^{-5}M) > haloperidol (2.5×10^{-5}M). The blocks induced by these drugs were markedly enhanced when the holding potential was set to more positive values, in agreement with the shifts of steady-state inactivation curves in the negative direction. The concentration-response curves for all three drugs were best fit by a 2:1 stoichiometry for cardiac sodium channels whereas sodium channels in neuroblastoma cells exhibited a Hill coefficient of 1. All these drugs produced an additional block of sodium channels during a train of depolarizing voltage clamp pulses. This use-dependent block occurred even by a brief (10 msec) depolarizing pulse and a long (up to 2 sec) interpulse interval. Such a prominent use dependence was due to the extremely fast onset of drug-induced block and slow repriming of sodium channels from drug-bound inactivated state. The blocking action of psychotropic drugs was not affected by low temperature (12°C). The action of psychotropic drugs on cardiac myocytes is characterized by a tonic decrease of sodium channels which are available to open on membrane depolarization and a phasic decrease due to use-dependent block. These actions which occur at concentrations of therapeutic range and could be pronounced by pathological conditions such as excessive membrane depolarization, presumably underlie cardiac side effects commonly observed during a treatment by the psychotropic drugs.

Supported by NIH Grant NS14144.

29.14 INVESTIGATION OF HYDROGEN SULFIDE TOXICITY IN MOUSE NEUROBLASTOMA CELLS USING PATCH-CLAMP TECHNIQUE. M.W. Warenycia, J.A. Steele*[1], E. Karpinski*[1] and R.J. Reiffenstein. Departments of Pharmacology and Physiology[1], Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Hydrogen sulfide (H_2S) and its soluble alkali salts are extremely toxic. Every year many cases of fatal exposure occur in the seventy or more occupational settings where H_2S is routinely encountered. Inhaled concentrations of greater than 500 ppm are lethal (717 ppm = 1 mg/L). The cause of death appears to involve abolition of respiratory drive in brainstem nuclei. The exact mechanism underlying respiratory failure has not been elucidated. Since H_2S has lipophilic properties, being five times more soluble in ether than water, it seemed of interest to examine membrane currents in cells poisoned with sodium hydrosulfide (NaHS), a precursor of H_2S in vivo. The cells chosen for these experiments were mouse N1E-115 neuroblastoma cells, a cell line with well-studied sodium channels. Cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) fetal calf serum under an atmosphere of 95% O_2/5% CO_2 and were used on the second day postplating. Culture media was removed and replaced with a HEPES-buffered (10 mM) physiological salt solution. Cells were voltage-clamped using the giga-seal method employing micro-electrodes filled with 135 mm CsCl, 20 mM TEA, 10 mM HEPES and 2 mM EGTA. Membrane potential was maintained at -70 mV and sodium currents were examined at different test-potentials in the absence (control) or presence of 9 mM NaHS. This concentration of NaHS represents a 50-fold excess of the amount comprising the LD_{50} in mice (Reiffenstein, R.J. and Warenycia, M.W., Br. J. Pharmac., in press, 1987). The results of ten experiments did not reveal any significant changes in sodium currents. Current-voltage relationships appeared unaltered in poisoned cells as compared to controls. Similarly, channel kinetics also appeared to be unaffected.

These results in a model system suggest that alteration of sodium channel function in neuronal cell membranes does not appear to underlie the mechanism(s) leading to loss of central respiratory drive. However, it is also possible that smaller neurons, having higher respiratory rates, are selectively more vulnerable to poisoning with H_2S than N1E-115 cells, especially if in such cells resting membrane potential is highly dependent on Na^+-K^+-ATPase. In addition, it is distinctly possible that alteration of other ionic channel systems by H_2S, such as chloride, could still be a significant determinant in disrupting neuronal activity in brainstem respiratory centers.

Supported by the Occupational Health and Safety Heritage Grant Program, Alberta Department of Community and Occupational Health.

29.15 Myelination: optimization, conduction velocity in small fibers and resolution of a paradox. M.V.L. Bennett, A. Einstein Coll. Med., Bronx, NY 10461, and D.H. Perkel (Univ. Cal., Irvine, CA 92717).

Rushton (J. Physiol. 115: 101, 1952) concluded that "a very small fiber must conduct faster if it is non-myelinated than if it is myelinated", but also cited Huxley and Stampfli's demonstration (ibid. 108: 315, 1949) that there is "an optimum internodal length for a maximum conduction velocity" with monotonic decrease in conduction velocity on either side of the maximum. Thus it appears, paradoxically, that shortening internodes of a very small myelinated fiber should both increase and decrease its conduction velocity. Rushton's conclusion derives from the relations between conduction velocity and diameter. For myelinated fibers velocity is proportional to diameter, as observed for large fibers and derivable from dimensional analysis provided the fibers are "similar", which means that their nodal membrane has the same capacitance and channel densities and that they observe certain geometric relations. Unmyelinated fibers of different diameters but with constant membrane properties are "similar", and velocity is proportional to the square root of diameter. Thus sufficiently small myelinated fibers of any "similarity" class conduct more slowly than unmyelinated fibers of the same diameter but of a different similarity class. The paradox is resolved as follows: Shortening the internodes of a Rushton fiber below the optimum length does decrease its conduction velocity whatever its diameter, but shortening the internodes to zero does not make it an unmyelinated fiber. In a Rushton fiber nodal length is negligible with respect to internodal length and as internodal length decreases, the number of nodes per unit length increases without limit; thus a Rushton fiber without myelin has infinite admittance and conducts with zero velocity. We calculated velocity for myelination with finite nodal length using the Merson numerical integration algorithm and a model with Hodgkin-Huxley channels and compartments of 0.25 and 0.125 length constants. We find that velocity can be increased by myelin of even a single wrap, which is optimum thickness for fibers of about 0.1 um diameter. Thinner myelin cannot occur, although permitted in a Rushton fiber. However, "myelination" by inserting plasma membrane without channels (a myelin sheath of zero thickness) can slightly increase conduction velocity, because nodal membrane has a greater than optimum density of channels for maximum velocity in an unmyelinated fiber (A. Hodgkin, Phil. Trans Roy Soc. Lond. B270: 297, 1975). Of course the same velocity could be achieved by uniformly distributing the channels. We conclude that myelination can increase conduction velocity down to the diameter permitted by the constraints of myelin structure.

ACTION POTENTIALS AND ION CHANNELS II

30.1 **CALCIUM DEPENDENCE OF VOLTAGE SENSITIVITY IN ADENOSINE 3',5'-CYCLIC PHOSPHATE-STIMULATED SODIUM CURRENT.** **Daniel J. Green and Rhanor Gillette.** Department of Physiology & Biophysics, and the Neural & Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

Iontophoretic injection of cAMP into a voltage clamped neuron of the mollusc Pleurobranchaea caused a transient slow inward current (I_{si}) whose amplitude was enhanced by depolarization. Na^+-replaced salines suppressed the current, placing it with cAMP-stimulated Na^+ currents of other gastropod species.

I_{si} amplitudo was suppressed by extracellular Ca^{2+}. The amplitude increased up to 4-fold at holding potentials of -50 mV in nominally zero-Ca^{2+} saline. Ion substitutions showed that Ca^{2+} suppressed the I_{si} more effectively than Mg^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Ba^{2+}, or Sr^{2+}.

Voltage sensitivity of I_{si} was abolished both by low Ca^{2+} salines, by the Ca^{2+} current blocker Co^{2+} and by substitution of Ba^{2+} or Sr^{2+} as Ca^{2+} channel current carriers. In such salines the I_{si} showed no appreciable change in amplitude at holding potentials between -70 to -25 mV.

Intracellular injection of the Ca^{2+} chelator EGTA augmented both the amplitude of the current and its duration. EGTA injection failed to suppress the Ca^{2+}-dependent voltage sensitivity of the I_{si}. Intracellular injection of concentrated MOPS pH buffer to inhibit secondary, Ca^{2+}-dependent intracellular acidification also failed to suppress the voltage sensitivity, as did injections of mixed EGTA/MOPS solution.

While the data indicate a requirement for extracellular Ca^{2+} in conferring voltage sensitivity, they do not support a role for an intracellular action. An extracellular binding site for Ca^{2+} is likely to mediate the voltage sensitivity, either by local depolarization-dependent changes in extracellular Ca^{2+} concentration or through direct voltage-sensitive block of the I_{si} channel.

Conferral of voltage sensitivity to the cAMP-dependent Na^+ current by Ca^{2+} provides a novel positive feedback mechanism potentially involved in burst initiation and maintenance.

30.2 PROPERTIES OF THE RECTIFYING Cl^- CHANNEL IN CULTURED SWEAT GLANDS. M.E. Krouse* (SPON: J.J. Wine). Cystic Fibrosis Research Laboratory, Stanford University, Stanford CA 94305.

Single 45 pS Cl^- channels were found in 1/3 of the patches made on confluent monolayers of cultured human sweat glands. The channels showed outward rectification with a slope conductance of 30 pS at -75 mV and a slope conductance of 70 pS at +75 mV in symmetrical 150 mM NaCl Ringer at 20°C. The channels are selective for Cl^- over Na^+ by at least 4:1. The open probability of these channels is voltage dependent both in cell attached and excised configurations. The mean open time is 32 ms at +50 mV and increases e-fold for every 65 mV depolarization. The mean open time is almost identical in cell attached and excised patches. The mean closed time in the cell attached patch is 1100 ms which decreases to 24 ms in the inside-out patch (measured several minutes after excision). These mean closed times are also voltage dependent, decreasing e-fold for every 65 mV depolarization. Indirect evidence suggests that the open probability is Ca^{2+} dependent on cell and immediately upon excision, when the open probability excedes 0.9 (not analyzed). There is no Ca^{2+} sensitivity several minutes after excision, when the open probability equals .57 at +50 mV. One interpretation of these results is that Ca^{2+} increases the dissociation rate of a channel blocking molecule, which is loosely associated with the cytoplasmic face of the channel.

30.3 NONSELECTIVE CATION CHANNELS AND GENERATION OF BURSTING ACTIVITY IN CULTURED PITUITARY CELLS. R.E. Sheridan and M. Adler. Neurotoxicology Branch, USAMRICD, APG, MD 21010 and Dept. of Pharmacol., Georgetown University, Washington, DC 20007.

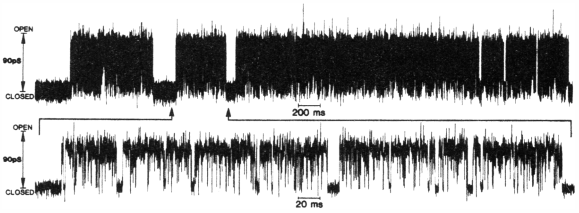
The clonal pituitary cell line AtT-20/D16-16 produces rhythmic bursts of spikes in response to certain neurotransmitters or after culture in dibutyryl cAMP. The current that initiates these bursts is unknown. We have identified a novel, nonselective cation channel in the AtT-20 cell line that has the necessary characteristics to act as the generator current for initiating burst activity.

AtT-20/D16-16 cells were grown in Ham's F12 medium supplemented with 10% fetal bovine serum and 1 mM dibutyryl cAMP. Cells were then transferred to HEPES buffered physiological salines of various cationic and anionic compositions for patch-clamp recording. In cell-attached patches an inward current was observed at the resting potential which reversed near 0 mV. The same channel could be seen in excised, inside-out patches and was equally permeable to Na^+ and K^+ but essentially impermeable to Cl^-. The channel had a conductance of 25 pS in symmetric solutions of 155 mM NaCl at 20° C. At least two open states were observed with lifetimes of 7.7 ms and 290 ms in the presence of 200 μM free Ca^{2+} at 20° C and -60 mV. However, unlike similar nonselective cation channels that have been described before, both lifetimes were voltage-dependent, increasing e-fold per 20 mV of depolarization. Also, the AtT-20 cation channel was active at sub-micromolar concentrations of intracellular Ca^{2+} and showed some short duration openings even in 10 nM free Ca^{2+}.

The characteristics of this depolarizing cation channel suggest that it could be responsible for initiating spike bursts in AtT-20 cells. Thus the activity of this nonselective cation channel, in conjunction with that of the Ca-activated K^+ channel, may be responsible for setting the level of excitability in these and other spontaneously active cells. Drugs that modify the nonselective cation channel could therefore change the firing patterns of such spontaneously active cells.

30.4 TETANUS TOXIN FORMS TRANSMEMBRANE CHANNELS IN LIPID BILAYERS AT NEUTRAL pH. F. Gambale* and M. Montal (SPON: R. Wurzburger). Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110.

Tetanus toxin from *Clostridium tetani* forms channels in planar lipid bilayers. The channel forming domain was assigned to the amino terminus of the heavy chain (apparent Mr ~ 45,000). Channel formation proceeds at neutral pH. However, an acidic pH and a positive voltage in the compartment containing the toxin are required to detect the presence of channels in the membrane rapidly and effectively. The toxin channel is cation selective with significant intracation selectivity (K^+/Na^+ = 1.35). Acid pH markedly lowers the single channel conductance: for phosphatidylserine (PS) at 0.5 M KCl γ = 89.01 ± 0.30 pS at pH 7.0 while at pH 4.8, γ = 30.12 ± 0.33 pS. The toxin channel is voltage dependent: for PS membranes (see Figure), the probability of the channel being open is significant at positive voltage both at neutral and acidic pH. Single channel conductance histograms are best fitted with a sum of three gaussians with relative frequency of occurrence for PS of 38% for the closed state, 18% for the open subconductance state and 44% for the fully open state. The subconductance state is readily identifiable in the single channel recordings (see Figure, lowest panel). Probability density analysis of the open and closed dwell times of the toxin channel indicate the existence of a single open state with a lifetime, τ_O, of about 1 ms and two closed states with lifetimes $\tau_{C1} \leq 0.5$ ms and $\tau_{C2} \approx 1$ ms.



Legend: Single tetanus toxin channel currents at an applied voltage of 80 mV and at pH 7.0. The records were filtered at 3 kHz. The lower record displays the section delimited by the arrows at higher time resolution.

30.5 ANTERIOR PITUITARY CELLS POSSESS A CALCIUM-DEPENDENT CHLORIDE CONDUCTANCE THAT IS REGULATED BY GTP-ANALOGS. M.A. Rogawski, S. Suzuki*, K. Inuoe* and J.L. Barker. Neuronal Excitability Section, Medical Neurology Branch and Lab. of Neurophysiology, NINCDS, NIH, and FDA, Bethesda, MD 20892.

Voltage and/or Ca^{2+}-dependent Cl^- conductances have recently been identified in a variety of excitable cell types. In the present study, we characterized a Ca^{2+}-dependent Cl^- current in GH_3 cells, a clonal cell line derived from anterior pituitary. Whole cell voltage clamp recordings were made from GH_3 cells under conditions in which outward currents were substantially blocked by external tetraethylammonium (TEA; 20 mM) and internal CsCl or N-methylglucamine Cl (NMG). Depolarizing voltage clamp steps from a holding potential of -50 mV activated transient (decay time <100 msec; T-type) and sustained (L-type) inward Ca^{2+} currents. In addition, prolonged depolarization (>1 sec) invariably elicited a slowly activating inward current that persisted with maintained depolarization, and deactivated slowly (τ~1 sec) upon repolarization, resulting in a prominent inward tail current. The amplitude of the tail increased as the step duration was lengthened to a plateau at 6 sec. The tail current nulled at 0 mV with symmetrical Cl^- and showed a negative reversal potential with nominally Cl^--free internal solution. In addition, the tail current could be elicited under conditions where Ca^{2+} and Cl^- were the only permeant ions (symmetrical NMG). Ba^{2+} substituted for Ca^{2+} as a carrier of inward current, but did not activate the tail current. These observations suggest that Cl^- ions are the charge carrier of the slow inward tail current. The voltage-dependency for activation of the slow tail current was U-shaped with a peak at about -10 mV. This closely paralleled the voltage-dependency of the Ca^{2+} currents. The slow tail current was eliminated upon superfusion with the Ca^{2+} channel blocker Cd^{2+} or with Ca^{2+}-free medium. These results demonstrate that the current is dependent upon Ca^{2+} influx and it is therefore referred to as $I_{Cl(Ca)}$. In a high proportion of cells, $I_{Cl(Ca)}$ rapidly diminished in amplitude upon repeated activation. This rundown could be slowed by addition of the non-hydrolyzable GTP analogs GppNHp (100 μM) or GTPγS (100 μM) to the pipette solution; GTP, ATP, 8-bromo cyclic AMP or GTPβS did not prevent rundown. Neither pertussis toxin nor cholera toxin prevented the GTP analogs from slowing rundown or influenced $I_{Cl(Ca)}$. These results demonstrate that clonal pituitary cells possess a slowly activating and deactivating Ca^{2+}-dependent Cl^- current. The current may serve to dampen excitability under conditions of large rises in free intracellular Ca^{2+}, such as occurs during activation by secretagogues.

30.6 NOVEL Cl^- CHANNEL SEEN IN PLANAR BILAYERS AND PATCH-CLAMPED LIPOSOMES MADE USING SARCOPLASMIC RETICULUM PURIFIED FROM MAMMALIAN SKELETAL MUSCLE. G.D. Hals* and P.T. Palade* (SPON: D. Brunder). Dept. of Physiol. & Biophys., UTMB, Galveston, TX 77550.

We wish to report observations of a high conductance Cl^- channel seen in purified sarcoplasmic reticulum (SR) subfractions from New Zealand White rabbit skeletal muscle. The SR was prepared according to Saito, A., et al., J. Cell. Biol., 99:875, 1984, fractions R3 and R4. Both liposomes and planar bilayers were made from phosphatidylethanolamine and phosphatidylserine in a 50:50 ratio. This channel has been observed with similar frequency as SR K^+ channels using standard planar bilayer techniques. We have also modified a published method for obtaining SR K^+ channels in liposome patches (Tomlins, B. and Williams, A.J., Pflugers Arch., 407:341, 1986) to allow observation of SR Cl^- channels as well. The difference in our method is that the liposomes were made with SR that was not detergent treated. The Cl^- channel seen in liposome patches has a slope conductance of 462 ± 25 pS with 200 mM KCl in the bath, 100 mM KCl in the pipette. The channel is fairly selective for Cl^- ions over K^+ ions, with a $P_{Cl}/_K$ = 22, is not voltage-dependent, and normally remains open nearly all the time with frequent transitions to substate levels. We have also seen a 100 pS SR Cl^- channel similar to those previously reported (Smith, J.S., et al., Biophys. J., 50:921, 1986), but we observe this channel much less frequently. Other high-conductance Cl^- channels seen in muscle surface membrane (Blatz, A.L. and Magelby, K.L., Biophys. J., 43:237, 1983), and the mitochondrial anion channel (VDAC) have similar conductances , but are both strongly voltage-dependent, which makes these channels different from the SR channel we see. 660 μM 4,4' Diisothiocyanostilbene-2,2' Disulfonic acid (DIDS) added to the cytoplasmic surface of the channel in planar bilayer experiments results in a complete block of the channel after a period of 1 to 3 minutes.

30.7 STRETCH-ACTIVATED CHANNELS IN HUMAN FIBROBLASTS. L.L. Stockbridge and A.S. French, Department of Physiology, University of Alberta, Edmonton, CANADA T6G 2H7.

Stretch-activated channels were studied with single channel recordings from cultured human epidermal fibroblasts (Detroit 551) in the cell-attached and inside-out patch clamp configurations. Patch pipettes contained (mM): KCl 140, $MgCl_2$ 2, EGTA 11 and HEPES 10 (pH 7.2). The bath solution contained (mM): NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1 and HEPES 10. Excised patches were exposed to isotonic KCl solutions containing (mM): KCl 140, HEPES 10 and various desired $CaCl_2$ concentrations made by buffering calcium with EGTA. To determine mechanosensitivity, 0-70 mm Hg suction was applied to the patch pipette with a 1 cc syringe and monitored with a pressure transducer.

Mechanosensitive channels were found only on the borders of cells. Although up to 18 channels have been seen in one patch, most patches contained no more than two channels and the probability of getting a channel in any given patch was low (p=0.21 in 71 patches). There appeared to be more than one type of mechanosensitive channel with slope conductances in the cell-attached configuration ranging from 30 pS to 204 pS. The channels of low conductance appeared to be rapidly adapting, closing 2-5 seconds after the onset of activity with stretch-activation. The larger channels did not appear to adapt and generally were less active than the smaller channels. All channels tested were selective for potassium over sodium, with some being only permeable to potassium. The permeability to calcium has not yet been directly tested. However, no stretch-activated channels have been observed under conditions where strontium has been the only permeant ion. There was no apparent calcium sensitivity or voltage sensitivity. The probability of a channel being open increased with increasing suction. The increased openings appeared to be due to an increase in the length of time a channel was open rather than a decrease in the closed times between channel openings.

Fibroblasts respond to mechanical stimulation with a hyperpolarization (Nelson et al., *J. Gen. Physiol.* 60:58-71, 1972). It is possible that these stretch-activated channels are the primary transducers for this response.

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30.8 DYNAMIC ANALYSIS OF THRESHOLD CHANGES IN AN INSECT SENSORY NEURON REVEALS TWO COMPONENTS OF RAPID ADAPTATION. A.S. French, Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7.

Rapid sensory adaptation in the cockroach femoral tactile spine can be demonstrated by electrical stimulation of the single sensory neuron in the base of the spine, showing that it occurs during action potential encoding, not transduction. The threshold for action potential production in the neuron is labile, increasing with depolarization and decreasing with hyperpolarization. It always exceeds the membrane potential of the neuron, preventing continuous firing at any stimulus level. Threshold behavior is very sensitive to chemicals which affect sodium channel inactivation, but insensitive to many other channel modifiers.

Threshold levels in cockroach tactile spine neurons were determined as rheobasic currents, using an extracellular current stimulus applied to the neuron at the axosomatic junction (French, *J. Comp. Physiol.* 159:757-764, 1986). Threshold levels were measured at intervals up to 1500 ms after step changes in polarizing current. Decreasing current steps were easier to apply because they did not elicit bursts of action potentials. However, threshold increases were also measured following current steps small enough to prevent firing by the stimulus.

Threshold changes as functions of time after step changes in polarizing current were always well-fitted by a relationship having two exponentially decaying components. The two time constants varied strongly with the initial polarization level of the cell membrane, but not with step size. The rapid component was maximal close to the resting condition at about 170 ms, while the other component was approximately 10 times slower. Minimum values of about 50 ms and 500 ms were obtained with strong initial depolarizations. The amplitude of the slow component varied approximately linearly with different step sizes, but saturated at the strongest hyperpolarizations. In contrast, the rapid component reversed strongly with initial hyperpolarizations. Reconstruction of the total threshold from the two components agreed well with static measurements, and indicated that the two components contribute approximately equally to the threshold under depolarized conditions but the slow component is dominant with hyperpolarization. Future experiments will attempt to identify the mechanisms responsible for the two components.

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30.9 EFFECTS OF A LOCAL ANESTHETIC (QX-222) ON GATING PROPERTIES OF MOUSE-TORPEDO HYBRID ACETYLCHOLINE RECEPTORS EXPRESSED IN XENOPUS OOCYTES. R.J. Leonard, L. Yu*, C. Labarca*, N. Davidson*, and H.A. Lester. Divisions of Biology and Chemistry, California Institute of Technology, Pasadena, CA. 91125.

cDNA clones are available for all four subunits of the nicotinic acetylcholine receptor (AChR) from several species. The mRNAs transcribed in vitro from those cDNAs can be injected into Xenopus oocytes; the receptors synthesized and inserted into the membrane are amenable to electrophysiological recording. This approach promises to improve our knowledge of the structural correlates of AChR function. In an attempt to identify portions of the subunits' structure which may be involved in formation of the ion channel of the AChR, we are comparing the effects of a local anesthetic (QX222) upon the gating parameters of 'hybrid' AChRs constructed by mixing the individual subunit mRNAs derived from mouse and Torpedo cDNAs. Because the site of action of local anesthetics is presumed to lie within the ion channel, the sensitivity of hybrid, chimeric, or mutated AChRs to QX-222 may prove to be an additional probe for the assignment of particular regions or even individual residues to discreet parameters of channel function. So far, comparisons of mouse-torpedo hybrid channels indicate that some hybrids differing in mean open time in the absence of local anesthetic exhibit very similar open times in the presence of blocker, thus indicating that the forward blocking rate of QX-222 is only weakly influenced by subunit composition. On the other hand, the residence time of QX-222 (measured by the time constant of closings within an open channel burst) does appear to be influenced by subunit composition. (Supported by NIH Grant NS-11756 and an MDA grant. R.J.L. is an MDA Postdoctoral Fellow)

30.10 CHANNEL DURATION MAINLY DETERMINES VOLTAGE SENSITIVITY IN MOUSE-TORPEDO ACETYLCHOLINE HYBRIDS. L. Yu,* R. J. Leonard, C. Labarca,* N. Davidson* and H. A. Lester (SPON: J. F. Olavarria). Div. of Biol., Calif. Inst. of Technology, Pasadena, CA 91125.

At many nicotinic acetylcholine receptors, the agonist-induced conductance varies with the membrane potential, decreasing at more positive voltages. Our previous study with two-microelectrode voltage-clamp recording demonstrated that the β subunit plays the most important role in determining the equilibrium in mouse-Torpedo AChR hybrids expressed in Xenopus oocytes after the injection of mRNA synthesized in vitro from cDNA clones encoding the AChR subunits. The δ subunit plays a secondary role, interacting with the β subunit (Yoshii et al., Biophys. J. 51: 60a, 1987).

In the present work, we have examined voltage sensitivity at the level of single-channel recording with the following hybrid receptors: $\alpha_M\beta_M\gamma_M\delta_M$, $\alpha_M\beta_M\gamma_T\delta_M$, $\alpha_M\beta_T\gamma_T\delta_T$, $\alpha_T\beta_T\gamma_T\delta_M$, and $\alpha_T\beta_T\gamma_T\delta_T$. In outside-out patches, all combinations showed linear single-channel current-voltage relationship; but open duration depended on voltage. Average channel duration, τ, was measured at -60 mV and +60 mV. The extent of the voltage dependence of each combination was estimated by the ratio of $\tau_{-60mV}/\tau_{+60mV}$, and the results were in agreement with our previous conclusions from the macroscopic measurements. The voltage sensitivity of the closed times was assessed either by directly measuring the closed duration (in favorable cases where desensitization was minimal) or by subtracting the voltage sensitivity of the equilibrium conductance from that of the open duration. According to either method, closed times had less than 1/5 the voltage sensitivity of the open times. Thus, of the three possible parameters underlying voltage sensitivity (channel conductance, opening rate, and closing rate), the latter makes the major contribution to the voltage sensitivity of the macroscopic conductance of the AChR.

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30.11 MULTIPLE CONDUCTANCE CLASSES OF ACETYLCHOLINE RECEPTOR CHANNELS IN LARVAL AMPHIBIAN SKELETAL MUSCLE. J.L. Owens, G.S. Hartig*, F.A. Laitinen* and R. Kullberg. Biology Department, University of Alaska, Anchorage, AK, 99508.

Two classes of ACh receptor channels with conductances of about 40 and 60 pS have been described in previous studies of amphibian and mammalian skeletal muscle. In addition to the 40 and 60 pS channels, we have often observed in nonjunctional recordings from larval Xenopus muscle two classes of smaller events, having amplitudes of about 1/3 and 2/3 of the 40 pS channel. These smaller events appear to represent the opening of ACh receptor channels since they were abolished by alpha-bungarotoxin. The frequency of observing them was variable. In some patches they were entirely absent while in others they were the predominant channels. We have seen them in all varieties of muscles which we have studied (myotomal, extraocular, and interhyoideus), and so far we have not noticed any relation between frequency and development. The smaller events do not appear to be subconductance states of the other two channels, since we have never observed transitions directly from the 40 or 60 pS openings to the lower conductance states, and in some patches only smaller events were observed. The larger of the two small channels had a slope conductance of 25±5 pS (n=17 sites) between 40 and 120 mV (applied pipette potential) and had an extrapolated reversal potential which was comparable to that of the 40 and 60 pS channels. The gating kinetics of this channel appear to be similar to those of the embryonic 40 pS channel in myotomal muscle. The apparent mean open time at resting potential was 2.7±1.2 msec (n=15 sites), and the open time increased e-fold for about every 80 mV of hyperpolarization. In a sample of 5 recordings, the smallest channel had a slope conductance of 10 to 15 pS. We have not yet examined its gating kinetics. The functional importance of these channels in larval muscle is not clear. We do not yet know whether they are exclusively nonjunctional or whether they play any role in neurotransmission.

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30.12 KINETIC SCHEME FOR GABA-ACTIVATED CHANNELS IN CULTURED CHICK NEURONS. D. S. Weiss*, J. J. Hablitz+, and K. L. Magleby. Dept. of Physiol. and Biophys., Univ. of Miami Sch. of Med., P.O. Box 016430. Miami, FL 33101, and+Dept. of Neurology, Sect. of Neurophysiol., Baylor Coll. of Med., One Baylor Plaza, Houston, TX 77030.

In order to gain insight into the activation kinetics of a GABA-gated chloride channel, currents from single channels activated with 0.5-2.0 micromolar GABA were examined in patches of membrane from cultured chick cerebral neurons. Stability of channel kinetics was assessed by plotting the mean durations of every 50 consecutive open and shut intervals against interval number. Subsequent analysis was limited to periods over which these mean open and shut durations remained stable. The distribution of open and shut intervals of the GABA channel were both described by the sum of three significant exponential components, suggesting a minimum of three open and three shut kinetic states. Five different six state models were found which could accurately predict the observed interval distributions, so additional criteria were used to evaluate the different models. A plot of the mean duration of open intervals adjacent to shut intervals of specified durations revealed that, on the average, openings of shorter durations were adjacent to closings of longer durations. The distributions of open intervals adjacent to shut intervals of either short or long durations were both described by three exponential components with time constants similar to those observed in the distribution of all open intervals. The areas of the different components, however, were changed such that the open interval distribution adjacent to shut intervals of short duration was dominated by the component of open intervals with intermediate duration, and the open interval distribution adjacent to shut intervals of long duration was dominated by the component of open intervals with short duration. These observations suggest multiple pathways and gateway states between open and shut states. Two of the five proposed models did not predict the adjacent interval data. Two of the remaining models predicted that the channel should switch between two modes of behavior with markedly different mean open and shut intervals. The stability plots indicated that such moding behavior was not a characteristic of the channel. The single model left predicted the distributions of all open and shut intervals, the inverse relationship between durations of open and shut intervals, the distributions of open intervals adjacent to shut intervals of specified durations, and the stability of the channel kinetics with time. (Supported by NIH grants: NS11535, AM32805, NS08138, and a grant from the Muscular Dystrophy Association.)

30.13 SINGLE CHANNELS ACTIVATED BY GLUTAMATE AND ASPARTATE. J.R. Howe*, D. Colquhoun* & S.G. Cull-Candy* (SPON: C. Marshall). Dept. of Pharmacology, University College London, WC1E 6BT, U.K.

Glutamate-receptor channels in cerebellar Purkinje and hippocampal neurons possess multiple open levels and the proportion of openings to each level depends on the agonist used (Cull-Candy & Usowicz, *Nature* 325:525, 1987; Jahr & Stevens, *Nature* 325:522, 1987). We have obtained similar results in rat cerebellar granule neurons (Cull-Candy, Howe & Ogden, *J. Physiol., in press*). We now describe further the single channel currents produced by L-glutamate (Glu: 3-300μM) and L-aspartate (Asp: 10-100μM) in outside-out patches from these cells. Granule cells were maintained in culture. Solutions were nominally Mg^{2+} free. The amplitude and duration of single channel transitions were measured by fitting the signals (low pass filtered 2-5kHz, -3dB) with the known step response functions of the recording system. Up to 5 discrete peaks could be identified in amplitude histograms of Glu- or Asp-activated currents (results for both agonists were very similar and were pooled unless stated). Current-voltage (I-V) plots for each respective peak were linear between +60 and -100mV, and conductances of 8, 18, 34, 42 and 50pS were determined for the 5 open levels. At potentials negative to -100mV, large conductance openings (>40pS) showed significant inward rectification. Currents recorded at -150mV were ≈25% larger than expected from extrapolation of the linear portion of the I-V plots. The relative proportions of openings to each level were: 8pS, 2.3%; 18pS, 1.7%; 34pS, 4.9%; 42pS, 13.2%; 50pS, 78%. Of 7705 fully resolved direct transitions (time at each level >2.5 filter rise times), 62.2% were between the 50pS and shut levels. Transitions between the 42pS and 50pS levels and the 42pS and shut levels represented 12.6 and 9.4% of all transitions, respectively. Transitions between the 34pS and the 42 and 50pS levels comprised 4.9%; all other open level-open level transitions were <0.4% of the total number. There was no evidence for temporal asymmetry in any of the transitions.

Shut time distributions for each agonist were fit by the sum of 4 exponentials. The briefest two components, 63μs and 590μs for Glu (n=6) and 32μs and 660μs for Asp (n=10), appeared to represent shuttings within activations of one channel and were defined as gaps within bursts of openings. Distributions of burst lengths were fit by 3 exponentials. Time constants of 2.4 and 12.4ms for Glu (n=5) and 2.0 and 10.1ms for Asp (n=6) were determined for the slowest two components in the burst length distributions at potentials of -60 to -130mV and concentrations of 3-30μM. These time constants agree well with the two time constants we estimated from noise analysis of whole cell currents produced by these agonists. They were little changed if openings to levels other than the 50pS level were excluded. Thus they appear to reflect the gating kinetics of 50pS openings.

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30.14 EFFECT OF 5-HT ON IONIC CHANNEL OF NG108-15 NEUROBLASTOMA CELLS BY PATCH-CLAMP STUDY.

C.J.V. Fueri* (1) and P.N.R. Usherwood* (SPON: European Neuroscience Association). Department of Zoology, University of Nottingham, Nottingham NG7 2RD, U.K.

Cells of the neuroblastoma glioma NG108-15 which have been known to have cholinergic properties (Hamprecht, B., Int. Rev. Cytol., 49:99, 1977; Nirenberg, M. et al., Science, 222:794, 1983) contain 5-HT and synthesize it (Furuya, S. et al., Brain Res., 361:77, 1985). The serotoninergic degrading enzyme, monoamine oxydase (Nagatsu, T. et al., 3:137, 1981) was detected in these cells.

In our work inward single channel currents were recorded from differentiated NG108-15 with giga-ohm patch clamp technique (Hamil et al., 1981) in cell attached, inside out and outside out patches. In each experiment the records were made in absence and in presence of 5-HT 10^{-6}M applied by perfusion procedure.

Channel opening and closing were modified by the presence of 5-HT. By potential variation applied to the tip of the pipette we measured a channel conductance of 130 pS. The reverse current, in cell attach conformation was estimated at the zero membrane potential. In inside out and outside out patches, the reverse current value was 0 mV, when the same ionic solution was used in the bath and in the pipette.

In absence of 5-HT, three exponential components to the open time distribution and four exponential components for the close time distribution could be fitted. In presence of 5-HT 10^{-8}M only three components to the close time distribution could be fitted and we observed change in the exponential components number to the open time.

A functional model for the ionic channel associated to the 5-HT receptor is proposed.

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31.1 ANTIBODIES TO THE ALPHA-SUBUNIT OF SKELETAL MUSCLE VOLTAGE-SENSITIVE CALCIUM CHANNELS REGULATE PARATHYROID CELL SECRETION. H. Chin, L. Fitzpatrick*, M. Nirenberg, and G. Aurbach* Lab. of Biochemical Genetics, NHLBI and *Metabolic Diseases Branch, NIDDK, NIH, Bethesda, Maryland, 20892.

Dihydropyrydine (DHP)-sensitive Ca^{2+} channels regulate hormone secretion in many neuroendocrine tissues. The parathyroid cell is relatively unique in that opening of the Ca^{2+} channels by DHP agonists results in inhibition of parathyroid hormone (PTH) release, while closing the channels by antagonists stimulates parathyroid cell secretion (Fitzpatrick, L. *et al.*, Biochem. Biophys. Res. Comm. 138: 960, 1986). We prepared polyclonal, monospecific mouse antibodies against highly purified preparations of the α-subunit of voltage-sensitive calcium channels from rat T-tubules and tested the effects of the antibodies on parathyroid cell secretion. The three mouse antisera tested inhibited PTH release in medium containing 0.5 mM Ca^{2+}, but had little or no effect in medium containing 2.0 mM Ca^{2+}. In medium with 0.5 mM Ca^{2+}, the extent of inhibition of PTH release from parathyroid cells by antibody MC-4 (100- to 100,000-fold dilution) was a function of antibody concentration. No inhibition was found with MC-4 that previously had been incubated with rat T-tubule calcium channel α-subunit protein immobilized on nitrocellulose membranes. Incubation of dispersed bovine parathyroid cells with antibody MC-4 resulted in a marked increase in $^{45}Ca^{2+}$ uptake by the cells. Immunoblot analysis of bovine parathyroid membrane proteins fractionated by SDS-PAGE showed that MC-4 antibodies bind to a protein with an apparent M_r of 150,000. We conclude that: 1) bovine parathyroid cell membranes contain a 150,000 M_r protein similar to the α-subunit of voltage-sensitive Ca^{2+} channels from other excitable tissues. 2) monospecific antibodies bind to the 150,000 M_r protein and acitvate the Ca^{2+} channels. 3) the marked increase in Ca^{2+} uptake by parathyroid cells upon incubation with MC-4 antibody parallels inhibition of PTH release.

31.2 **Monoclonal Antibody Identifies a 200 kDa Subunit of the Dihydropyridine-Sensitive Calcium Channel** Mary E. Morton* and Stanley C. Froehner. Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03756.

A monoclonal antibody, mab 1A, that immunoprecipitates the [^{3}H]PN200-110 binding complex from rabbit skeletal muscle has been used to study the subunit structure of the voltage-activated, dihydropyridine-sensitive calcium channel. Digitonin solubilized [^{3}H]PN200-110 binding component, purified by wheat germ agglutinin (WGA) chromatography, sediments as a 21S complex. The sedimentation coefficient of the complex is increased to about 24S after incubation with mab 1A and remains unchanged after incubation with control mab. Four polypeptides with apparent molecular weights under non-reducing conditions of 220,000, 200,000, 61,000, and 33,000 daltons co-sediment with the 21S complex. Mab 1A recognizes the M_r 200,000 polypeptide, as shown by Western blotting analysis.

[^{3}H]PN200-110 complex purified by WGA chromatography followed by immunoaffinity chromatography on a mab 1A column is comprised primarily of the same four polypeptides. When analyzed by SDS gel electrophoresis under reducing conditions, the M_r 220,000 protein migrates as a polypeptide of M_r143,000; the mobility of the M_r 200,000 protein recognized by mab 1A is unaffected by reduction of disulfide bonds.

In order to ascertain which component(s) of the [^{3}H]PN200-110 complex bind WGA, 1A immunoaffinity-purified material was examined for lectin binding ability. Nitrocellulose replicas were incubated with biotinylated-Con A or with biotinylated-WGA, followed by streptavidin, and detected with radiolabeled anti- streptavidin antisera. Both the M_r 220,000/143,000 and the M_r 200,000 polypeptides are labeled with Con A. In contrast, only the M_r 220,000/143,000 protein is labeled by WGA. Neither the M_r 61,000 nor the M_r 33,000 polypeptides are labeled by WGA. These results suggest that the M_r 200,000 protein is purified by WGA chromatography by virtue of its association with the M_r 220,000/143,000 polypeptide. Thus, the M_r 200,000 polypeptide appears to be a previously undescribed component of the dihydropyridine binding complex and, in association with the other polypeptides, may comprise the voltage-sensitive calcium channel.

31.3 MONOCLONAL ANTIBODY THAT IMMUNOPRECIPITATES SKELETAL MUSCLE 1,4-DIHYDROPYRIDINE RECEPTORS. H. Smilowitz, R.J. Chang* and C. Bowik*. Dept. of Pharmacology, Univ. of Conn. Health Center, Farmington, CT 06032.

Rabbit skeletal muscle membranes (10 pmole 1,4-dihydropyridine binding sites/mg protein) were pre-labeled with the dihydropyridine antagonist ^{3}H PN200-110, extracted with digitonin and subjected to wheat germ affinity chromatography (approximately 100-150 fold purification) followed by Sephacryl S-400 chromatography (approximately 2-fold purification). Generally 500 mg of starting membrane protein was used. When an eight protease inhibitor cocktail was used, about 0.5 to 1 mg of protein (40-50% 1,4-dihydropyridine receptor) was obtained in about 20 hours. SDS PAGE under non-reducing conditions (iodoacetamide) revealed two major broad α bands upon coomassie staining at about 142-155 Kd and 155-170 Kd as well as less prominent bands at ~57 Kd and ~30 Kd. When only four protease inhibitors were used, the lower α was selectively lost following Sephacryl S-400 chromatography. Under reducing conditions, most of the α material ran at 135-145 Kd while some of the protein stain remained in the 145-170 Kd region of the gel.

Wheat germ purified material (about 1000 pmole 1,4-dihydropryidine binding sites/mg protein) was used as immunogen. Mice were repeatedly injected until their serum gave a 1/2 maximum signal on ELISA at about a 1/5000 dilution. Spleen cells were fused with NSO myeloma cells. Screening of hybridoma supernatants was first by ELISA. Positives were screened again by immunoblot. Several hybridoma supernatants that immunoblotted to a ~170 Kd polypeptide were obtained and the cells were cloned. One such clone, antibody #78 (IgG_1), has been purified on Protein A agarose and investigated in greater detail. It has been found to:

(a) immunoblot to a 150-170 Kd polypeptide under both non-reducing and reducing conditions;

(b) immunoprecipitate 20-30% of the ^{3}H PN200-110 labeled material extracted from prelabeled skeletal membranes.

We are currently testing the hypothesis that antibody #78 binds to the skeletal muscle 1,4-dihydrcpyridine sensitive calcium channel. Supported by NIH grant HL33026.

31.4 OMEGA-CONOTOXIN BLOCKS DIHYDROPYRIDINE-INSENSITIVE CALCIUM CHANNELS IN THE INTACT NERVE TERMINALS OF THE FROG NEUROHYPOPHYSIS. A.L. Obaid*, R. Flores*, and B.M. Salzberg. (SPON: R.O. Davies) Dept. of Physiol., Univ. of Penn., Phila., PA 19104.

Multiple Ca^{++} channel types have been described in a variety of neuronal preparations and speculation has centered on their role in controlling release at nerve terminals. However, it has not yet been possible to characterize directly Ca^{++} channels in the intact nerve terminals of a vertebrate.

We have been using optical techniques that employ extrinsic potentiometric probes to record rapid changes of membrane voltage in the intact nerve terminals of a vertebrate neurohypophysis (Salzberg et al., 1983; Obaid et al., 1985), in order to establish the ionic basis of their action potential. We have shown, in the frog neurohypophysis, that Ca^{++} entrance contributes to the upstroke, and the after-hyperpolarization results from the activation of a very prominent Ca^{++}-dependent K^+-conductance. We have also demonstrated that Cd^{++} (200uM) eliminates the undershoot of the normal action potential, and completely abolishes the regenerative Ca^{++} response obtained in the presence of 1 uM TTX/5 mM TEA. We report here our attempts to define the pharmacological profile of the Ca^{++} channels present in the frog neurohypophysis. We used two dihydropyridine compounds (Nifedipine and Bay-K 8644) at concentrations ranging from 2 to 5 uM (0.1 to 0.25% EtOH), and the synthetic omega-conotoxin GVIA (Peninsula Labs, Inc., Belmont, CA) (1-3.3 uM). Neither of the dihydropyridine compounds had any effect on the Ca^{++}-dependent components of the action potential and the regenerative Ca^{++} response, as these spikes remained absolutely unchanged for up to 1 hr in the presence of Nifedipine and Bay-K 8644. Omega-conotoxin GVIA eliminated in 3 mins the after-hyperpolarization of the normal action potential, and dramatically reduced the height of the upstroke and the size of the undershoot in the regenerative Ca^{++}-spike.

These findings demonstrate that the type of Ca^{++} channel that dominates the behavior of intact vertebrate nerve terminals is blocked by omega-conotoxin and is insensitive to dihydropyridines.

Supported by USPHS grant NS 16824; NATO and Philippe Foundation fellowships to RF(INSERM). We thank M. Morad and G. Cota for their gifts of DHP compounds and to T.D. Parsons for helpful discussions.

31.5 OMEGA TOXIN PEPTIDE FROM CONUS MAGUS BLOCKS NEUROMUSCULAR TRANSMISSION AND VOLTAGE-GATED CALCIUM CHANNELS IN NEURONS. D.H. Feldman, Z. Bagabaldo*, B.M. Olivera*, and D. Yoshikami. Department of Biology, University of Utah, Salt Lake City, UT 84112.

Omega Conus geographus toxin (ωCgTX/GVIa), a 27 amino acid peptide from the venom of the marine snail Conus geographus, blocks synaptic transmission in the frog [Kerr & D.Y (1984) Nature 308, 282-4] and blocks calcium channels in cultured dorsal root ganglion (DRG) neurons from chick [D.H.F. & D.Y (1985) Soc Neurosci Abstr 11, 517; D.H.F. et al. (1987) FEBS Lett, in press]. The omega toxin from C. magus (ωCmTX/MVIIa) is a 25 amino acid peptide with a primary structure quite distinct from that of ωCgTX. Their structures are homologous however, particularly with regard to the positions of the six cysteine residues which are all disulfide-linked [B.M.O. et al. (1985) Science 230, 1338-43]. We report here that ωCmTX, like ωCgTX, also blocks neuromuscular transmission and neuronal Ca channels.

Extracellular recording of population endplate currents was used to monitor the effects of ωCmTX on frog cutaneus pectoris and 19-day embryonic chick biventer cervicis nerve-muscle preparations. In frog, ωCmTX reversibly inhibited synaptic transmission with a K_i of about 2 μM, and a half-life of block < 10 min. In contrast, the block by ωCmTX was essentially irreversible in chick.

Three clases of Ca currents (T, N, and L) have been identified in cultured chick DRG neurons [Nowyky et al. (1985) Nature 316, 440-3]. As previously observed with ωCgTX [McCleskey et al. (1986) Biophys J 49,431a (abstract); PNAS, in press], ωCmTX (10 μM) blocked 20-30% of the T current, and upon washout of toxin, T current recovered within 1 min. Also like ωCgTX, ωCmTX blocked N and L currents with a rate constant of block within a factor of two of 5000 M^{-1} s^{-1}, corresponding to a half-time for block of ~2 min for 1 μM ωCmTX. However, whereas ωCgTX blocks these currents relatively irreversibly, about one third of the current blocked by ωCmTX recovered readily upon washout of toxin (half-life ~15 min.), with both N and L components recovering at the same rate. It is not yet known why only partial recovery from ωCmTX-block is observed.

In summary, although the effects of ωCmTX and ωCgTX are similar, the toxins can affect Ca channels in various tissues of diverse animal species differently; this may be a reflection of changes in the structure of the Ca channels over the course of evolution.

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31.6 **CHARACTERIZATION AND VISUALIZATION OF [^{125}I]OMEGA-CONOTOXIN GVIA BINDING SITES IN RAT BRAIN.** J.A. Wagner, A.M. Snowman, P.F. Worley, B.M. Olivera, A. Biswas, J.M. Baraban, S.H. Snyder. The Johns Hopkins Univ. Sch. of Med. and the Univ. of Utah.

Omega-conotoxin GVIA (CgTx GVIA) inhibits calcium movement through N and L channels, while dihydropyridine and phenylalkylamine calcium antagonists only inhibit L channel activity. N channels appear predominantly localized to nerve terminals, while L channels appear more restricted to nerve cell bodies. Characterization and visualization of [^{125}I]CgTx GVIA binding sites in brain may clarify the role of different calcium channels in neural function.

Monoiodinated CgTx (synthesized by R. Garlick, DuPont) bound to rat brain membrane homogenates and tissue sections saturably, reversibly, and with very high affinity. Homogenate binding was linear with protein concentration in the range from 0.1 to 10 ug/ml. Two sites were discriminated by Scatchard analysis (K_d 50 pM and 2 nM; B_{max} 3 and 30 nmoles/mg protein). The high and low affinity binding sites corresponded closely to high and low affinity inhibition by CgTx GVIA of calcium flux into synaptosomes (Reynolds et al, **PNAS 83:** 8804, 1986). Association kinetics were rapid (K_{on} = 1.3 min^{-1}) at 22°C. Dissociation induced by excess ligand was slow (K_{off} = 0.028 min^{-1}). Omega-conotoxin MVIIA also completely inhibited [^{125}I]CgTx GVIA binding.

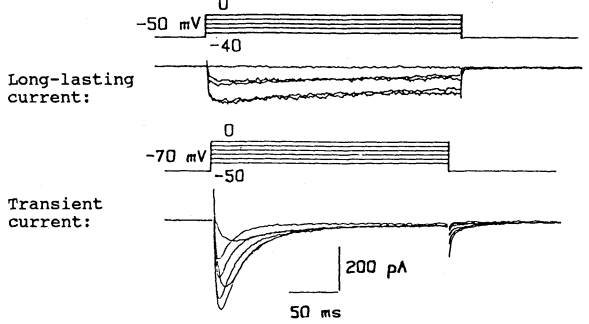
Slide mounted 10 uM coronal sections of rat brain were incubated in a 50 mM HEPES/0.2% BSA, pH 7.4 buffer with 20-35 pM [^{125}I]CgTx for 30 min at 22°C in the presence or absence of 200 nM cold CgTx GVIA. Sections were washed twice for 30 min at 4°C, dried, and exposed to film for 24 hrs.

[^{125}I]CgTx GVIA binding to rat brain sections was similar to homogenates. High grain densities were seen in the frontal cortex, hippocampus, and substantia nigra with highest levels in the superficial cortex. Low grain densities were seen in the striatum and thalamus. Specific binding was absent in white matter tracts such as the corpus collusum. The distribution of [^{125}I]CgTx GVIA binding sites differs from dihydropyridine or phenylalkylamine calcium antagonist binding sites , supporting the idea that [^{125}I]CgTx GVIA binds both to N and L channels.

31.7 CALCIUM CHANNELS IN FRESHLY-DISSOCIATED RAT CEREBELLAR PURKINJE CELLS. L.J. Regan* Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115 (SPON: B.P. Bean).

I have used the whole-cell patch-clamp technique to record calcium currents from cerebellar neurons tentatively identified as Purkinje cells. 3 wk postnatal cerebellar cortices were dissociated in 20 U/ml papain for 1.5 hr at 34-37°C. Electrodes contained (in mM): 120-140 TEA- or Cs-glu, 10 EGTA, 5 $MgCl_2$, 3 Mg-ATP, 0.3- 0.8 GTP, 10 HEPES, and sometimes 12 phosphocreatine and 42 U/ml creatine phosphokinase. External solutions contained 10 Ba^{++} or Ca^{++}, 154 TEA-Cl, 2 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.4, 22°C.

Two components of calcium current have been identified. One current is transient (T_h= 30 ms at -30 mV), activates at potentials more positive than -60 mV, peaks at -30 mV, and has an inactivation range from -60 mV to at least -90 mV. Its properties are similar to those of the "T" current in peripheral neurons (Nowycky, Fox, and Tsien 1985, Nature, vol. 316). The second component is long-lasting, activates at potentials more positive than -40 mV, peaks at -20 to -10 mV, and has a range of inactivation which extends from -20 to at least -90 mV. The properties of this component are like those of the "L" current, except that its inactivation range is much broader. The possibility that the long-lasting component consists (at least in part) of a very slowly inactivating "N"-like current is being explored.



31.8 SPATIALLY RESOLVED CALCIUM CHANGES IN OSCILLATING NEURONS OF THE CRAB STOMATOGASTRIC GANGLION MEASURED WITH ARSENAZO III AND FURA-2. Katherine Graubard, Galen Eaholtz, William N. Ross and John A. Connor. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195; Dept. of Physiology, New York Medical College, Valhalla, NY 10595; Dept. of Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

Calcium transients in many regions of crab stomatogastric neurons were monitored with arsenazo III and a photodiode array, and with fura-2 and digital imaging. Measurements with the 10x10 array had time resolution in the millisecond range and (with signal averaging) could follow calcium oscillations in the neuropil correlated with membrane potential oscillations in the soma. Digital imaging (Connor, PNAS 83:6197, 1986) had higher spatial resolution and could reveal absolute levels of calcium, but had poorer time resolution.

As previously reported (Graubard and Ross, PNAS 82:5565, 1985), intrasomatic depolarizing current pulses elicited arsenazo-detectable calcium transients from the neuropil and somatic regions of stomatogastric neurons. When cells were loaded with fura-2 by iontophoretic injection of the dye into the soma, similar depolarizing pulses caused changes in fura-2 fluorescence indicating an increase in calcium concentration. In the first few seconds after stimulation, the increase in intracellular calcium was largest at the edge of the soma near the neurite hillock and was less in the center of the soma. As the calcium concentration recovered to resting levels (over a period of 60 to 90 s), the edge of the soma recovered more rapidly than the center. We could also detect calcium changes in the primary neurites, but changes in the finer processes were unresolvable with this simple protocol. Calcium concentrations in resting somata were in the range of 100 to 180 nM (indicator K_b = 800 nM). Intense repetitive stimulation drove levels into the micromolar range. Calcium levels in the primary neurites were about the same as somatic levels, both for resting and for stimulated neurons.

Calcium changes in spontaneously-oscillating pyloric neurons were analyzed with arsenazo III, a photodiode array, and an improved signal averaging algorithm. For many cells, step increases in calcium could be observed in the neuropil corresponding to each action potential. For some cells, it appeared that all of the rising phase of the calcium oscillation could be attributed to these steps. In other cells, there was clearly a contribution from the graded depolarization since the rise in calcium began before the action potentials. In some cells, no contribution from the action potentials could be detected. Careful examination of the calcium oscillations indicated that, for a few neurons, the relative contribution of action potential- and graded potential-evoked signals was different in different neuritic regions of the same cell.

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31.9 CALCIUM CURRENTS IN EMBRYONIC CULTURES OF DROSOPHILA NEURONS
H.-T. Leung* and L. Byerly. Section of Neurobiology, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371.

The electrical excitability of Drosophila neurons is being studied in the hope that genetic tools can be used to analyze the control of membrane channels. Embryonic cultures were prepared by dissociating whole gastrulating embryos as described by Seecof et al. (Exp. Cell Res 69:161, 1971). Whole-cell patch clamp studies were performed on neurons 20-72 hours after plating the cells. Na and K currents were eliminated by replacing external Na^+ and K^+ with $Tris^+$ and using Cs^+ for the internal cation. Aspartate was used as the major internal anion. Cells rapidly became leaky when Cl^- was the major internal anion, and the Ca current disappeared within 2-3 minutes with internal F^-. The neuronal cell bodies studied were about 5 µm in diameter and had one to three processes. Although the spacial control of voltage was clearly poor in some cells, in other cells the Ca current appeared well clamped, first activating at about -45 mV and reaching a maximum near -10 mV. Cells were usually held at -73 mV; more negative holding potentials did not increase the magnitude of the Ca current or shift the potential at which the inward current activated. Typically the current activated in about 5 ms and exhibited very little inactivation during a 100 ms depolarizing pulse. There was no evidence to suggest the existence of more than one type of Ca current.

The identity of the Ca current was confirmed by demonstrating that the inward current persisted when external Ca^{2+} was replaced by Ba^{2+} and was eliminated reversibly by replacing Ca^{2+} with Mg^{2+} or adding 0.1 mM Cd^{2+} to the external solution. The average value of the maximum Ca current with 5 mM external Ca^{2+} was 40 pA, ranging from 0 to 150 pA in individual cells. 10 µM nifedipine or 100 µM diltiazem had no blocking effect while 100 µM verapamil blocked about 30% of the Ca current. The Ca current "washed out" when the pipet solution contained only EGTA and other inorganic ions, typically dropping 50% in 5 minutes. Addition of ATP to the internal solution usually slowed the rate of washout and completely prevented washout in some cells.

Studies of on-cell patches with electrodes containing 120 mM Ba have found no currents through Ca channels. This suggests that either there are very few Ca channels on the upper side of the cell body of the neuron or the single-channel current of these Ca channels is less than 0.2 pA.

This work is supported by NS15341.

31.10 CALCIUM CHANNEL TAIL CURRENTS IN CHICK SENSORY NEURONS.
D.Swandulla* and C.M.Armstrong* (SPON: J.Kaltenbach) Dept. of Physiol., School of Med., Univ. of Pennsylvania, Philadelphia, PA, 19104.

We have studied deactivation of calcium channel currents in dissociated chick DRG cells which were free of processes 6 to 10 h after plating. Whole cell currents carried by Ca or Ba ions were recorded from single cells using an improved patch clamp circuit (Armstrong and Chow, Biophys.J., in press). Voltage steps were imposed on the membrane in 10-15µs and changes in membrane current could be measured 30µs after the initiation of the step. Tail currents, associated with the closing of Ca channels, decayed in two phases. The tails were fit with the sum of two exponentials. The fast time constant (τ_f) was near 160µs and the slow one about one order of magnitude larger when repolarizing to -80mV at 20°C. The slow component inactivated nearly completely as the pulse duration increased up to 200ms. Changing the holding potential to more positive values or adding 100-200µM Ni^{2+} to the external solution strongly reduced the slow tail component without affecting the decay kinetics of the fast one. These findings indicate that the slow tail component is due to deactivation of the low voltage activated (LVA) Ca channel current described in this preparation (Carbone and Lux, Biophys.J., 46, 1984, 413; Carbone et al., Pfluegers Arch.,408, 1987, R234). The fast tail component was fully activated with 10ms steps from -80 to +20 mV and inactivated to 40% in 100ms and 15% in 500ms. Deactivation kinetics (τ_f) did not change significantly as the channels activated with short steps, or inactivated with long ones. τ_f was voltage dependent and became progressively faster down to -100mV (35mV for an e-fold change in τ_f). 20 second long prepulses to potentials up to -40mV decreased the tail current amplitude presumably due to steady state inactivation of Ca channels but had no effect on the decay kinetics of the tail currents. Lowering the temperature from 20 to 10°C decreased τ_f by a factor of about 2.5. Our findings indicate that the fast tail current component is due to deactivation of current through a single population of Ca channels which can be activated from holding potentials as high as -40mV.

C.M.Armstrong supported by NIH grant NS 12543. D.Swandulla supported by a grant of the Max Kade Foundation.

31.11 QUATERNARY AMMONIUM COMPOUNDS BLOCK CALCIUM CURRENTS IN PERFUSED NEURONS OF **LYMNAEA STAGNALIS.**
B. Yazejian, S.D. Hess & G.J. Augustine. Neurobiology Section, Dept. Biological Sciences, Univ. Southern California, Los Angeles, CA 90089-0371.

There once were some clampers of squid
to isolate currents, they did
  have need of a blocker
  which, during a shocker
stopped current through channels well hid.

As noted by Charlton, Horn and GJA (Biol. Bull. 171:489)
tetrapentylammonium ion, TPA
  blocked Ca current in squid
  so to follow, we did
turn to neurons of snails in LA

With the method of internal perfusion
to avoid other currents of confusion
  we used cesium as we clamped
  methods of Byerly & Yazejian, revamped (J. Physiol. 370:631)
to prevent Ca channel contusion

Blockade of Ca current we did see
with a 50-100 micromolar K_D
  washing out TPA
  caused block to go 'way
suggesting reversibility

The blockage is voltage dependent
at +40 mV more resplendent
  so now we confide
  the drug works from inside
and senses the potential gradient

In addition, ethylammonium four (TEA)
did not slam the Ca channel door
  we think TPA selective
  but need more work, detective
for statements in faith we'll have more

We implore you to come view our data
for which we will, somewhat later
  have numbers, kinetics
  and graphs, bon aesthetics
for questions from those who are greater!

Supported by NIH grants NS 15341 and NS 21624.

31.12 CHARACTERIZATION OF THE BINDING OF THE DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKER [^{3}H]PN200-110 TO RINm5F MEMBRANES AND INTACT CELLS.
G. C. Yaney*, J. D. Henstenburg* and G.A. Weiland. Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

The RINm5F is an insulin-secreting cell line derived from a pancreatic β-cell tumor induced in the NEDH rat by X-ray irradiation. This cell line has been used extensively as a model system for the study of the mechanism of insulin secretion. Although the cells do not respond to glucose, insulin secretion is stimulated by glyceraldehyde, K^+-induced depolarization. The presence of voltage-sensitive calcium channels on these cells has been demonstrated electrophysiologically and biochemically, and these channels appear to blocked by calcium antagonists. Based on these findings, the binding of the dihydropyridine calcium channel antagonist [^{3}H]PN200-110 to RINm5F membranes and intact cells was characterized.

Membranes were incubated with [^{3}H]PN200-110 for 1 hr in 10 mM MOPS, 1 mM $CaCl_2$ (pH 7.4) and free and bound radioligand were separated by vacuum filtration. Nonspecific binding was determined in the presence of 1 µM nitrendipine. Saturation isotherms of the specific binding of [^{3}H]PN200-110 demonstrated a single population of high affinity binding sites (K_d ~ 150 pM; B_{max} ~ 70 fmoles/mg protein, ~2000 sites/cell). Specific binding was displaced by the dihydropyridines nitrendipine, nisoldipine, and nimodipine with K_i values of approximately 1 nM. Specific [^{3}H]PN200-110 binding was Ca^{++} dependent; 2 mM EGTA or La^{+++} decreased specific binding by 60% and 80% respectively. Verapamil inhibited specific binding by less than 100% with an apparent IC_{50} of 0.3 µM, reflecting a 2-fold increase in the K_d. In the presence of EGTA, diltiazem increased specific binding with an apparent EC_{50} of 0.1 µM, reflecting a 2-fold decrease in the K_d. From these results we conclude that [^{3}H]PN200-110 binds to L-type calcium channels on RINm5F membranes.

Intact cells were incubated with [^{3}H]PN200-110 for 45 min in normal K^+ or high K^+ HEPES-buffered Krebs/Ringer/bicarbonate. Bound and free ligand were separated by vacuum filtration. [^{3}H]PN200-110 binding to the cells in either buffer was displaced by nitrendipine with an apparent IC_{50} of 1 nM. Specific binding to the cells was significantly lower in low K^+ *vs.* high K^+ buffer. The affinity of [^{3}H]PN200-110 binding to depolarized cells was similar to that in membranes (K_d ~ 100 pM). In polarized cells the affinity was at least 3-fold lower. Further characterization of this binding is currently underway.

These results demonstrate the potential usefulness of the RINm5F cell line as a model system to study the mechanism of action of calcium antagonists as well as calcium channel function.

Supported in part by an NIH grant (RO1-HL35467-02) to GAW.

31.13 PHENOBARBITAL BLOCK OF VOLTAGE-DEPENDENT CALCIUM CHANNELS IN NEUROBLASTOMA CELLS. D.A. Twombly*, M.D. Herman*, and T. Narahashi (SPON: S.-C. Cheng). Depts. of Pharmacol. and Neurosurg., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Phenobarbital, a barbiturate effective in the treatment of many seizure disorders, has been shown to interfere with calcium fluxes through neuronal membranes. Calcium uptake into depolarized synaptosomes is inhibited by phenobarbital, and calcium action potentials of dorsal root ganglion cells are shortened in duration. However, the direct effects of phenobarbital on different types of voltage-dependent calcium channels have yet to be defined. This study examined the nature of calcium channel block in N1E-115 neuroblastoma, cells which express both inactivating (type I) and non-inactivating (type II) calcium channels during the process of differentiation.

Calcium channel currents of cultured cells were recorded with the whole-cell voltage clamp technique. Barium (50 mM) was included in the external medium to serve as charge carrier through calcium channels. Under control conditions (standard external solution, 35 - 37°C), type I currents activated at potentials positive to -45 mV; maximum amplitudes were obtained with test pulses to -20 mV or -10 mV. Current inactivation followed a single exponential time course (time constant, 10 - 30 ms). Type II currents activated at potentials positive to 0 mV, and displayed little or no inactivation during 200 ms test pulses. Their amplitudes were generally largest at potentials of +10 mV. Addition of 30 uM - 1 mM concentrations of phenobarbital to the external medium resulted in dose-dependent suppression of calcium channel currents. In this concentration range, both type I and type II currents were blocked to a similar extent. The lowest effective dose of 30 uM reduced both current types by approximately 15%. At 100 uM and 300 uM phenobarbital, currents were decreased by 36% and 40%, respectively. Although the amplitudes of calcium channel currents were suppressed by phenobarbital, the time courses of the two current types were not altered. Moreover, the voltage dependence of channel activation was not shifted, even in the presence of 1 mM phenobarbital.

Studies of CSF and brain tissue from epileptic patients have indicated that the therapeutic range of phenobarbital concentrations is 10 - 215 uM. Block of type I and type II calcium channel currents by phenobarbital therefore occurs at clinically relevant drug levels. Since voltage-dependent calcium channels are suspected to participate in epileptiform bursting activity, the channel blocking action of phenobarbital may constitute an important aspect of its anticonvulsant action in the nervous system. (Supported by the American Epilepsy Society, the Epilepsy Foundation of America, and N.I.H. grant NS14144).

31.14 PENTOBARBITAL REDUCES THE LARGE TRANSIENT AND SLOWLY INACTIVATING CALCIUM CURRENTS IN MOUSE SENSORY NEURONS BY DIFFERENT MECHANISMS. R.L.Macdonald and R.A.Gross, Department of Neurology, University of Michigan, Ann Arbor, MI, 48104, U.S.A.

Barbiturates reduce calcium currents in invertebrate neurons and dorsal root ganglion (DRG) neurons (Werz and Macdonald, Mol Pharmacol 28: 269, 1985). This block of calcium entry may underlie the suppression of neurotransmitter release by barbiturates. Three distinct calcium currents have been described in DRG neurons (Nowycky, Fox, and Tsien, Nature 316:440, 1985; Gross and Macdonald, PNAS, in press). Pentobarbital (PB) had selective effects on these currents, apparently by different mechanisms.

Intracellular recordings were obtained with micropipettes filled with 3M CsCl (20-30 MΩ) from mouse DRG neurons in culture. Recording medium (pH=7.3-7.4) suppressed Na and K currents and contained (in mM): Tris 13.0; choline-Cl 67; KCl 5; TEA 100; $CaCl_2$ 2.0; glucose 5.6; $MgCl_2$ 0.8. A single electrode voltage clamp was used to record calcium currents with a switching frequency of 6-8 kHz and a 70-30 duty cycle. Voltage steps were generated and digitized data stored (2-6 kHz sampling) using a microcomputer. PB and Cd^{2+} were applied by diffusion from blunt-tipped (10-20 µm) micropipettes. Three calcium currents were recorded. A small transient current was recorded at -60 to -50 mV when evoked from negative holding potentials (-100 to -90 mV) (T-type). A moderate very slowly-inactivating current was recorded at potentials positive to -20 mV when evoked from holding potentials of -50 to -40 mV (L-type). A large transient current (N-type) was recorded with the L-type current at potentials positive to -40 mV when evoked from holding potentials of -80 mV.

PB (500 µM) did not affect the T-type current. At all voltages tested, PB produced a 50-100% reduction in the L-type current throughout the voltage command. In combined N- and L-type currents, PB reduced the peak current and produced a more rapid rate of current decay. When evoked from holding potentials negative to -80 mV, peak reduction of current by PB was due to reduction of the L-type current. When evoked from holding potentials positive to -80 mV, peak suppression of current by PB was greater due to a -10 to -15 mV shift in the voltage-dependence of steady-state inactivation of N-type current. There was a voltage-dependent reduction in the inactivation time constants of all three currents above -30 mV; PB produced a further reduction of the N-type current inactivation time constant, accounting for the more rapid current decay. These effects may explain barbiturate suppression of neurotransmitter release and suggest specific roles for the N- and L-type calcium channels.
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31.15 EFFECTS OF SPIDER TOXINS ON L AND N CNS CALCIUM CHANNELS: INHIBITION AND ENHANCEMENT OF BINDING. L.M. Kerr, J.K. Wamsley, F. Filloux, T.N. Parks and H. Jackson (SPON: R. Mullen). Departments of Psychiatry and Anatomy, Univ. of Utah Medical Center, Salt Lake City, UT 84132.

Two novel spider toxins with putative calcium antagonist effects were tested using filtration and in vitro quantitative autoradiography against radiolabeled forms of the know Ca channel blockers [3H]-nitrendipine [125I]-omega conotoxin. The former binds primarily to brain L-type Ca channels whereas the latter binds to both N- and some L-type channels (see Miller, Science 235:46). With autoradiography, the conotoxin shows a diffuse pattern of binding throughout rat brain with increased grains over synaptic areas such as the caudate-putamen and the molecular layer of the cerebellum. This is consitent with its action as a presynaptic Ca-channel blocker. The first spider toxin, AG1, blocks this binding in a dose-dependent manner with an EC50 of about 100 nM (all concentrations estimated from protein contents of partially-purified venom fractions). AG1 is physiologically similar but not identical to omega conotoxin (H. Jackson, unpub. observ.; R. Llinas and M. Sugimori, pers. comm.). The other spider toxin, AG2, also affects omega conotoxin binding. In contrast to AG1, however, AG2 enhances rather than suppresses conotoxin binding, producing an increased grain density over that seen with conotoxin alone. This enhancement is dose dependent with an EC50 of about 3 uM. AG2 is very different from both AG1 and omega conotoxin in its physiological effects; it blocks Ca currents in cardiac muscle unaffected by AG1 or conotoxin and its suppression of synaptic transmission in brain is readily reversibly in contrast to the other two toxins. It is possible that AG2 blocks both neuronal and cardiac L-type channels or that it blocks a type of Ca channel not previously described.

AG1 and AG2 were tested using a rat brain membrane preparation for effects on [3H]-nitrendipine binding. AG2 suppressed nitrendipine binding in a dose-dependent fashion with an EC50 of about 3 uM. This is consistent with an action at L-type channels. Conversely, AG1 enhanced nitrendipine binding with an EC50 of about 250 nM. Scatchard analyses of specific nitrendipine binding with and without AG1 demonstrated that AG1 caused an increase in Bmax (from 63 to 84 fmols/mg tissue; $p<0.05$, n=4) without changing the KD (0.07 mM). This suggests that AG1 allows nitredipine to bind to new sites either by uncovering sites or by changing sites that do not normally bind nitrendipine. These results raise the possibility that a given channel can express either N- or L-type characteristics in the presence of particular modifiers and that N- and L-type Ca channels are therefore not anatomically distinct entities.

31.16 **Calcium currents differ in FACS-sorted prolactin and growth hormone pituitary cells**

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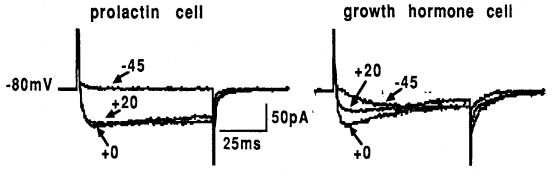
Adult rat anterior pituitary cells were enzymatically dissociated, sorted with a fluorescence-activated cell sorter (FACS), and cultured for 2-6 days. Prolactin cells were sorted with anti-prolactin antibodies, and growth hormone cells with ß subunit of cholera toxin. The whole-cell patch clamp technique was used to record voltage-dependent calcium currents, I_{Ca}. Cells were recorded in 10mM extracellular Ca^{2+} at 22°C. After recording, hormonal contents of cells were identified by immunohistochemical techniques.

Growth hormone cells had a fast inactivating I_{Ca} which activated at -50mV. This I_{Ca} showed faster activation with more postive potentials and reached peak amplitude at -20mV. This fast inactivating I_{Ca} could be completely inactivated at a holding potential of -40mV. A second I_{Ca} activated at more positive potentials and showed little inactivation. Prolactin cells exhibited a fast inactivating I_{Ca} which activated between -40 and -30mV and could be completely inactivated at a holding potential of -40mV. Prolactin cells expressed a second I_{Ca} which activated at more positive potentials, showed little inactivation, and reached a peak at +10mV. The peak of the I/V differed; growth hormone I/V peaked at -20mV and prolactin, at +10mV. This suggests that growth hormone cells have a greater proportion of a low threshold, fast inactivating I_{Ca}, and that prolactin cells have a greater proportion of high threshold, sustained I_{Ca}. This is further suggested by the effect of Bay K 8644, an agonist for the sustained calcium current, which greatly increased I_{Ca} amplitude of prolactin cells but had little effect on I_{Ca} of growth hormone cells. The calcium currents here differ from a recent report of I_{Ca} in the same cell types, gradient-separated and plaque-identified (S.A. DeRiemer and B. Sakmann, *Exp. Brain Res.* 14:139, 1986).



31.17 VOLTAGE-DEPENDENT CALCIUM CURRENTS IN CULTURED EMBRYONIC RAT HIPPOCAMPAL NEURONS.

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Voltage-dependent calcium currents (VDCC) in cultured embryonic (E18) rat hippocampal cells (HPCs) were investigated using the whole-cell patch-clamp technique. VDCC were isolated by removing K^+ from both internal and external solutions. Solutions contained (mM): (External) Ca^{++} or Ba^{++} 2, Mg^{++} 1, Na^+ 5, HEPES/Na OH 5 (pH 7.3) plus 1μM TTX; (Internal) N-methyl D-glucamine (NMG) methanesulphonate 110, NMG Cl 10, BAPTA or EGTA (Cs salts) 5, MgATP 5, Tris phosphocreatine 20, creatine kinase 50 U/ml, HEPES/CsOH 5 (pH 7.3).

Depolarizing commands from holding potentials (HP) between -100 and -40mV elicited inward currents that were increased by raising $[Ca^{++}]_o$ to 10mM and abolished by lowering $[Ca^{++}]_o$ to 0.1mM or by addition of 5mM Co^{++}. The leak subtracted I-V curve showed that maximal inward curent was obtained at -10mV in 2mM Ca^{++} and declined at more positive potentials. During steps from HP = -100mV to -50mV, a small inward current was elicited that decayed completely within 100ms. Commands to more depolarized potentials elicited a relatively sustained inward current.

After 24hrs in culture, maximal I_{Ca} was small (<20pA) and decayed completely during a 200ms depolarising command to -10mV from -60mV. After 72hrs in culture, maximal I_{Ca} ranged from 50-100pA and underwent only 40-60% decay during a 200ms command. The I_{Ca} in cells which had been in culture for more than 6 days showed no consistent differences. Maximal I_{Ca} was 200-400pA. The results reported below are from cells grown for 7-14 days in tissue culture.

When EGTA was used as the internal Ca buffer, I_{Ca} showed little decay during a 200ms step from HP = -40mV to -10mV but I_{Ca} decayed by 20-30% during a 200ms step from HP = -80mV to -10mV; maximal inward current was greater from HP = -80mV than from HP = -40mV. Replacement of 4 mM Ca^{++} with 2 mM Ba^{++} resulted in a substantial decrease in decay of the inward current during a 200ms step from -80 to -10mV. When BAPTA was used as the internal Ca buffer, mean decay during such steps was reduced from 26% to 8% ($n_1 = n_2 = 12$).

1μM BAY K 8644 invariably enhanced peak I_{Ca} (50-100%). 1μM nifedipine appeared to reduce I_{Ca} in a voltage-dependent manner. From HP = -40 mV, block was complete. From -60 and -80mV the maximum block was 60%. When significant decay was present, nifedipine produced a greater reduction in peak current compared to the current at the end of the step. The remaining current was blocked by 5mM Co^{++}.

The transient current may be similar to that already described ('T type' or low voltage-activated) in other preparations. The other type of VDCC in HPCs decays with time and this decay appears to be related to the rise of internal Ca^{++} during prolonged activation of VDCC, as suggested by the results with Ba^{++}_o or $BAPTA_i$.

31.18 WHICH TYPE(S) OF Ca CHANNELS ARE PRESENT IN MAMMALIAN BRAIN SYNAPTOSOMES?

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Three types (T,N,and L) of the voltage sensitive Ca channels (VSCC) have been distinguished in vertebrate neurons (1,2). Based on ^{45}Ca flux measurements, the properties of presynaptic VSCC in brain synaptosomes appear to be dissimilar from those associated with either T or L type channels. It has been suggested that the presynaptic Ca channels may be of the N-type (2). The N channels recorded in neuronal soma have high activation threshold (> -10 mV) and are strongly blocked by the neurotoxin ω-CgTx (1,2). In this work we have examined voltage dependence, and sensitivity to ω-CgTx of the K^+-depolarization dependent ^{45}Ca influx in rat brain synaptosomes. Ca influx was measured in synaptosomes suspended in Na-deficient (Ch-substitution) media, during 1 sec exposures to 5-130 mM $[K^+]_o$; synaptosomal membrane potential was estimated as described previously (3). The results indicate that Ca influx in synaptosomes activates at about -50 mV, is half maximal at about -40 to -30 mV, and reaches maximum at about -10 to 0 mV. This voltage dependence of Ca influx is different from that which might be expected from mediation by the N-type channels. Preincubation of synaptosomes with ω-CgTx resulted in partial inhibition of Ca influx. 40% of Ca influx was blocked by ω-CgTx ($I_{50} \simeq 3.3$ μM); 60% of Ca influx is not inhibited by the toxin at concentration up to 10 μM. This suggests the presence in synaptosomes of two pharmacologically distinguishable, i.e., moderately CgTx-sensitive Ca channels as well as CgTx-insensitive channels. In conclusion, although single channel recordings of identified presynaptic VSCC are needed to establish this, the currently available evidence suggests that the properties of the presynaptic (P) channels inferred from ^{45}Ca influx measurements in rat brain synaptosomes, i.e., (1) low activation threshold, (2) slow inactivation (3) insensitivity to dihydropyridine drugs, and (4) moderate or lack of sensitivity to ω-CgTx, do not fully coincide with any of the extrasynaptic (E) channel subtypes (E_T, E_N, or E_L).

References: (1) R.W. Tsien (1987) ch.11, *in* Neuromodulation, Kaczmarek and Levitan, eds. Oxford Univ. Press; (2) R.J.Miller (1987) Science, 235:46; (3) J.B. Suszkiw et.al. (1986) J. Neurosci. 6:1349.

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31.19 ELECTROPHYSIOLOGICAL, PHARMACOLOGICAL AND SPATIAL SEPARATION OF THREE CALCIUM CURRENTS IN RAT DENTATE GRANULE NEURONS.

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Using the single electrode voltage-clamp technique (Axoclamp II), we investigated calcium currents in dentate granule neurons in the in vitro rat hippocampal slice preparation. Intracellular recordings were performed with microelectrodes (40-80 MΩ) filled with 3 M CsCl. The perfusion media included (in mM): 4 Ca^{2+}, 2 Mg^{2+}, 0.001 TTX, 10 TEA, 5 4-aminopyridine and 3 CsCl.

Three different voltage-dependent calcium currents could be distinguished by their waveform and activation characteristics, pharmacology and regional sensitivity. Voltage commands (1 sec) to -50 mV or more from a holding potential (V_h) of -70 to -80 mV activated a transient (<200 msec) inward current with an amplitude of 0.2-0.6 nA. Further depolarization to -30mV evoked a large (amplitude 0.8-6.0 nA), often difficult to clamp, slowly decaying inward current whose peak amplitude occurred at -15mV. This current was readily blocked by repetitive activation. Holding at more depolarized V_h's (≥-40mV) blocked the large current and revealed a smaller, non-inactivating inward current whose peak amplitude was evoked at +10mV. A similar non-inactivating inward current (? the same) was elicited at -50 to -60 mV from more negative V_h's. All currents were blocked in zero Ca^{2+}, 200 μM Cd^{2+}, 4 mM Mn^{2+} or 1 mM Ni^{2+} perfusate. These three calcium currents resemble the T, N and L currents described by Nowycky et al. (Nature, 316: 440, 1985).

Nimodipine (1 μM) perfusion selectively blocked the persistent inward current and Cd^{2+} (50 μM) decreased the N- and L-type currents with little effect on the transient current. Focal applications of 10-100 mM Ca^{2+} or cation blockers further separated these currents. The large N-type current was enhanced mainly by Ca^{2+} application on the mid- to distal dendrites. The persistent L-type current was increased by Ca^{2+} (or blocked by Cd^{2+}) ejected only at the soma, while the transient current was affected at all parts of the neuron tested (soma, dendrites and axon). Over 96% of the neurons (n=87) exhibited the full complement of all three calcium currents.

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31.20 MULTIPLE CALCIUM CHANNEL TYPES IN ASCIDIAN OOCYTES.

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Properties of macroscopic currents in oocytes from 5 species of ascidians have been studied using the two-microelectrode voltage-clamp technique. Transient inward calcium currents have been identified in the oocytes of all 5 species. The kinetics, ion selectivity, saturability, and mode of inactivation of these calcium currents varied even between oocytes of closely related species. However, a possible phylogenetic trend in the presence or absence of low threshold calcium channels has been observed. Oocytes from ascidians of the order phlebobranchia (Ascidia, Ciona) have low threshold calcium currents while oocytes from ascidians belonging to the order stolidobranchia (Boltenia, Pyura, Styela) do not.

Low threshold currents observed in Ascidia and Ciona oocytes reached peak current at steps to -30 mV (V_{hold} = -80 mV), but time to peak current differed by a factor of 2 (ca. 32 msec for Ascidia, and ca. 70 msec for Ciona). Both currents were enhanced by elevation of external Ca^{2+}; however, reduction of external Na^+ also reduced both currents. Possible co-existence of a Ca^{2+}-activated Na^+ current has not been discounted. Preliminary experiments with Ciona oocytes have shown that reduction of external Na^+ can also change the apparent divalent ion selectivity of the low threshold current. With normal external Na^+ (460 mM) substitution of Ba^{2+} for Ca^{2+} (10 mM) increases peak current ca. 2-fold: with reduced external Na^+ (8 mM) peak current is not significantly changed when Ba^{2+} is substituted for Ca^{2+} (10 mM).

High threshold calcium currents (peak current at ca. 0 to +10 mV) have been studied primarily in Boltenia, Pyura, and Styela oocytes since the low threshold currents in these species are Na^+-selective and easily blocked and because no large outward currents exist for the voltage range studied. The Boltenia oocyte Ca^{2+} current has the apparent divalent selectivity of Ca < Sr < Ba and shows Ca^{2+}-dependent inactivation. Substitution of either Ba^{2+} or Sr^{2+} for Ca^{2+} results in no significant inactivation for 1 sec duration pulses. In contrast Pyura and Styela oocyte Ca^{2+} currents show the apparent divalent ion selectivity of Ca < Ba < Sr and appear to undergo voltage-dependent inactivation. Times for peak current vary (ca. 12 to 40 msec) between the 3 species and with the divalent ion used. Relaxation of the Styela Ca^{2+} current requires several seconds to reach steady state in contrast to the more rapidly decaying Ca^{2+} currents of Boltenia and Pyura.

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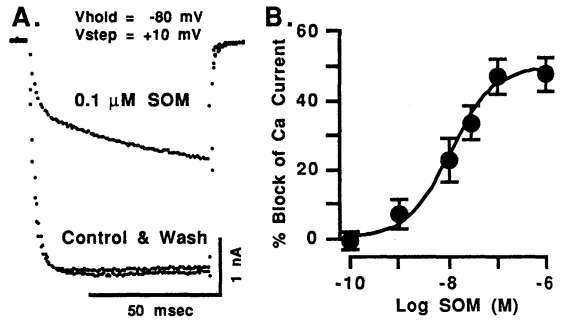
32.1 **SOMATOSTATIN BLOCKS A CALCIUM CURRENT IN ACUTELY ISOLATED ADULT RAT SUPERIOR CERVICAL GANGLION NEURONS.** Stephen R. Ikeda, Geoffrey G. Schofield and Forrest F. Weight. Section of Electrophysiology, Lab. of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

It has been reported that somatostatin (SOM) blocks voltage-dependent Ca^{2+} channels and secretion in clonal cell lines (Lewis *et al., PNAS* **83**:9035-9039, 1986; Tsunoo *et al., PNAS* **83**:9832-9836,1986) and that SOM-like immunoreactivity exists in the somata and processes of neurons in the rat superior cervical ganglion (SCG) (Ariano, M.A. & Kenny, S.L., *Brain Res.* **340**:181-185,1985). Thus, we have examined the effect of SOM on Ca^{2+} currents in neurons isolated from the adult rat SCG.

Neuronal isolation and whole-cell patch-clamp recording techniques were similar to those previously described (Ikeda, *et al., Soc. Neurosci. Abs.* **12**:1343,1986). Superfusion of [D-Trp^8] SOM resulted in a rapid, concentration-dependent and reversible reduction of the Ca^{2+} current elicited by depolarizing voltage pulses from a holding potential of -80 mV. In addition, SOM slowed the rising phase of the Ca^{2+} current, especially at more depolarized potentials (fig A). The concentration-response relationship for SOM could be fit to a single-site binding curve with an apparent dissociation constant of 11 nM (fig B). We conclude that SOM blocks Ca^{2+} currents in postganglionic neurons of sympathetic ganglia and thus may play an important role in the modulation of neuronal excitability and/or neurotransmitter release.



32.2 SUBSTANCE P AND NEUROKININ A AUGMENT A CALCIUM CURRENT IN VOLTAGE-CLAMPED SPINAL DORSAL HORN NEURONS OF THE RAT. K. Murase and M. Randić. Information and Computer Sciences, Toyohashi University of Technology, Tempaku, Toyohashi 440 Japan, and Veterinary Physiology and Pharmacology, Iowa State University, Ames, IA 50011 USA.

Spinal dorsal horn neurons of 2-4 week-old rats in a slice preparation were studied by using the single-microelectrode sample-and-hold voltage-clamp technique. After Na spikes were blocked by 0.2-1µM TTX, the cells were held at -75 to -55mV. In the normal Krebs solution containing 2.4mM Ca, 1sec depolarizing commands to between -55 and -35mV induced slow inward current relaxations and inward current tails. Commands to -35mV, or above, evoked slow outward relaxations and outward current tails. The outward relaxations and outward tails appear to be due to the activation of a Ca-dependent K conductance, because they were substantially reduced in 10-20mM TEA or zero-Ca containing solution, by replacing Ca with Ba or Co, or by use of CsCl-filled pipettes.

The inward relaxations and inward tails evoked by voltage commands to between -55 and -35mV were clearly enhanced in the presence of TEA or by replacing Ca by Ba in the bath. The following results obtained in the Ba-containing solution indicate that the inward relaxations and tails are likely to be due to the activation of a Ca current since they were; increased in a dose-dependent manner when Ba replaced Ca in the bath; blocked by removing Ba, or replacing Ba with Co as well as by 10uM of nifedipine; only slightly modified when Na was replaced by Tris, or Cl with isothionate; and were inward in response to wide range of commands (-150 to -35mV). Raising the external K concentration from 3.1 to 10mM did not change the inward direction of the relaxations and tails. The current appears to activate at membrane potentials between -60 and -50mV and reaches the full activation at -45mV. Both on- and off-relaxations were not adequately described by a single exponential, two time constants obtained by the peeling-off method being in the range of 2.5 to 5s and 0.2 to 0.4s. Some properties of the current, such as the graded activation to command voltage and duration, the voltage dependency, and the slow time course of the activation and inactivation, resemble those of the persistent slow Ca current found in bursting neurons of the Helix pomatia and Aplysia, and in hippocampal and olfactory neurons of the guinea pig. However, determination of the Ca-channel type will await future single-channel recordings from dissociated spinal cord neurons.

Substance P, neurokinin A and cholecystokinin octapeptide enhanced the Ca current in a dose-dependent manner. The effect seemed to coexist with two other effects, namely an increase of an outward current having similar voltage dependency to the Ca-dependent K current and an increase in a voltage-insensitive inward current, the latter probably carried by Na and Ca ions.

Supported by JMEC (#61750357) to KM, and by JSPS and NSF to MR.

32.3 SOMAN INCREASES POTASSIUM STIMULATED CALCIUM INFLUX IN RAT BRAIN CORTICAL SYNAPTOSOMES. M.G. Hamilton* and C. Posavad* (SPON: A. Miller) Biomedical Defence Section, Defence Research Establishment Suffield, Ralston, Alberta, Canada, T0J 2N0.

The potent organophosphorus (OP) acetylcholinesterase (AChE)inhibitor soman (pinacolyl methylphosphonofluoridate) causes severe clonic convulsions and eventual death by respiratory arrest. The convulsive episodes following soman challenge produce electrical changes in brain activity similar to those observed in epileptogenic phenomena. These types of seizures are known to alter the normal balance of many ions, including Ca^{++}, suggesting that OP AChE inhibitors may alter normal Ca^{++} homeostasis in a manner that could mediate at least some of the OP-induced hyperexcitability. Synaptosomes were isolated from the cortex of rats 1, 2, 3 or 7 days following poisoning with one or four LD_{50}'s of soman. (Animals challenged with four LD_{50}'s of soman were also treated with the cholinolytic atropine (17 mg/kg) and the oxime HI-6 (125 mg/kg) which prevented death, but not the convulsions). Both resting (5mM K^+) and depolarized (15 - 60mM K^+) Ca^{++} influx was estimated in 200 µL aliquots of synaptosome suspension using $^{45}Ca^{++}$ as a tracer. At various times from 1 to 240 seconds, influx was terminated by rapid dilution and filtration through 0.45 µM membrane filters and the radioactivity remaining was determined by liquid scintillation spectroscopy.

Beginning 24 hours after a challenge with 1 LD_{50} of soman (110 µg/kg, s.c.), K^+ - stimulated synaptosomal Ca^{++} influx was significantly elevated above that observed in control animals. This elevation of neuronal Ca^{++} influx was evident for the duration of the experiment and was apparently specific for depolarization-induced influx as there was no effect on resting Ca^{++} accumulation. The increases in Ca^{++} influx were observed with all depolarizing stimuli and at all time periods up to 4 minutes. Results obtained with synaptosomes isolated from animals challenged with 4 LD_{50}'s of soman were essentially identical to those collected from animals which had received a single LD_{50} dose. Ca^{++} influx in synaptosomes from animals treated with atropine and HI-6 alone was not significantly different from untreated rats. It is noteworthy that this increase in depolarization-induced Ca^{++} influx occurs at a time when there is a significant degeneration of certain central neurons.

32.4 INCREASING THE INTRACELLULAR CONCENTRATION OF cAMP REDUCES CA-DEPENDENT INACTIVATION OF CA CHANNELS. D. Kalman*, P. O'Lague and D. Armstrong*. Department of Biology, UCLA, Los Angeles, CA 90024.

Patch-clamp studies of a cell line (GH_3) derived from a rat pituitary tumor have revealed a class of voltage-activated Ca channels that inactivate reversibly when channel opening results in Ca ion entry and accumulation inside the cell (Kalman et al., Biophys.J. 51:432a, 1987). Like the Ca channels in many other cells, these dihydropyridine-sensitive channels stop responding to depolarization altogether when the cytoplasmic side of the membrane is exposed to standard saline solutions. Both inactivation and the washout that occurs in dialyzed cells can be slowed, but not prevented, by buffering the concentration of intracellular Ca ions below 0.1 µM. Given the similarity in their dependence on Ca, and the observation that washout in cell-free patches from GH_3 cells can be reversed by cAMP dependent phosphorylation (Armstrong & Eckert, PNAS 84: 2518 2522, 1987), we have investigated the effects of altering cAMP levels on the Ca-mediated inactivation of Ca current in GH_3 cells. Whole-cell recordings of the dihydropyridine-sensitive Ca current were obtained during voltage steps from -40 mV with patch clamp pipettes filled with 140 mM CsCl, 10 mM HEPES (pH 7.2) and 2 mM $MgCl_2$. Increasing the effective intracellular concentration of cAMP, either 'directly' with dibutyryl cAMP (2 mM), or indirectly by activating adenylate cyclase with forskolin (10-50 µM) or vasoactive intestinal peptide (VIP, 100 nM), quickly increased the peak Ca current. Although the Ca current continued to wash out, currents of equal magnitude inactivated much more slowly after cAMP levels had been increased. Thus, a roughly equal number of Ca ions entering the cell produced less inactivation when cAMP levels were elevated. In contrast to its effects on Ca current, forskolin had a much smaller effect on the current carried by barium, which does not inactivate. The phosphorylation-dependence of Ca channel activity demonstrated here provides the cell with a dynamic mechanism for regulating Ca fluxes in response to neurotransmitters, fluctuations in the concentration of intracellular Ca, and the metabolic state of the cell. It also supports the hypothesis that Ca ions promote both inactivation and washout by stimulating calcineurin, an endogenous Ca and calmodulin-dependent phosphatase, to dephosphorylate the channel (Chad & Eckert, J.Physiol. 378:31-51, 1986). This work was supported by USPHS grant NS-08364 to Roger Eckert, GM 07185 and the Greater Los Angeles Affiliate of the American Heart Association.

32.5 OPIOID AND ADRENERGIC MODULATION OF CALCIUM CURRENTS IN NEURONS FROM THE RAT MYENTERIC PLEXUS. M. Sturek*, L.D. Hirning* and R.J. Miller (SPON: B.H. Wainer) Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, 947 E. 58th St. Chicago, IL 60637

Neurons of the myenteric plexus contain a multitude of neurotransmitter /neuromodulator substances. Several of these, including the opioid peptides and norepinephrine, have been suggested to have prominent roles in the inhibitory tone of the intestine. The modulation of Ca^{++} influx into nerves has been proposed as a mechanism of action by which neuromodulators alter release of neurotransmitters. In the present studies, neurons from the myenteric plexus of neonatal rats were dissociated from the small intestinal longitudinal muscle using a collagenase/dispase enzyme treatment (variation of the method of Nishi and Willard, Neuroscience 16:187, 1985). These neurons usually grew as single cells with a single large process and were specifically chosen to maintain consistent morphology. Ionic currents were studied using the patch clamp technique in the whole cell configuration. When depolarized to 0 mV from a holding potential (HP) of -100 mV in a 2 mM Ca^{++}, Tyrode's solution, these cells displayed a large fast inward Na^+ current and a prominent outward K^+ current. The K^+ current appeared to have two phases, a transient outward current which resembled the I_A of molluscan neurons and a more sustained delayed rectifier type current. The I_A was not TEA sensitive but was blocked by 4-AP (5 mM). Approximately 50% of the delayed outward current was also shown to be Ca^{++} activated as indicated by the reduction in the current after addition of 20 μM Cd^{++} to the external solution. After replacement of K^+ in the pipet with Cs^+ only the inward ionic currents were observed. Substitution of TEA for Na^+ abolished the fast inward Na^+ current. The remaining inward current was completely blocked by the addition of 20μM Cd^{++} in the external solution, confirming that this was a Ca^{++} current. The current appeared to have two components, an inactivating portion evident from HP=-100 mV and a sustained portion which was more prominent at more depolarized HP (-50 mV). These are very similar in appearance to the 'N' and 'L' currents described by Nowycky et al., (Nature 316:440, 1985). Nitrendipine (5 μM) had no effect on the calcium currents evoked from a HP of -100 mV. Surprisingly, from a HP of -50 mV, the currents evoked by test pulses to 0 mV were inhibited by only 10 ± 8% (mean ± S.E.M.) by 5 μM nitrendipine. Only when the HP was -30 mV (or more positive) was a significant effect of nitrendipine observed. However, ω-conotoxin (1 μM; 3 min exposure) had dramatic inhibitory effects on both phases of the whole cell calcium current. The inactivating phase was inhibited by 75 ± 7% while the more sustained current was inhibited by 47 ± 12%. The most remarkable characteristic of the currents in these cells was the modulation by neurotransmitters. Addition of the opioid peptide dynorphin (1-17) (3 μM) to the external solution produced a 61 ± 5% inhibition of the inactivating phase of the current. The sustained current appeared slightly less sensitive but was modestly inhibited 29 ± 8% when compared to control. These results are suggestive of the presence of κ-opioid receptors on these neurons. Similarly, norepinephrine (50 μM) inhibited the inactivating current by 43 ± 9% whereas the sustained current was only inhibited by 18 ± 10%. For both dynorphin and norepinephrine, it is obvious that the inactivating ('N' type) current is predominantly affected. Our previous studies have demonstrated that the 'N' type current contributes most of the calcium required for transmitter release in sympathetic neurons. The present data suggest that norepinephrine and the opioids may alter intestinal motility by inhibition of Ca^{++} influx via 'N' type channels into myenteric plexus nerve cells thus reducing neurotransmitter release.

32.6 G-PROTEIN ACTIVATION MODIFIES CALCIUM CHANNEL LIGAND ACTION ON WHOLE CELL CALCIUM CHANNEL CURRENTS. R.H. Scott* and A.C. Dolphin* (SPON: I. Harrow) Dept Pharmacology, St George's Hospital Medical School, London SW17 ORE, UK.

Voltage-dependent calcium channel currents were recorded (using Ba^{2+} as charge carrier) from cultured rat dorsal root ganglion neurones using patch pipettes (1-3MΩ) containing, inter alia, 2mM ATP Mg and 0.5mM GTP-γ-S. We have previously shown that the relatively non-hydrolysable GTP analogue (GTP-γ-S) inhibits calcium channel currents (J. Physiol. (1987) 386 1-17). The transient component appears to be particularly sensitive, and the sustained calcium channel current remaining in the presence of GTP-γ-S can be potentiated by Bay K 8644 (1μM), suggesting L-type channel activity underlies this slowly activating non-inactivating (T_{inact}>400ms) current.

The inhibitory actions of nifedipine (5μM), D_{600} (10μM) and diltiazem (30μM) on calcium channel currents activated from V_H -80mV, were masked in the presence of internal GTP-γ-S. Calcium channel currents modified by GTP-γ-S were enhanced by the calcium channel ligands. D_{600} induced an initial 4.3 fold (n=7) increase in the calcium channel current in the presence of intracellular GTP-γ-S. This decayed to a 2.9 fold (n=5) increase after 5 minutes application of D_{600}. This potentiation was prevented by pretreating the neurones with pertussis toxin. Depolarizing V_H from -80mV to -30mV resulted in control calcium channel currents being more rapidly inhibited by the calcium channel ligands. At V_H -30mV calcium channel ligands induced a biphasic action on GTP-γ-S modified currents. The slower inhibitory action which followed the initial potentiation was rapidly reversed by hyperpolarizing V_H to -80mV, subsequent currents were potentiated relative to those prior to application of the ligands.

In conclusion all three classes of calcium channel ligand have receptor sites which appear to be functionally linked with the site where activated G-protein associates with calcium channels. Interaction of activated G protein with L calcium channels may induce a functional switch, promoting the agonist action of calcium channel ligands.

32.7 THE ACTION OF PHOTOACTIVATABLE GTP ANALOGUES ON CALCIUM CHANNEL CURRENTS IN CULTURED DRGS J.F. Wootton*, D.R. Trentham*, A.C. Dolphin* and R.H. Scott* (SPON: D. Tapper) Dept of Pharmacology, St George's Hospital Medical School, London; Div. Physical Biochem, NIMR, London, U.K. and Dept of Physiology, Cornell University, Ithaca, N.Y.

Photoactivatable (caged) analogues of GTP were synthesized using the 1(2-nitrophenyl)diazoethane method (J. Physiol. 377, 100P). Photolysis by a 0.5ms flash (300-350nm) from a xenon arc lamp produced 1% conversion of caged GTP-γ-S to free GTP-γ-S with a $t_{\frac{1}{2}}$ of about 10ms.

Caged GTP-γ-S (2mM) was included in Cs acetate patch solution used to fill pipettes of 1-3MΩ resistance. Prior to photolysis, caged GTP-γ-S did not alter the characteristics of whole cell calcium channel currents recorded using 2.5mM Ba^{2+} as charge carrier. Calcium channel currents were then activated every 30 to 60 s following a single flash; the currents were reduced in amplitude with the $t_{\frac{1}{2}}$ for reduction of the peak current being 1.5±0.2 min (n=5). The peak and sustained currents were inhibited 5 minutes after the flash by 70% and 50% respectively, and the transient component of the peak current was usually completely abolished. Illumination of the cell in the absence of caged compound had no effect on the calcium channel currents, and the action of photochemically released GTP-γ-S was inhibited by preincubating the DRG neurones with pertussis toxin.

Photochemically liberated GMP-PNP also abolished the transient component of the calcium channel current activated by a voltage step command from -80mV to 0mV; the maximum current activated from V_H -30mV was reduced to a smaller extent.

In conclusion, caged guanine nucleotide analogues offer useful tools to examine the role of guanine nucleotide binding (G) proteins in regulating ion channels. Data from this study suggests that G-proteins with different affinities for GTP analogues or different GDP dissociation rates may be associated with different classes of calcium channel. The N-type channels which underlie the transient component of the maximum calcium channel current appear to be more sensitive to inhibition by guanine nucleotide analogues, but it is likely that in DRGs the L channels underlying the sustained component of the current are also inhibited by G protein activation.

32.8 AUGMENTATION OF BURSTING PACEMAKER ACTIVITY BY THE APLYSIA NEUROPEPTIDE ELH IS MEDIATED BY A CYCLIC AMP-DEPENDENT INCREASE IN CALCIUM AND POTASSIUM CURRENTS. Edwin S. Levitan, Richard H. Kramer*, and Irwin. B. Levitan. Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254

Neurotransmitters and hormones often cause complex changes in the pattern of electrical activity of neurons. However, the ionic and intracellular mechanisms of these changes are not well understood. For example, it has been shown previously that release of the neuropeptide egg-laying hormone (ELH) from Aplysia bag cell neurons augments the endogenous bursting pacemaker activity of neuron R15. ELH increases the number and frequency of spikes during bursts of action potentials, and also increases the amplitude of the interburst hyperpolarization of R15 (Mayeri et al., (1985) J. Neurosci. 5:2060-2077).

We have studied the ionic mechanisms underlying the effects of ELH in voltage-clamped R15 neurons. Both electrical discharge of the bag cells, which releases endogenous ELH, and application of synthetic ELH on cell R15, result in an increase in two discrete ionic currents. One of these currents activates with hyperpolarization, reverses near the K^+ equilibrium potential, is sensitive to the external K^+ concentration, and is blocked by addition of 5 mM Rb^+ or 1 mM Ba^{2+} to the bathing medium. This current appears to be identical to the inwardly rectifying K^+ current, I_R. The other current activates with depolarization, and is blocked by replacement of external Ca^{2+} with Co^{2+} or Mn^{2+}. This current appears to be a voltage-gated Ca^{2+} current, I_{Ca}. Hence ELH enhances two ionic currents, a Ca^{2+} current and a K^+ current. We propose that the ELH-induced increases in the depolarizing and the hyperpolarizing phases of bursting activity are due to the regenerative activation of I_{Ca} and I_R, respectively.

I_{Ca} and I_R in R15 have previously been shown to be enhanced by the neurotransmitter serotonin (5-HT), acting via intracellular cyclic AMP. We now report that increasing cyclic AMP in R15, by applying either serotonin, or the adenylate cyclase activator forskolin, mimics and occludes the action of ELH on neuron R15. Furthermore, application of ELH increases the cyclic AMP content of single R15 neurons by 71% over untreated R15 neurons, as measured by radioimmunoassay. Finally, the effects of ELH on I_{Ca} and I_R are potentiated by isobutylmethylxanthine, a phosphodiesterase inhibitor. These results suggest that the effects of ELH on neuron R15 are mediated by cyclic AMP.

This work was supported by NIH grant NS17910 to IBL.

32.9 PROTEIN KINASE C INHIBITORS PREVENT THE PHORBOL ESTER-INDUCED INCREASE IN CALCIUM CURRENT IN APLYSIA BAG CELL NEURONS. P. J. Conn, J. A. Strong, and L. K. Kaczmarek, Departments of Pharmacology and Physiology, Yale Univ. Sch. of Med., 333 Cedar Street, New Haven, CT 06510.

Stimulation of an afferent input to the peptidergic bag cell neurons of Aplysia induces a 30 minute afterdischarge of action potentials. This results in secretion of peptides which initiate egg-laying behavior. The afterdischarge is accompanied by enhancement of height and width of action potentials and by increases in cAMP concentrations and phosphoinositide hydrolysis.

Previous work has shown that activators of protein kinase C (PKC) and injection of PKC enhance the height of evoked action potentials in bag cell neurons grown in primary culture. Whole cell voltage clamp experiments showed that PKC activators increase Ca currents (ICa) without altering K currents. Cell attached single channel studies indicated that the increased ICa is mediated by expression of a previously covert Ca channel. Control cells have single class of Ca channel with a conductance of 12 pS. After treatment with PKC activators such as 20 nM TPA (12-O-tetradecanoyl-phorbol-13- acetate) or dioctanoyl glycerol, a second class of channel appears with a conductance of 24 pS. Control and TPA-induced ICa have very similar voltage dependence, kinetics, and pharmacological profiles.

We now present evidence that the small and large conductance channels may be differentiated on the basis of their sensitivity to two structurally distinct PKC inhibitors, H-7 (1-[5-isoquinolinesulfonyl]-2-methylpiperazine) and sphinganine. In cells impaled with a single microelectrode, TPA increased the height of stimulated action potentials. Subsequent addition of H-7 (100 μM) reversed the effect of TPA within 30 minutes. Furthermore, H-7 and sphinganine inhibited the TPA-induced increase in ICa measured under voltage clamp. Using the whole-cell patch clamp technique in cells internally dialysed with TEA- and Cs-aspartate, we observed voltage-activated ICa which peaked at 20 mV. Pretreatment with TPA increased ICa at all potentials. Prior addition of sphinganine (10 μM) inhibited TPA's effect (peak ICa densities (nA/nF): control= 6.6±1.0; TPA= 15.4±1.5; TPA ± sphinganine= 6.1±0.9). H-7 (100 μM) also inhibited TPA's effect (control= 8.3+1.2; TPA= 18.4+2.7; H-7 + TPA= 12.1±0.9). In the absence of TPA, ICa was not affected by H-7 (control= 7.0±1.1; H-7= 6.0±1.4) or sphinganine (control= 6.1±0.8, sphinganine= 5.7±1.5). This is consistent with the finding that the large conductance Ca channels were never observed in control cells, and suggests that basal levels of PKC activity are very low in these neurons.

These data support the hypothesis that the unmasking of the covert calcium channel is mediated by PKC. We are currently using these inhibitors to investigate the role of PKC induced calcium channels in the prolonged changes in excitability that occur in bag cell neurons.

32.10 INOSITOL 1,4,5-TRISPHOSPHATE (IP3) REDUCES THE VOLTAGE-DEPENDENT CALCIUM CURRENT OF APLYSIA BURSTING NEURONS. J. H. Byrne and K. P. Scholz, Department of Neurobiology and Anatomy, The University Texas Medical School, Houston, TX. 77225.

The left upper-quadrant bursting (LUQB) neurons of Aplysia display a regular burst-firing pattern of activity which is associated with fluctuations in the level of intracellular Ca. Because of the sensitivity of this bursting rhythm and several membrane conductances to intracellular Ca, neurotransmitters that alter the homeostasis of intracellular Ca could have profound effects on the generation of pacemaker activity. In view of this, we have begun to study the membrane currents modulated by intracellular pressure injection of IP3, a second messenger implicated in the release of Ca from intracellular stores.

Injection of IP3 into LUQB cells voltage-clamped within the pacemaker potential region (-35 to -50 mV) yielded a biphasic response which lasted 5-15 minutes and consisted of an early inward current followed by a slow outward shift in holding current. The outward current corresponds to a slowing of the bursting frequency and hyperpolarization in unclamped neurons. Control injections of D-myo-Inositol yielded no consistent response. Furthermore, the response was not due to contamination of the IP3 sample by Ca ions, since the response to injection of Ca did not mimic the time course of the response to IP3.

We have studied the slow outward current in over 100 cells at constant holding potentials and with small depolarizing pulses. The IP3-induced slow outward current was abolished by intracellular iontophoresis of EGTA but was insensitive to high concentrations of TEA that blocks I(K,Ca) in these cells. However, it is blocked by divalent cations (Co, Ni) that block the Ca current. These results suggest that IP3 causes release of intracellular Ca which inactivates the steady-state Ca current present within the pacemaker range of potentials.

To test this hypothesis further, small depolarizing pulses were elicited in cells that had been axotomized within 200 um of the soma and bathed in a solution that blocks outward currents. Depolarizing pulses (4 mV) yielded non-inactivating calcium-currents. This voltage-dependent Ca current was reduced following injection of IP3. These effects of IP3 were also abolished by intracellular EGTA. In addition, both the voltage-dependent inward current and the response to IP3 were blocked by ASW containing 15 mM Ni or 30 mM Co. These data indicate that IP3 decreases the Ca current of the LUQB cells by releasing intracellular stores of Ca and inducing Ca-dependent inactivation of the Ca current. The decreased Ca current appears to be responsible for the slowing of the bursting frequency that is seen in response to IP3 in unclamped cells.

32.11 EFFECTS OF INOSITOL TRISPHOSPHATE (IP3) AND ACTIVATORS OF PROTEIN KINASE C (PKC) ON TAIL MOTOR NEURONS OF APLYSIA. M. Sawada*, L. Cleary and J. H. Byrne (SPON: J. Wood). Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

In the nervous system of Aplysia, the modulatory effects of cAMP have been established in several monosynaptic reflex pathways. Little is known, however, regarding the function of other second messenger systems in these reflexes. We have begun to examine a possible role for the inositol phospholipid pathway in motor neurons mediating the tail withdrawal reflex. The effects of both IP3 and an analogue of diacylglycerol (DAG) on membrane conductance were examined after intracellular injection.

Injection of 1.4 mM IP3, but not 10 mM myo-inositol, into motor neurons voltage-clamped at their resting potential produced an outward current that was associated with an increase in input conductance. The voltage-dependence, reversal potential and sensitivity to alterations of extracellular K suggested that the response to IP3 was due to an increased conductance to K.

One mechanism that could account for the outward current is activation of the Ca-dependent K current (Ik,ca) by Ca released from intracellular stores. Indeed, injection of 10 mM CaCl2 produced an increased conductance outward current virtually identical to that produced by IP3. Moreover, extracellular application of TEA at concentrations known to block Ik,ca (5mM) blocked these outward currents.

The other product of inositol phospholipid hydrolysis is DAG. Injection of 30 μM 1-oleoyl-2-acetylglycerol (OAG), an analogue that mimics DAG's effects on PKC, produced an inward current that did not appear to alter membrane input conductance. A similar effect was produced by injection of 200 uM phorbol 12,13-dibutyrate (PDBu), another activator of PKC. Since IP3 and DAG are generated together, one might expect that their effects are interrelated. To test this possibility, either OAG or PDBu was injected in short pulses that did not affect holding current. This treatment reduced the amplitude of the outward current produced by subsequent injection of either IP3 or Ca. Therefore, PKC appears to inhibit the ability of Ca to activate Ik,ca.

These results indicate that changes in phospholipid metabolism may modulate the properties of motor neurons mediating the tail withdrawal reflex. The receptor-mediated hydrolysis of inositol phospholipids would result in a hyperpolarization produced by IP3. Activation of PKC by DAG would tend to oppose that hyperpolarization.

32.12 **IP3 AND IP4 MIMIC ANTIGEN ACTIVATION OF A DEPOLARIZING CONDUCTANCE IN A RAT MAST CELL LINE.** S.V.P. Jones*, J.R. Cunha-Melo*, M.A. Beaven* and J.L. Barker. (SPON: P.A. St. John). Lab. of Neurophysiology, NINCDS and Lab. of Chemical Pharmacology, NHLBI, National Institutes of Health, Bethesda, MD 20892.

Stimulation of IgE-primed rat basophillic leukemia cells (RBL2H3) by antigen (DNP/BSA 10 ng/ml) induces time-dependent changes in inositide metabolism, cytoplasmic Ca^{2+} concentration and secretion of histamine. Using the whole-cell patch-clamp technique, DNP was shown to activate a time-dependent change in membrane conductance in RBL2H3 cells at room temperature. A similar membrane conductance was activated in the absence of antigen stimulation when inositol-1,4,5-trisphosphate (IP3) (10 μM) or inositol-1,3,4,5-tetrakisphosphate (IP4) (10 μM) was included in the patch pipette recording solution. Control recordings did not demonstrate any significant conductance changes over a 1 hour period.

Under current-clamp conditions, with KCl-filled patch pipettes, DNP stimulated and inositol loaded cells depolarized over a 5-10 minute period from resting membrane potentials of -65 to -75 mV to potentials in the range -15 to 0 mV. The depolarization persisted for a further 10-15 minutes, followed by repolarization to near resting potential.

Under voltage-clamp conditions, either DNP, IP3 or IP4 induced a time-dependent, reversible inward current at resting potentials. Current-voltage (I-V) plots showed that the current reversed polarity between -45 and -75 mV when recorded with patch pipettes containing K^+-gluconate and at around -10 mV when recorded with KCl-filled pipettes. Although these results suggest that the current is carried predominantly by Cl^- ions, replacement of nearly all of the Cl^- ions on both sides of the cell membrane with methane-sulphonate did not depress the current response.

Increasing the calcium concentration in the bathing medium from 1to 10 mM increased the magnitude of the DNP- or inositol-induced current responses. The current was blocked by 5 mM $CoCl_2$ or by 5 mM $BaCl_2$ but was insensitive to 20 mM TEA or 2 mM 4-AP. Replacement of K^+ in the pipette solution by N-methyl glucamine did not alter the current characteristics nor did reduction of the Na^+ concentration in the bathing medium to 5 mM.

Thus, DNP activates a conductance in which calcium and chloride are implicated as charge carriers. IP3 and IP4 are probably the mediators of the DNP-induced activation of the above conductance, which may be important in secretion of histamine from RBL cells.

32.13 CADMIUM ANTAGONIZES SODIUM CHANNEL AGENT-MEDIATED PHOSPHOINOSITIDE BREAKDOWN IN SYNAPTONEUROSOMES. F. Gusovsky, E. McNeal* and J.W. Daly. LBC, NIDDK, NIH, Bethesda, Md. 20892

Agents that increase intracellular concentrations of Na^+ stimulate phosphoinositide breakdown in guinea pig cerebral cortical synaptoneurosomes. Scorpion venom (Leiurus quinquestriatus) and pumiliotoxin B, which induce increases in influx of $^{22}Na^+$ in synaptoneurosomes, stimulate phosphoinositide breakdown and both effects are inhibited by tetrodotoxin (TTX). Batrachotoxin (BTX) and veratridine (VT), which cause a large increase in influx of $^{22}Na^+$ through activation of voltage-dependent sodium channels, induce a dose-dependent increase in phosphoinositide breakdown that is shifted to the right in the presence of 5 μM TTX. BTX- and VT-elicited influx of $^{22}Na^+$ into synaptoneurosomes is virtually completely blocked by 5 μM TTX. Agents that block voltage-dependent calcium channels, such as D-600, nifedipine and Co^{2+}, do not inhibit either influx of $^{22}Na^+$ or stimulation of phosphoinositide breakdown elicited by scorpion venom, pumiliotoxin B or BTX. Cadmium ions (200 μM), which are known to block TTX-resistant sodium channels, block phosphoinositide breakdown induced by agents that activate sodium influx through sodium channels. Cadmium blocks BTX-induced phosphoinositide breakdown with an IC_{50} value of 48 μM, while blocking BTX-induced $^{22}Na^+$ influx in synaptoneurosomes with lower potency (IC_{50}: 650 μM). However, in the presence of 0.5 μM TTX, the IC_{50} for Cd^{2+} inhibition of BTX-induced $^{22}Na^+$ influx is 70 μM. Neither TTX nor Cd^{2+} antagonize neurotransmitter- or monensin-induced phosphoinositide turnover. It appears likely that BTX-induced phosphoinositide turnover in guinea pig synaptoneurosomes is dependent primarily on activation of TTX-resistant, Cd^{2+}-sensitive sodium channels that account for only a small fraction of the total sodium influx induced by BTX in synaptoneurosomes.

32.14 THE EFFECTS OF INOSITOL PHOSPHATE DERIVATIVES ON MEMBRANE DEPOLARIZATION IN THE XENOPUS OOCYTE. B.J. Stith* and W.R. Proctor. Dept. of Pharmacology, Univ. of Colo. Hlth. Sci. Cntr., Denver, CO 80262

Inositol phosphate compounds are now recognized as important second messengers in many cell systems. For comparison of inositol phosphate action in an intact cell, a cell was required that readily withstands multiple impalements by microelectrodes. The Xenopus oocyte was chosen because of its large size (1.3 mm in diameter) and well-studied membrane characteristics.

Oocytes were prepared by manual removal of the follicular layer and maintained at 22-23°C in modified Ringer's media (O-R2). Stock concentrations (100-300 uM) of inositol trisphosphate (IP3), inositol tetrakisphosphate (IP4), and cyclic-inositol trisphosphate (cIP3, gift from P. Majerus) were pressure-injected (Picospritzer II, General Valve) into oocytes while the membrane potential was continuously monitored via an intracellular recording microelectrode. Calibration curves were constructed for each micropipette by varying the time (5-5000 msec) of pressure application and measuring the resulting drop diameter. This was done before and after oocyte impalement to allow calculation of the intracellular inositol phosphate concentration.

All three inositol phosphate compounds produced an early (D_1) and late (D_2) membrane depolarization. The introduction of one of these derivatives, IP3, into oocytes has been reported to cause similar early and late depolarizations, which were determined to be the result of an increase in intracellular calcium and subsequent opening of calcium-dependent chloride channels. In the present investigation, the dose-response relationship of each inositol phosphate was determined by the intracellular concentration (EC_{50}) that produced a half-maximal late depolarizing response (D_2). The mean EC_{50} ± s.e.m. and the number of cells (n) tested for each compound were as follows: IP3, 88 ± 16.5 nM (n=10); cIP3, 86 ± 29 nM (n=7); and IP4, 3440 ± 1230 nM (n=7).

Microinjection of calcium chloride mimicked the early and late depolarizations. Coinjection of EGTA with each inositol phosphate derivative blocked these responses. Injection of inositol 1-phosphate (IP) alone produced no detectable membrane potential changes. Therefore, these results suggest that IP3, cIP3 and IP4 can each trigger an increase of intracellular calcium that results in an increase in chloride efflux. Although IP4 is capable of producing this effect, it must be present at a 40-fold higher concentration than IP3 or cIP3.

32.15 EVIDENCE THAT DIACYLGLYCEROL MODULATES ACTION POTENTIAL FREQUENCY IN GH_3 CELLS STIMULATED WITH THYROTROPIN RELEASING HORMONE: CORRELATIVE BIOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES. C.M. Gammon*, G.S. Oxford*, A.C. Allen*, K.D. McCarthy*, P. Morell. Departments of Biochemistry, Physiology, Pharmacology, and the Biological Sciences Research Center, Univ. of North Carolina, Chapel Hill, NC 27514

Thyrotropin Releasing Hormone (TRH) elicits a transient hyperpolarization followed by increased action potential frequency in GH_3 pituitary tumor cells. The time course of the electrophysiological response is correlated with increased intracellular calcium and prolactin secretion.

Cultured GH_3 cells were radiolabeled for 24 hours with either [^{3}H] myo-inositol (24 uCi/ml, to label phosphoinositides and inositol phosphates) or [^{3}H] arachidonate (0.2 uCi/ml, to label diacylglycerol and phosphatidic acid). Cells were harvested, washed to remove unincorporated radiolabel, and resuspended in tissue culture medium. Aliquots of the cell suspension were stimulated with 1.0 uM TRH for time intervals from 5 seconds to 3 minutes. Stimulation was terminated by organic extraction of lipids and inositol phosphates. Mono-, di-, and tri-phosphoinositides, diacylglycerol, and phosphatidic acid were resolved from each other and from other lipids by thin layer chromatographic (TLC) procedures. Inositol phosphates were separated by anion exchange high performance liquid chromatography. Stimulation with TRH resulted in significant increases in inositol 1,4,5-trisphosphate within seconds with a return to control levels by 1 minute. Increases in other inositol phosphates were also observed. Phosphoinositide analysis revealed corresponding decreases in phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate. In contrast to the transient increase in inositol 1,4,5-trisphosphate, stimulation with TRH also resulted in significant increases in diacylglycerol and phosphatidic acid which were sustained for several minutes. The time course of diacylglycerol and phosphatidic acid accumulation correlates with that observed for increased action potential frequency after TRH stimulation.

In order to investigate the role of diacylglycerol as a mediator of the TRH induced electrophysiological response, 1-oleoyl-2-acetyl-glycerol (OAG) (60 uM) and phorbol 12,13-dibutyrate (2 uM) were administered to GH_3 cells by a U-tube apparatus during patch clamp recording in the current clamp, whole-cell configuration. Both OAG and the phorbol ester increased action potential frequency in a manner similar to TRH. These results suggest that diacylglycerol produced in GH_3 cells in response to hormone acts to increase action potential frequency through a protein kinase C mediated event.

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32.16 MULTIPLE EFFECTS OF ACTIVATION OF PROTEIN KINASE C IN THE RAT SPINAL DORSAL HORN. G. Gerber*, P. D. Ryu* and M. Randic (SPON: N. Myslinski). Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011.

Protein kinase C (PKC), a calcium and phospholipid-dependent kinase is present in high concentration in the nervous system, where it has been shown to phosphorylate its substrates at the low levels of Ca ions present in resting cells. Kinase C activity is dependent upon intracellular levels of the second messenger, diacylglycerol (Nishizuka, Y., Science 225:1365, 1984). This action of diacylglycerol is mimicked by membrane-permeant, tumor-promoting phorbol esters. Since PKC activation can be mediated directly by phorbol esters, in the absence of phosphoinositide breakdown, we used these agents to examine the effects of such activation on electrophysiological properties of rat spinal dorsal horn neurons and fast and slow excitatory synaptic transmission.

Rats 14-20 days were used. Transverse or horizontal spinal cord slices, the latter with attached dorsal roots and ganglia were made. Cells were activated either directly with current injection via the bridge circuit, or synaptically by electrical stimulation of a lumbar dorsal root or dorsal root ganglion neurons. 4β-Phorbol-12, 13-dibutyrate and 4 -Phorbol-12, 13-diacetate, two phorbol analogues known to activate PKC, and synthetic diacylglycerol (1,-oleoyl-2 acetylglycerol, OAG) were applied by bath perfusion. Standard techniques were used for intracellular recording.

Phorbol esters (10^{-7} to 10^{-6}M for 10-20 min) produced a small, but prolonged membrane depolarization accompanied by an increase in input resistance and frequency of presumptive spontaneous synaptic potentials. In a smaller number of cells slow spontaneous action potential firing was also elicited. Low concentrations of phorbol esters reduced the amplitude of the slow phase of afterhyperpolarization and markedly increased rate of neuronal firing in response to a prolonged depolarizing pulse. With higher concentrations of phorbol esters, however, the dorsal horn neurons showed an enhancement in accommodation as manifested by a decline of the frequency of spike discharge. In the presence of TTX and TEA, phorbol esters or OAG produced a reversible decrease in the duration of calcium spikes. In addition, phorbol esters caused a marked and long-lasting increase in the amplitude and duration of both fast and slow excitatory postsynaptic potentials. The increase in the e.p.s.p.s often occurred in the absence of any change in membrane potential or when corrections were made for the change in resting membrane potential. Our results suggest that both the synaptic transmission and some of the membrane properties of rat dorsal horn neurons might be regulated through the C-kinase system and this modulation may have physiological relevance.

Supported by NSF and the USDA.

32.17 A NOVEL CALCIUM CURRENT IS ACTIVATED BY HYPERPOLARIZATION IN PARAMECIUM TETRAURELIA. T.M. Hennessey*. Dept. of Biol. Sci., State Univ. of N.Y. at Buffalo, Buffalo, N.Y. 14260. (SPON. Dr. M. Hudecki).

A new type of Ca++ current, a hyperpolarization-induced Ca++ current, is the Ca++ source for the Ca++-dependent K+ current which produces the "anomolous rectification" seen in response to hyperpolarizations in a solution containing 4.0mM K+ and 1.0mM Ca++. The hyperpolarization activated K+ current can be eliminated by: 1. EGTA injection, 2. A mutant with a defective Ca++-dependent K+ current (pant A), 3. Substitution of Mg++ for Ca++ in the bath, or 4. Removal of K+. Therefore, the K+ current seen upon hyperpolarization is a Ca++-dependent K+ current.

The hyperpolarization-induced Ca++ current is isolated for voltage clamp analysis by the use of 2M CsCl electrodes and $Ca(OH)_2$ only in the bath. Replacement of external OH^- with Cl^- does not affect this current. This inward Ca++ current is transient, peaking at $2.9\pm.90$ nA during the first 50-100 msec. of a 500 msec. voltage step from -40 to -100mV, but a sustained inward current of $-1.2\pm.45$ nA persists at 500 msec. Neither component of this Ca++ current is blocked by verapamil, nifedipine, diltiazem, or W-7 but both are blocked completely by high Mg++. Twin 100 msec pulses were used to show that the time constant for this inactivation is about 780 msec. The inactivation is removed by EGTA injection, suggesting a Ca++-dependent inactivation process. Two different Ca++ channels have been well described in Paramecium, an anterior mechanosensory and a ciliary voltage dependent Ca++ channel. The first is not blocked by Mg++ and is on the body membrane while the second is on the cilia and is eliminated by deciliation or by the pwB mutation. The hyperpolarization-induced Ca++ current is present in both the pwB mutant and in deciliated wild type. This suggests that this Ca++ current is on the body but it may also be on the ciliary membrane. This inward Ca++ current represents a new route for Ca++ entry in Paramecium. The role of the hyperpolarization-induced inward Ca++ current may be to help maintain a steady resting membrane potential by activation of the repolarizing Ca++-dependent K+ current during large hyperpolarizations.

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PAIN MODULATION I

33.1 VAGAL AFFERENT MODULATION OF A NOCICEPTIVE REFLEX IN RATS. Ke Ren, Alan Randich and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

The vagus nerve is composed of approximately 80% afferent fibers. The physiological significance of these afferents is not completely understood, although accumulating evidence suggests a role in the modulation of nociception. In this study, modulation of the nociceptive tail flick (TF) reflex by vagal afferent stimulation (VAS) was parametrically characterized and spinal neurotransmitters mediating the descending inhibition examined.

Rats were anesthetized with pentobarbital (50 mg/kg) for cannulation of the femoral vein and artery and isolation of the right cervical vagus nerve via a dorsal approach. Following surgery, rats were subsequently maintained in a lightly anesthetized state (corneal and flexion reflexes present) with pentobarbital (3-6 mg/kg/hr iv). The proximal portion of the cut vagus nerve was electrically stimulated; pulse width (0.01-2.0 ms), frequency (1-50 Hz), intensity (5-100 μA) and the duration of continuous VAS were systematically varied to determine optimal parameters of VAS for inhibition of the TF reflex.

VAS at 2 ms pulse width and 20 Hz inhibited the TF reflex at lowest intensities of stimulation. The mean inhibitory threshold (T) intensity of VAS @ 2 ms, 20 Hz was 60 ± 3.3 μA. Subthreshold VAS (0.2-0.5 T) facilitated the TF reflex while 16 T was required to inhibit the TF reflex at 0.01 ms, 20 Hz VAS. The effect of VAS at T outlasted stimulation by 10-20 s. VAS generally produced a depressor effect (20.5 mmHg, n=43), but occasionally produced a pressor effect (20.7 mmHg, n=6) or no change in blood pressure (n=3). Subdiaphragmatic VAS also inhibited the TF reflex and always produced a pressor effect. Thus, inhibition of the TF reflex by VAS is independent of its effects on blood pressure.

The intensity of VAS to inhibit the TF reflex was significantly increased by the intrathecal administration of naloxone in a dose-dependent manner (5-20 μg) or a combination of phentolamine and methysergide (30 μg each), but not by phentolamine (15-30 μg) or methysergide (15-30 μg) given alone.

These results suggest that vagal afferents have a modulatory influence on nociception that may be either facilitatory or inhibitory depending on the intensity of stimulation, perhaps reflecting an influence on different fibers in the nerve. VAS engages a spinal opioid system and may simultaneously coactivate descending serotonin and noradrenergic systems. Supported by NS 19912 and DA 02879.

33.2 PERIAQUEDUCTAL GREY (PAG) INHIBITION OF CARDIAC INPUT TO T_2- T_6 SPINAL CELLS IN CATS. M.J. Chandler, D.W. Garrison, T.J. Brennan and R.D. Foreman, Depts. of Physiology & Biophysics and Allied Health Education, Univ. of Okla. HSC, Okla. City, OK 73190.

Stimulation of the periaqueductal grey (PAG) area of the brain produces analgesia and inhibits activity of lumbar cord neurons that respond to noxious somatic input. The present study examined the effects of PAG stimulation on thoracic cord dorsal horn neurons that responded to visceral input. Our hypothesis was that stimulation of the PAG will inhibit thoracic cord neurons that are activated by noxious input from thoracic somatic fields and from the heart.

Adult cats were anesthetized with α-chloralose and paralyzed with pancuronium. The grey matter of the upper thoracic spinal cord (T_2-T_6) was searched with microelectrodes for neurons antidromically activated from the medullary reticular formation (RF) and for spontaneously active spinal neurons. All cells received excitatory viscerosomatic convergent input from the left forelimb triceps and from cardiopulmonary (CP) sympathetic afferents. Electrical stimulation of the PAG or the midbrain RF (100 Hz, 100 μsec, 50-500 μA) inhibited spontaneous cell activity (36 of 44 cells) and cell activity that resulted from pinching the left triceps (40 of 44 cells). Visceral input to the neurons was elicited during electrical stimulation of CP sympathetic afferent fibers and after injection of the algesic compound, bradykinin, into the left atrium. Electrical stimulation of CP sympathetic afferents increased cell activity; stimulation of the PAG and adjacent RF reduced this response (38 of 47 cells). Intracardiac injection of bradykinin excited 29 of 46 cells; electrical stimulation of the PAG or midbrain RF reduced this response to bradykinin (28 of 29 cells). The inhibitory effects of electrically stimulating these midbrain areas could have resulted from activation of fibers of passage. Therefore, the effect of glutamate microinjections into the PAG or adjacent midbrain regions was tested against spontaneous activity of 9 cells. Spontaneous activity of 5 neurons was inhibited after glutamate was injected into the PAG. Injection of glutamate into adjacent midbrain regions did not inhibit spontaneous cell activity of 4 cells.

These results demonstrate that electrical stimulation of the PAG and adjacent midbrain regions inhibits noxious visceral input from the heart as well as noxious somatic input. Responses of thoracic cord cell activity to midbrain glutamate injections suggest that a portion of this descending inhibition may be mediated by cell bodies in the PAG. (Supported by NIH grants HL 22732, HL07430 & NS08150 & OUHSC Faculty Senate Award 43038700).

33.3 VISCEROSOMATIC NOXIOUS INHIBITION (VNI) OF PRIMATE T_2-T_5 SPINOTHALAMIC TRACT (STT) CELLS: NON-LINEAR REGRESSION OF THE INHIBITION VERSUS SPONTANEOUS ACTIVITY RELATION. T.J. Brennan, U.T. Oh* and R.D. Foreman. Dept. of Physiology & Biophysics, Univ. of Oklahoma HSC, Okla. City, OK, 73190.

Our laboratory has previously shown that upper thoracic STT neurons receive convergent excitatory input from the cardiopulmonary sympathetic afferents and somatic fields of the left triceps-chest region. In this series of experiments attention was focussed on the inhibitory inputs from the urinary bladder and hamstring regions of the hindlimbs; this convergent inhibition has been named viscerosomatic noxious inhibition (VNI).

Forty-one T_2-T_5 STT cells were antidromically activated from the contralateral thalamus in 20 anesthetized monkeys. Urinary bladder distension (UBD) inhibited 33 of 41 (80%) cells, right hindlimb pinch reduced the activity of 32 of 39 (82%) and left hindlimb pinch inhibited 21 of 38 (55%). While examining these inhibitory responses, it was apparent that greater decreases in activity were observed in cells with higher spontaneous activities and at very high levels of background activity a maximal inhibitory effect was elicited. These inhibitory inputs were analyzed by plotting the decrease in activity (expressed in imp/s) elicited by a stimulus (y-axis) versus the level of spontaneous activity (x-axis) for all cells. The relation was subjected to curve-fitting analysis to quantify the inhibition versus activity relation. The data fit the equation $y=a - be^{-kx}$ (r); a represents the maximal inhibitory effect, k is the rate constant for the curve, a-b is the y-intercept, and r is the correlation coefficient.

The inhibition versus activity relation for right hindlimb pinch was described by the equation $y=19-25e^{-0.089x}$ (r=0.70) and for left hindlimb pinch by the equation $y=13-13e^{-0.065x}$ (r=0.52). Overall, right hindlimb pinch produced a greater maximum inhibitory effect and a more homogeneous response (greater r value) than left hindlimb pinch. The rate constants were similar. Curves for UBD to 40, 60, and 80 cmH_2O were described by the equations $y=24-22e^{-0.015x}$ (r=0.55), $y=22-22e^{-0.039x}$ (r=0.73) and $y=25-26e^{-0.044x}$ (r=0.81), respectively. These curves suggest that as the magnitude of the distension increases a higher rate constant and a more homogeneous response were produced. Also, further analysis showed that, for a given level of spontaneous activity, higher distending pressures produced greater inhibition.

These curvilinear relations indicate that the amount that cell activity is decreased by VNI depends upon the spontaneous firing rate of the neuron. Comparisons among the curves show apparent differences between inhibition produced by left and right hindlimb pinch and inhibitory responses caused by increasing the intensity of UBD. Supported by NIH Grants HL22732.

33.4 SOMATOSENSORY AND CARDIOVASCULAR RESPONSES TO ISCHEMIC PAIN IN HEALTHY VOLUNTEERS. W. Maixner, G.R. Bloodworth*, J.S. Smith* R.H. Gracely* and J.R. Zuniga*. Dental Research Center, Univ. of North Carolina, Chapel Hill, N.C. 27514 and Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

The outcomes of a variety of studies support the view that peripheral hemodynamic responses can modify pain perception (Randich & Maixner Neurosci. Biobeh. Rev. 8:343,1984; ANYAS 467:385,1986; Zamir and Maixner ANYAS 467:371,1986). However, few clinical studies have evaluated the relationship between pain and alterations in peripheral hemodynamics. In the present studies, we have evaluated the relationship between peripheral hemodynamic responses to acute ischemic pain with the perception of sensory and affective components of ischemic pain.

For these studies, five healthy male volunteers were instrumented for cardiovascular monitoring: Right ankle arterial pressure (systolic = SP; diastolic = DP; mean = MP); heart rate (HR); peripheral venous pressure (VP), measured from a superficial vein in the antecubital region of the dominant forearm; forearm blood flow (FAF) and venous capacitance (VC) measurements obtained from the dominant forearm using conventional strain gauge plethysmographic procedures. Following the measurement of baseline cardiovascular parameters, ischemic pain was induced in the non-dominant arm via the submaximal effort tourniquet procedure. Cardiovascular and somatosensory responses were obtained at two minute epochs during the tourniquet procedure. Somatosensory responses were also obtained by having the subjects select weighted verbal descriptors (Gracely et al., Science 203:1261,1979) that most closely described the sensory (i.e. intensity) and affective (i.e. unpleasantness) aspects of their perceived pain. The subjects also verbalized a number between 0 and 100 that most closely described their overall pain.

Following the application of the tourniquet, there was a progressive increase in pain (intensity, unpleasantness, overall). Arterial blood pressure (SP, DP, MP), HR, and forearm vascular resistance (MP-VP/FAF) increased during the tourniquet procedure while FAF and VC decreased. There was a significant correlation between various peripheral hemodynamic responses with the sensory, affective and overall estimates of pain.

This study demonstrates that there are unique and reproducible hemodynamic responses to acute ischemic pain that co-vary with particular dimensions of pain perception. The specificity of these changes as they relate to pain and the ability of these cardiovascular responses to modulate pain perception is currently under investigation.

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33.5 IS SUBJECTIVE PAIN SENSATION CORRELATED WITH THE FLEXION REFLEX IN MAN? M. Dallaire* and C.W.Y. Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada H3G 1Y5.

The quantification of experimental pain in human subjects has always presented researchers with a formidable challenge. Psychophysical methods such as verbal report, numerical and descriptive scaling as well as analog scales are often used to provide a subjective measure of pain. Other studies favor the use of various physiological correlates to try to quantify pain sensation in an objective manner. Keeping in mind the complexity of pain experience, we felt the need to bridge the gap between psychophysical and physiological correlates of pain in this study.

The two pain indices we chose to compare are the visual analog scale (VAS) for its simplicity and sensitivity, and the lower limb flexion reflex (FR), known for its high correlation with pain sensation. Subjects (7 females, age 23 to 30) were seated comfortably in a semi-reclined position with right ankle and knee joints fixed in slight extension by partial casts to enhance FR excitability. The stimulus used to elicit the flexion reflex consisted of a 30 msec duration train of 1 msec square pulses at 200 Hz, delivered to the median arch of the right foot. Surface electromyographic (EMG) recordings were taken from the right biceps femoris (BF) muscle and processed on-line through an averaging program. Stimulus intensities were then determined for each subject's perceptual threshold and pain tolerance. The range in between was divided in 10% increments with respect to the normalized anchors (0%=perceptual threshold, 100%=pain tolerance). Subjects were then instructed to estimate stimulus intensity by moving a cursor along a VAS connected to a linear potentiometer. Each subject received 110 randomized stimuli (ie. ten stimuli at each 10% increment of the stimulation range).

Mean VAS ratings and mean FR EMG areas were computed at each stimulation level for each subject, and normalized with respect to her maximum value (=100%). The stimulus-response curves for subjective pain estimates and FR area were both found to increase linearly with increasing stimulus intensity, having correlation coefficient values of r=0.85 and r=0.95 respectively, as well as identical slopes. Interestingly, the cross-correlation between FR area and subjective estimates also yielded a high value of r=0.85.

These data therefore tend to support the existence, under our experimental paradigm, of a high correlation between subjective pain sensation and the flexion reflex in man. Further experiments will determine if the parallel relationship seen between these two pain indices still holds when subjected to peripheral pain modulation techniques such as vibration.

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33.6 MAGNITUDE SCALING OF CHEMOGENIC PAIN AND HYPERALGESIA IN HUMANS. D.A. Simone, T.K. Baumann, C.N. Shain, and R.H. LaMotte. Dept. of Anesthesiology, Yale University Sch. of Med., New Haven, CT 06510

Although chemical sensitization of nociceptors may contribute to the hyperalgesia that follows tissue injury, there have been few psychophysical studies of the relationship between the concentration of algesic chemicals and measures of pain and hyperalgesia. In a study of the magnitude of chemical pain and hyperalgesia, different doses of capsaicin (CAP) were injected intradermally into the forearm in humans. CAP produced burning pain, a flare, local hyperalgesia to heat, and a larger surrounding area of mechanical hyperalgesia. Each subject judged the magnitude of pain produced by an injection of vehicle (Tween-saline) and CAP doses of 0.01, 0.1, 1, 10, and 100 ug in 10 ul.

The lowest dose of CAP which produced pain was 0.1 ug. The magnitude of pain increased with dose, yielding a power function with an exponent of 0.17. The duration of pain also increased as a function of dose and ranged from 1.2 to 16.7 min.

Both the magnitude and duration of heat hyperalgesia (lowered pain threshold) and the area of surrounding mechanical hyperalgesia (to light skin stroking) increased with CAP doses of 1 to 100 ug.

Measurement of pain response latency revealed that a light stroke within the area of mechanical hyperalgesia evoked two sensations of pain. The first was brief and immediate and was followed after a delay by a second pain lasting 5 to 20 sec. Latencies were consistent with conduction in fast and slow conducting C-fibers. An ischemic block of conduction in A-fibers with loss of touch and loss (or delay) of cool sensation did not alter these two components of pain. However, as the block progressed, the first pain disappeared, whereas the second pain persisted together with normal pain reactivity to an additional CAP injection.

We hypothesize that the two components of mechanically-induced pain following CAP are mediated by separate classes of C-fiber afferents. One mediates the first component of mechanical pain while the other mediates the second component as well as contributes to the chemically-mediated pain to CAP injection.

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33.7 ANTINOCICEPTION FROM PERIPHERAL NERVE STIMULATION IN RATS AND HUMANS. B. Pomeranz . Departments of Zoology and Physiology, University of Toronto, Toronto, Ont., M5S1A1.

We studied the roles of spinal cord endorphins and GABA in mediating electroacupuncture(EA) antinociception in rats, using low frequency/high intensity EA. In addition we developed a transcutaneous electrical nerve stimulation(TENS) device called Codetron for human patients using similar parameters (low frequency/high intensity), and tested it against conventional EA in patients with chronic pain.

Rats were anesthetized with continuous infusion of pentobarbital. Low frequency (4 Hz) high intensity (25x threshold) EA was applied for 15 minutes via needles to the 1st dorsal interosseus muscle, in order to suppress tail flick reflexes elicited by radiant heat. An intrathecal cannula was used to apply drugs to the cord. Naltrexone given before EA treatment prevented EA antinociception, but naltrexone given after EA failed to reverse the EA effect. Picrotoxin blocked EA effects, while diazepam enhanced EA antinociception.

In the human study, patients with chronic pain (N=171) were randomly assigned to one of two treatment groups: one receiving Codetron (TENS) treatments twice a week for 6 weeks, the other receiving a similar course of EA (with needles). In the short term (6 weeks) Codetron performed as well as EA, but in the long term followup (4–8 months) Codetron outperformed EA by a wide margin.

We conclude in the rat studies that antinociception from low frequency/high intensity stimulation is mediated by spinal cord endorphins and GABA. Moreover the endorphin effect produces a modulation which is preventable but not reversible by naltrexone. In the human study, Codetron was a successful means of delivering low frequency/high intensity TENS. Moreover, Codetron outperformed EA perhaps because it was designed to give high intensity stimulation without undue discomfort to the patients.

33.8 THE LOCAL EFFECT OF TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION (TENS) AT LUMBAR SITES (L5-S1) ON SCALP SOMATOSENSORY EVOKED POTENTIALS (SEP): AFTER STIMULATION OF THE TIBIAL AND MEDIAN NERVES. S. Marchand* and L. Laurencelle* (SPON: A.M. Smith). Université du Québec à Trois-Rivières, C.P.500, Trois-Rivières, G9A 5H7 (Québec) CANADA.

The Gate Control theory by Melzack and Wall (1965) provided a theoretical rationale for the low intensity, high frequency transcutaneous electrical nerve stimulation (TENS) analgesia. In the present study, we investigated the local topographical effects of TENS analgesia.

The amplitude of the late component (P200) somatosensory evoked potential (SEP) seemed to be a good electrophysiological index of the analgesic effect. In the present study the localized effects of lumbar TENS were studied using the P200 component of the SEP amplitude. Scalp SEPS were obtained by stimulating the median nerve at the wrist and the tibial nerve at the popliteal fossa. SEP samples were taken before and after the application of TENS to a region of skin innervated by lumbo-sacral fibers (L5-S1).

The root mean square (RMS) voltage was calculated on each SEP in order to conduct a statistical analysis of the amplitude over time. The results showed that TENS produced a selective reduction of the SEP amplitude of the response evoked from the tibial nerve but no difference was found in the amplitude of the SEP evoked from the median nerve. In particular, TENS decreased the amplitude of the SEP surrounding the P200 component. The RMS voltage at a latency of 175-200 ms was compared with the PES evoked from stimulation of the median and tibial nerves with and without TENS. For the tibial nerve, there was a significant ($P<.050$) reduction in the SEP amplitude after TENS. For the median nerve, no difference was found between the two conditions.

The results are in accord with the assumption that TENS has a focalized topographical effect. The amplitude of the SEP of the tibial nerve was selectively reduced by the application of TENS; as predicted, the median nerve SEP was not affected.

33.9 PSYCHOPHYSICAL THRESHOLD OF THE HUMAN CORNEA TO CAPSAICIN. B. Dupuy,* H. Thompson,* and R.W. Beuerman. (SPON: L. Happel). LSU Eye Center, New Orleans, LA 70112.

Capsaicin is a pungent substance found in hot peppers. In rabbits, topical application of 1% capsaicin on the cornea leads to ocular irritation. Following repeated applications in the anesthetized rabbit twice a day for 5 days, the cornea showed no structural changes when observed by the slit-lamp, light or electron microscopy. Electrophysiologically, low capsaicin concentrations produced marginal responses, while higher concentrations reduced sensitivity. These results did not adequately explain the observed behavioral effects of capsaicin. Therefore, we sought to determine the psychophysical threshold of the human cornea to capsaicin in order to better describe its sensory effects on corneal free nerve endings.

Capsaicin (Sigma, 90% purity) in vehicle (10% ethanol, 10% Tween 80 in isotonic saline) was serially diluted in sterile isotonic saline to concentrations ranging from 3.3×10^{-8} M to 7.8×10^{-8} M. For testing, all solutions were warmed to 40°C and delivered as a 5 µl drop on the apex of the cornea. Five subjects were given diluted vehicle or capsaicin at one of the following concentrations: 3.3×10^{-8} M, 4.4×10^{-8} M, 5.8×10^{-8} M, 7.8×10^{-8} M in each eye. Each subject underwent two experimental sessions in which concentrations were presented in ascending order. Isotonic saline washes preceded and followed each stimulus. Subjects, unaware of the order or concentrations of the stimuli presented, were asked to report when a drop was irritating. Following each session, subjects were examined by slit-lamp.

The population probabilities of reporting irritation were as follows: vehicle, $P = 0.06$; 3.3×10^{-8} M, $P = 0.09$; 4.4×10^{-8} M, $P = 0.14$; 5.8×10^{-8} M, $P = 0.59$; 7.8×10^{-8} M, $P = 0.83$; ($n = 20$ at each concentration). By probit analysis, the ED_{50} (threshold) was calculated to be 5.9×10^{-8} M with 95% confidence limits ranging from 5.3×10^{-8} M to 6.6×10^{-8} M. Threshold is equivalent to 1.2×10^{-8} gm/5 µl drop. Slit-lamp examinations showed no epithelial staining or corneal changes in any of the subjects.

The extreme sensitivity of corneal free nerve endings is demonstrated by the low psychophysical threshold to capsaicin. Furthermore, determination of the threshold to capsaicin allows the implementation of more diverse experimental models such as JND and signal detection analysis. Lastly, the reliability of capsaicin to evoke a sensation at low concentrations and the absence of tissue damage following topical application suggest that capsaicin may prove to be useful in understanding corneal nociceptors.

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33.10 EFFECTS OF SYSTEMIC MORPHINE ON SECOND PAIN REACTIONS OF PRIMATES. D.C. Yeomans*, B.Y. Cooper and C.J. Vierck, Jr. Dept. of Neuroscience and Center for Neurobiological Scs., Univ. of Florida Col. of Med., Gainesville, FL 32610.

Previous human psychophysical studies have demonstrated that systemic morphine acts preferentially to inhibit second pain sensations attributable to activity in unmyelinated (C) peripheral afferents (Cooper et al., 1986). Therefore, a behavioral paradigm was devised to evaluate responses of monkeys to pain sensations elicited by C or A-delta afferent activity. Stimulus parameters that evoked only second pains were determined by human subjects who rated pain magnitudes evoked by 500 msec contacts of a preheated thermode with skin of the lateral calf. Within series of 7 contacts at a frequency of 0.33 Hz, pain sensations increased with successive contacts. Conduction velocities of 0.8 to 1.8 M/s were obtained from latencies of responses to proximal vs. distal stimulation. In addition, pain magnitudes were increased by topical capsaicin.

The paradigm for monkeys presented 3 trial types: A) 16 C pain trials in which a preheated thermode (50 or 56 deg C) contacted the lateral calf as described above. Escape periods (defined by a tone) occurred when the animals experienced second pain in the interstimulus intervals. B) 16 A-delta pain trials, involving a single, 4.5 sec contact of the thermode (48 or 50 deg C) or electrocutaneous stimulation. Escape periods coincided with peak A-delta pain. C) 8 avoidance trials in which non-pain-specific effects of morphine were examined. Systemic morphine suppressed C fiber pain responses at doses which did not affect responses to A-delta pain. Morphine at these doses also had no effect on avoidance responses or reflexive responses.

33.11 **USE OF A REPEATED-MEASURES DESIGN TO STUDY ANALGESIA PRODUCED BY OXOTREMORINE (OX) AND NICOTINE (NC) IN THE MOUSE TAIL IMMERSION TEST.** K.C. Retz and T. V. Tran*. Dept. of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

The feasibilty of using a repeated-measures factoral design to study the analgesia produced by oxotremorine (OX) and nicotine (NC) in the tail immersion test was investigated. Two separate groups of 20 male HSD:ICR(BR) mice, 2-4 mo., were further divided into five subgroups of four animals. To test for analgesia, each of the OX subgroups received saline vehicle, OX 0.005, OX 0.01, OX 0.02, and OX 0.04 mg/kg with a 3-4 day interval between each treatment. Latencies were measured immediately prior to injection and at 15 and 30 min post-injection using the 50°C tail immersion test (Sewell & Spencer, Neuropharmacology 15:683-688, 1976). Analgesia produced by NC was assessed by measuring latencies immediately prior to injection and at 2 and 5 min post-injection using saline vehicle, NC 0.32, NC 0.64, NC 1.25 and NC 2.50 mg/kg. To examine the effects of order, a 2-way ANOVA on the pre-injection latencies was conducted with subgroups (5 levels) as a between factor and order of presentation, the repeated measure, (5 levels) as a within factor. In the "OX" group neither subgroup main effect ($F(4,15)=1.3$, $p=0.32$) nor order main effect ($F(4,60)=2.1$, $p=0.09$) reached statistical significance. In the "NC" group neither subgroup main effect ($F(4,15)=1.4$, $p=0.28$) nor order main effect ($F(4,60)=1.7$, $p=0.16$) reached statistical significance. These findings implied that prior exposure to the test and/or administration of drug or vehicle had no appreciable effect upon the pre-injection performance on subsequent days. With regard to the drugs themselves, a dose-dependent and time-dependent increase in latency was observed following injection of both OX and NC. To verify the effects of each drug, a 2-way ANOVA on latencies was conducted on the data pooled from all subgroups with both dose (5 levels) and time (3 levels) as within factors. Following OX, main effects due to dose ($F(4,76)=8.2$, $p=6.9E-05$), time ($F(2,38)=11.9$, $p=2.3E-04$), and the dose x time interaction ($F(8,152)=4.6$, $p=1.3E-04$) were all highly significant, as were the simple main effects of the 0.005, 0.01 and 0.04 mg/kg doses. Following NC, main effects due to dose ($F(4,76)=80.1$, $p=2.1E-10$), time ($F(2,38)=81.1$, $p=3.1E-08$), and the dose x time interaction ($F(8,152)= 43.9$, $p=1.7E-11$) were also all highly significant, as were the simple main effects of the 1.25 and 2.50 mg/kg doses. These findings demonstrate the validity of using a repeated measures factoral design in the tail-immersion test to study the analgesia produced by OX or NC in mice. Supported by Texas College of Osteopathic Medicine grant #34107 awarded to K.C.R.).

33.12 INTERMITTENT SHOCK TITRATION IN THE RHESUS MONKEY. J.L. Bloss and D.L. Hammond. Department of CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077.

Several criticisms have been made of the shock titration paradigm (STP) used for analgesiometric determinations in the monkey. Foremost criticisms are 1) the animal's ability to avoid the stimulus and maintain shock intensity at less than painful levels, and 2) poor localization of the stimulus such that current densities are too low to be nociceptive. This study evaluated a modification of the discrete trial STP in which well-localized electric stimuli of suprathreshold intensity were interspersed among a sequence of titratable stimuli. The effect of morphine on response parameters was also examined.

Male rhesus monkeys were trained to terminate an electric stimulus (0-6 mA, 25 equal steps) delivered to the mid-lateral calf via two 10 mm^2 electrodes. A trial consisted of a train of 60Hz alternating constant current square wave pulses (40 msec on, 200 msec off) of 2.2 s duration delivered every 10 s. A lever press terminated the trial and decreased the intensity of the subsequent trial by one step (0.24 mA/mm^2). In the absence of a response, the intensity of the subsequent trial increased by one step. After every third titratable trial, an unavoidable suprathreshold stimulus of either step 10 (0.24 mA/mm^2) or step 18 (0.43 mA/mm^2) intensity was presented. These trials were also terminated by a lever press. Mean shock titration threshold and response latency, and percent response were calculated from 6 min segments of the record corresponding to 25 titratable trials and 4 presentations each of the two suprathreshold stimuli.

Sixty min after saline, mean titration threshold was step 2.6 ± 0.7 (0.07 mA/mm^2) and mean response latency was 0.74 ± 0.05 s. In contrast, mean response latencies to the suprathreshold stimuli were 0.46 ± 0.05 s (step 10) and 0.42 ± 0.05 s (step 18), significantly shorter than titration response latency. Presentations of step 10 and step 18 intensity were terminated 94% and 100% of the time, respectively. Morphine (0.3-6 mg/kg i.m.) produced a dose-dependent increase in mean titration threshold and decrease in % response. Responses to presentations of step 10 and step 18 intensity were reduced to 55% by 3.0 and 6.0 mg/kg, respectively. Morphine also produced a dose-dependent increase in response latencies to step 10 and 18 intensities with significant increases occurring at doses $\geq$ 3.0 mg/kg.

The significantly shorter response latencies and the virtual 100% response to presentation of step 10 and step 18 stimulus intensities suggest that these stimuli are suprathreshold for pain. With respect to the titratable stimuli, it appears that these stimuli are sufficiently localized to yield current densities in the range that elicit response latencies normally associated with nociceptive stimulation. Intermittent STP permits simultaneous evaluation of drug effects on titratable threshold stimuli and on response to suprathreshold nociceptive stimuli.

33.13 DEVELOPMENTAL CHANGES IN THERMAL NOCICEPTIVE THRESHOLD AND ITS RELATIONSHIP TO IMMUNOREACTIVE CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P AFTER NEONATAL ADMINISTRATION OF CAPSAICIN TO THE RAT. D.L. Hammond & M.A. Ruda. CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077 and Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Neonatal administration of capsaicin is reported to increase the threshold to nociceptive stimuli and to decrease the levels of calcitonin gene-related peptide (CGRP) and substance P (SP) in the spinal cord. Both these peptides are found in small diameter primary afferent axons. Although the depletion of CGRP and SP has been confirmed, the increase in threshold to noxious thermal stimuli has been disputed. This study examines the alteration in thermal nociceptive threshold between 10 days and 4 months after neonatal administration of capsaicin and correlates it with changes in the distribution of immunoreactive CGRP and SP in the lumbar spinal cord.

Sprague-Dawley rats were treated with either capsaicin (50 mg/kg, s.c.; Sigma) or vehicle on day 2 of life. Hot plate latency (HPL) was measured at 10 days, 2, 3, 4, 6, 8, 12 and 16 weeks of age in groups of 7-9 rats. After measurement of response latency, the rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Transverse sections of lumbar spinal cord were incubated in antiserum either to human CGRP (1:15,000, Peninsula) or SP (1:10,000, Immunonuclear) for 24-48 hrs at 4°C and processed for immunocytochemistry using standard PAP methodology.

HPL was significantly increased in capsaicin-treated (CAP) rats as compared to values in vehicle-treated rats (controls). At 8 weeks, HPL in CAP rats was 36.1 ± 2.9 s as compared to 7.0 ± 0.6 s controls. At 12 weeks of age, HPL in CAP rats had decreased to 24.2 ± 4.2 s, but was still significantly greater than in controls (9.3 ± 1.4 s). At 16 weeks of age, HPL in CAP rats had decreased further to 11.2 ± 1.4 as compared to 6.2 ± 0.7 s in controls. A reduction in CGRP and SP immunoreactivity in laminae I, II and V of the lumbar spinal cord was visible as early as 10 days after capsaicin administration. At 12 weeks of age, the reduction in CGRP immunoreactive axons in the superficial dorsal horn of CAP rats was less evident and an increase in CGRP axons migrating dorsoventrally through laminae III and IV was observed. These changes in CGRP immunoreactivity were also present at 16 weeks of age. In contrast, there was no apparent increase in SP immunoreactive axons in laminae III and IV.

These data indicate that there is a time dependent return of thermal sensitivity in CAP rats between 8 and 16 weeks of age, and that the reduction in HPL may be related to alteration in CGRP immunoreactive primary afferent axons.

33.14 EVALUATION OF THE ANALGESIC AND NEUROTOXIC ACTIONS OF THE CAPSAICIN ANALOG N-OCTYLHOMOVANILLATE. T. R. LaHann, J. Shafii*, S. Shetty*, G. S. Yost*, and W. S. Ritter. The Colleges of Pharmacy and Veterinary Medicine, Washington State University, Pullman, WA 99164.

Systemic administration of capsaicin produces a profound analgesia in a variety of different animal models of pain. The mechanism underlying this analgesic action is not known. However, since capsaicin is neurotoxic to sensory neurons, there is speculation that its analgesic activity may be the result of damage to sensory neurons. To evaluate the possibility that analgesia and neurotoxicity are dissociable, we have measured the analgesic and neurotoxic actions of the capsaicin analog n-octyl homovanillate (OHV). This compound has previously been reported to be neurotoxic when injected sc in neonatal rats (Jancso and Gabor, Brain Res, 210:83, 1981). Adult, male Sprague-Dawley rats were injected sc with capsaicin (100 mg/kg), OHV (200 or 400 mg/kg) or vehicle (ethanol/Tween 80/saline or DMSO/water/ethanol). The 55°C hot plate model was used to evaluate analgesia, and pain thresholds were measured prior to injection and at 1, 5 and 11.5 hours post-injection. Injection of capsaicin elicited significant analgesia (581% increase in pain threshold), while injections of OHV or vehicle were without effect (<5% increase in pain threshold). Twelve hours after the injections, rats were perfused with phosphate buffered saline and 4% paraformaldehyde and the spinal cords removed. After post-fixation in paraformaldehyde and 30% sucrose, the spinal cords were sliced on a cryostat and thaw-mounted on gelatin subbed slides. Neurodegenerative changes were detected by the Carlsen and DeOlmos cupric-silver technique. Rats treated with capsaicin showed clear signs of terminal degeneration in the substantia gelatinosa of the dorsal horns. Spinal cords of vehicle-treated rats showed no evidence of neurodegeneration. Spinal cords of HVO-treated rats showed little or no evidence of terminal degeneration. In adult rats, then, the sc injection of capsaicin produced both analgesia and degeneration of neurons which terminate in the substantia gelatinosa of the dorsal horn. SC injection of the capsaicin analog HVO failed to elicit either analgesia or neurodegenerative changes. Thus, HVO is not a suitable analog for demonstrating a dissociation of capsaicinoid induced analgesia and sensory nerve damage. While capsaicin is neurotoxic to either neonatal or adult rats, HVO appears to be neurotoxic only to the neonatal rat.

33.15 CHANGES PRODUCED BY LOCAL APPLICATION OF CAPSAICIN TO A PERIPHERAL NERVE ON INPUTS TO SPINOTHALAMIC TRACT CELLS IN THE MONKEY. J.M. Chung, J.S. Kim*, K.S. Paik*, S.C. Nam*, K. Chung and W.D. Willis. Marine Biomedical Institute and Departments of Anatomy & Neurosciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Capsaicin is a neurotoxin which produces selective degeneration of unmyelinated fibers when systematically injected into a neonatal animal. Topical application of capsaicin onto a peripheral nerve produces conduction block of unmyelinated fibers. The purpose of this study was to investigate a long term effect of topical application of capsaicin to a peripheral nerve on the responsiveness of spinothalamic tract (STT) neurons and the correlated anatomical change in the fiber population in the nerve.

Ten monkeys were anesthetized with sodium pentobarbital and capsaicin (1.5% solution) was applied onto the tibial and common peroneal nerves of one side for 30 min. The nerves on the other side in some monkeys were untreated or treated with vehicle only in other animals for the control. After a 3 to 4 week survival period, the monkey was reanesthetized and extracellular recordings were made from identified STT cells in the lumbosacral spinal cord. The responses of STT cells to graded mechanical, thermal and electrical stimuli applied to the receptive fields in the foot were tested. The responses were compared to those recorded from the opposite (control) side. After the experiment, about 2 cm lengths of the tibial nerves on both sides were taken out and processed for examination under the electron microscope.

As compared to the cells recorded from the control side, STT cells recorded from the experimental side showed much weaker noxious thermal responses. Electrical stimulation of peripheral nerve elicited smaller C fiber evoked responses. However, there was no obvious difference in responses to graded mechanical stimuli between the two sides. Preliminary results of the examination of the tibial nerve fiber population in electron micrographs indicated a significant reduction of unmyelinated fibers in the nerve treated with capsaicin.

These data suggest that a brief exposure of capsaicin onto a peripheral nerve produces a long-lasting, selective degeneration of unmyelinated fibers in primates. The most vulnerable peripheral fibers to capsaicin application seem to be those that carry noxious thermal inputs. Topical application of capsaicin or a similar agent may be a useful technique for selective, long-lasting peripheral nerve block in some clinical situations. (Supported by NIH grants NS09743, NS11255 and NS21266 and NIH RCDA NS00995).

33.16 PERIPHERAL PROSTAGLANDINS ENHANCE NOCICEPTIVE INPUT TO THE NEONATAL RAT SPINAL CORD IN VITRO. M.N. Perkins*, J.A. Bettaney and A. Dray. (SPON. A. Nistri) Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, UK.

We have used the neo-natal rat spinal cord-tail preparation in vitro (Yanagisawa et al Eur. J. Pharmacol. 106:231,1984) to investigate possible prostaglandin involvement in peripheral mechanisms of nociception.

The spinal cord with tail attached was isolated from 1-2 day old rat pups following decapitation, and the surface of the skin removed from the tail with forceps. The preparation was maintained at 22°C with the cord and tail perfused separately with artificial CSF. Extracellular recordings were made from one of the lumbar ventral roots (L3-L5) with stimulation of the corresponding dorsal root.

Capsaicin (caps. 0.5-1μM) or bradykinin (Bk 0.5-1μM), which specifically activate peripheral nociceptive nerve terminals, were applied to the tail for 10-12s. This resulted in a depolarizing response in the ventral root of up to 2mV. Similar responses were obtained by dropping hot CSF (>45°C) onto the tail for 5-10s.

Perfusion of the tail with CSF containing PGE_2, E_1, F_2 and D_2 at 0.6-25μM enhanced the amplitude and duration of the responses to Bk and caps varying extents. The response to noxious heat was enhanced in some cases. In addition, the prostaglandins produced a transient ventral root depolarization when applied to the tail. Arachidonic acid (0.7-1.4μM), nifedipine (20μM), BayK8644 (1μM) and $CoCl_2$ (1-2mM) applied to the tail had no effect on the response to Bk, caps or noxious heat. Indomethacin (100μM) had no consistent effect on the responses to Bk or caps. Perfusion of the tail with Ca^{2+} free CSF plus 100μM EGTA enhanced the responses to Bk and caps. Verapamil (2μM) also potentiated of the responses to Bk and caps. However, $CoCl_2$ (1-2mM) prevented or reduced the prostaglandin-induced enhancement of the noxious stimuli.

These results show that prostaglandins can directly stimulate peripheral nerve terminals and enhance peripheral nociceptive activity induced by noxious chemical stimuli. This enhancement may involve calcium in its mechanism of action.

33.17 THE DEAFFERENTATION SYNDROME IN THE RAT: EFFECTS OF INTRAVENTRICULAR APOMORPHINE. Mark A. Lyerly*,Eugene Rossitch,Jr.,M.D.*,Janice Ovelmen-Levitt,Ph.D., and Blaine S. Nashold, Jr.,M.D., Div. of Neurosurgery, Duke Univ. Med. Ctr., Durham, NC 27710

A deafferentation syndrome (DS) produced in rats is expressed as scratching of partially deafferented and/or biting of anesthetic limb areas. This self-mutilation may be objective evidence of dysesthesias, thus serving as an experimental model to study chronic dysesthesias and/or pain from deafferentation in man. This study included behavioral observations of the DS and the effects of intraventricular apomorphine (Apo-M) on its expression. Apo-M, a dopamine agonist, has been shown to produce analgesia in rats when administered intrathecally or intraventricularly, possibly mediated through the dopaminergic diencephalic pathway whose cell bodies localized periventricularly project through the dorsal lateral fasciculus to terminate in the dorsal horn at all spinal levels. Forty-eight female Sprague-Dawley rats underwent unilateral C5-T2 dorsal root ganglionectomies followed immediately by stereotactically guided cannulation of the right lateral ventricle in 30 of the rats. For 2 weeks continuously via an osmotic minipump, 10 rats received Apo-M (5ug/hr), 10 L-ascorbate (the Apo-M vehicle), and 10 lactated mammalian ringers(LR). Rats with ganglionectomies only (DO), as well as those receiving either L-ascorbate (ASC) or LR, demonstrated early onset, more severe and later onset, less severe biting groups ($P<0.05$). The more severe autotomy DO, ASC, and LR groups were of the same population with respect to bite onset and autotomy, while the late onset, less severe groups of ASC and LR fell outside 1 std dev of the mean bite onset of the late DO animals. Animals receiving Apo-M, irrespective of bite onset, correlated with only the late onset, less severe autotomy DO group regarding autotomy. The DO and ASC groups demonstrated an association of early onset of biting with more severe autotomy, while the Apo-M group failed to demonstrate any predictive value of bite onset as an indication of the autotomy to be reached.

This study has 2 major indications: One, the late onset groups of both ASC and LR rats having earlier bite onsets than late onset DO indicates that there may be stress from the presence of the minipump which accelerates biting; and two, that stereotactically applied Apo-M can affect the dysesthesias and/or pain referred to denervated limb areas as indicated by the absence of a severe Apo-M autotomy group and lack of relationship between bite onset and autotomy.

33.18 THE DEAFFERENTATION SYNDROME IN THE RAT: AN EVALUATION OF SEVERAL PARAMETERS. Eugene Rossitch,Jr.*, M.D., Mark A. Lyerly*, Jacob N. Young, M.D.*, Janice Ovelmen-Levitt, Ph.D., and Blaine S. Nashold, Jr., M. D. (SPON: P.G. Shinkman). Div. of Neurosurgery, Duke Univ. Med. Ctr., Durham, NC 27710.

A deafferentation syndrome (DS), produced in rats is expressedas scratching of partially deafferented and/or biting of anesthetic limb areas. This self-mutilation may be objective evidence of dysesthesias, thus serving as an experimental model to study chronic dysesthesias from deafferentation in man. There is evidence that genetic factors, CNS lesions, type of deafferentation, and location of the deafferentation may affect the DS expression. This study examines the effects of sex, age, cortical lesions, and type and locale of lesions on the expression of the DS in rats. A total of 71 Sprague-Dawley rats were involved in 4 protocols. In the first, 18 female and 14 male rats underwent unilateral C5-T2 dorsal root ganglionectomies. In the second, 13 female rats had C5-T2 dorsal root avulsions plus ganglionectomies. The third protocol involved 21 female rats with 1 of 4 surgical combinations: 5 with cortectomy only (C); 7, ganglionectomy only (GO); 6,C then GO; and 3, GO then C (the latter two types separated by 14 days). The cortectomy, at the level of bregma, 2-5mm lateral to the sagittal sinus, consisted of suction removal of 1mm cortex of S1 representation. In the fourth protocol 5 female rats had unilateral L2-S1 dorsal root ganglionectomies. In all protocols, the rats' behavior were quantified using an autotomy grading scale ranging from 0-19. Both female and male rats demonstrated early onset, more severe and later onset, less severe biting groups. Of all rats in the early onset, severe biting groups, the males bit significantly earlier than the females. Both sexes demonstrated an association of early onset of biting with high autotomy scores. The male group was examined with respect to age, which was found not to affect the DS. In avulsion (A) and ganglionectomy (GO) groups, onset of autotomy, autotomy score 10d after the onset of biting, and total autotomy scores were recorded. Both groups appeared to progress in autotomy in parallel fashion. Although the A animals had a decreased latency to autotomy onset (7.5d) vs ganglionectomy (21.8d)($p< 0.025$), the autotomy scores 10 days after the onset of biting were not significantly different. No cortectomy animals expressed autotomy, while the GO animals express the most. Animals having had both the cortectomy and GO deafferentation, in either order, showed markedly decreased autotomy vs GO animals ($p<0.05$). Comparing lumbosacral vs cervical ganglionectomy there appeared to be no difference in onset or degree of biting; however, no lumbosacral deafferentation rats scratched while this behavior occurred in 50% of cervical deafferentations. In conclusion no differences were found in the expression of the DS with respectto age and locale of lesions. However, sex, cortical ablations and type of lesion did alter the syndrome.

34.1 LOCAL AXON COLLATERALS FROM SPINAL LAMINAE I AND II NEURONS WHICH PROJECT TO THE BRAIN STEM. M.J. Sedivec, M.A. Dunnigan*, A.M. Kavookjian*, and A.R. Light. Dept. of Biology, Appalachian State University, Boone, NC, 28608 and Dept. of Physiology, UNC-Chapel Hill, Chapel Hill, NC, 27514.

Recent studies in our laboratories have demonstrated that many nociceptive neurons in laminae I and II have projecting axons which terminate at least as far rostral as the parabrachial region in the midbrain. Some of these neurons have local axon collaterals which terminate in laminae I,II and in deeper laminae (III,IV and V). In this study we have examined these collaterals with both the light and electron microscopes.

Single neurons were recorded in the spinal cords of anesthetized cats. Nociceptive neurons which were antidromically activated from the parabrachial region of the brain stem were intracellularly labeled with horseradish peroxidase. The tissue was perfused with buffered paraformaldehyde and glutaraldehyde, removed and postfixed, and then sectioned on a Vibratome at 50μm. Following a reaction with DAB + H_2O_2, selected sections were reacted with osmium and embedded in plastic between Teflon-coated coverslips. Neurons were reconstructed with a drawing tube at 1000X and selected portions were cut from the plastic wafers and recut with an ultramicrotome into serial sections. These sections were examined with a Zeiss 10C electron microscope unstained. Later these sections were post-stained with lead citrate and uranyl acetate.

Nociceptive neurons which projected to the parabrachial region were found in both laminae I and II. Local axon collaterals were found which terminated in laminae I and II and which continued deeper to terminate in laminae III and V. These local collaterals were unmyelinated and demonstrated many *en passant* and a few terminal varicosities. These varicosities were small synaptic boutons which were about 1μm in diameter. These synaptic boutons were Gray's Type I, in that they contained clear, round vesicles about 45 nm in diameter, a few large dense core vesicles approximately 85nm in diameter, and established one or two asymmetric contacts with dendritic profiles.

These results demonstrate that long projection neurons in laminae I and II that respond to nociceptive stimulation of their receptive fields have local axon collaterals which may be capable of exciting neurons in laminae I-V in the same spinal cord segment. Thus, these long projection neurons may be important in local integration of nociception. *Supported by NINCDS grants #NS16433, and #DA4420 to A.R.L. and a grant from the University Research Committee and Cratis D. Williams Graduate School at Appalachian State University to M.J.S.*

34.2 LATERAL VERSUS MEDIAL SPINOTHALAMIC NEURONS AND THEIR AXON COLLATERALS TO THE PERIAQUEDUCTAL GRAY AND MEDULLARY RETICULAR FORMATION IN THE RAT. Constance M. Pechura. Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, Md. 20814-4799.

Fluorescent, retrograde tracing studies were undertaken to compare laterally versus medially projecting spinothalamic tract neurons in terms of their axon collaterals to the periaqueductal gray (PAG) or medullary reticular formation (MRF). The tracers used for these double-label experiments were fast blue or fluorogold in combination with rhodamine-labeled latex microspheres. Under general anesthesia, the tracers were injected into the lateral thalamus and MRF (L-STT/MRF,N=6), lateral thalamus and PAG (L-STT/PAG,N=5), medial thalamus and MRF (M-STT/MRF,N=6), or medial thalamus and PAG (M-STT/PAG,N=5). After 7 days, the animals were deeply anesthetized and perfused transcardially with saline followed by 10% formalin. The brains and spinal cords were removed and processed according to standard protocols. Locations of single- and double-labeled STT neurons were plotted for the spinal enlargements, mid-thoracic and upper cervical segments.

In all segments examined, single- and double-labeled L-STT and M-STT neurons were commonly observed in contralateral laminae V-VIII and, at upper cervical levels, in ipsilateral laminae VII-VIII. The percentages of STT cells which were double-labeled (10-40%) in these areas were similar across the experimental conditions, but were generally highest in the upper cervical and lowest in mid-thoracic segments. The lateral spinal nucleus contained double-labeled STT neurons in all four experimental groups. More L-STT than M-STT neurons were observed in lamina I in the cervical enlargement and these were double-labeled only from the PAG. In the lumbar enlargement, many L-STT neurons, none of which were double-labeled, were seen in the ventromedial dorsal horn. These neurons have been shown to respond to both proprioceptive and cutaneous stimuli and, thus, have been proposed to function in transmitting information regarding locomotion to the thalamus (Menetrey, D., et al., J. Neurophysiol. 52:612,1984). In this context it is interesting that heavy labeling in this region was associated with involvement of the ventral lateral nucleus in the L-STT injection sites. Such populations of exclusively single-labeled L-STT neurons were also found at upper cervical levels in the internal basilar and lateral cervical nuclei. Heavy labeling in the internal basilar nucleus was associated with L-STT injections which impinged upon the posterior nucleus.

These data suggest that many L-STT and M-STT neurons issue axon collaterals to the PAG or MRF. Further, L-STT neurons which project directly to the thalamus without axon branches are located in restricted spinal regions and may function in highly specific types of sensory processing. (Supported by USUHS Grant FE5500.)

34.3 COLLATERALS OF SPINOTHALAMIC TRACT (STT) NEURONS TO THE PAG: A DOUBLE LABELING STUDY IN THE MONKEY. D. Zhang*, S.M. Carlton, L.S. Sorkin, P.A. Harmann* and W.D. Willis (SPON: D. Trevino). Dept. of Anatomy & Neurosciences and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

The STT is an important pathway transmitting nociceptive information to the brain. Electrophysiological evidence suggests that the STT also sends collaterals into the PAG. The present study employs retrograde transport of fluorescent tracers, granular blue (GB), diamidino yellow (DY) and rhodamine conjugated latex beads (RB) to identify the location and distribution of neurons in the spinal cord which project to both the thalamus and the PAG.

Two adult monkeys (M. fascicularis) received bilateral injections via Hamilton syringes of 5% GB (0.6μl each side) in the VPL thalamus and unilateral injections of 50% RB (0.2μl) or 2% (0.2μl) in the lateral PAG. After 10 to 15 days, animals were perfused with 4% paraformaldehyde in 0.1M phosphate buffer. The brains and spinal cords were removed and stored in 30% sucrose overnight. Cervical and lumbar segments and the injection sites were frozen sectioned (30 μm), mounted and air dried. Thalamic injection sites were centered in the VPLc. Midbrain injection sites were located in the dorsolateral PAG; however, in one animal, the ventral inferior colliculus was also included.

Single labeled GB neurons were found in all dorsal horn laminae and laminae 7 and 8 in both cervical and lumbar segments. However, the majority of GB cells were observed in lamina 5. Cervical lamina 1 contained a greater number of GB cells than lumbar lamina 1. However, the reverse was true for lamina 7, in that more GB cells were observed in lumbar lamina 7 than cervical lamina 7. Single labeled RB neurons were found almost exclusively in laminae 1 and 5 and the majority were contralateral to the PAG injection site. No DY labeled cells were found in the spinal cord. Several double labeled GB-RB neurons were observed in cervical and lumbar cord in both laminae 1 and 5. They were of a size similar to single labeled RB neurons. The actual percentage of double labeled neurons is under further investigation.

This preliminary study demonstrates that some STT neurons that send collaterals to the PAG have the same distribution as PAG-projecting neurons in the lumbar and cervical segments. The STT collaterals to PAG provide a mechanism for activation of descending, as well as ascending control of noxious input. (Supported by NS 11255 and NS 09743.

34.4 SPINAL AND TRIGEMINAL PROJECTIONS TO THE PARABRACHIAL AREA: AN ELECTRON MICROSCOPIC STUDY WITH REFERENCE TO PAIN PATHWAYS. Wu Ma* and Marc Peschanski (SPON. D. Forman), INSERM U 161, 2 rue d'Alésia, 75014 Paris, France.

Recent anatomical and electrophysiological studies have demonstrated that the parabrachial area of the pons (PB) receives projections from the spinal and trigeminal lamina I neurons, some of which are activated by noxious stimulation. This suggests that PB is a relay for a pain-related system running parallel to the well documented thalamic relays. In previous studies, we have indicated that the fine structure of spinal and trigeminal afferent terminals possibly involved in pain pathways are remarkably similar in the different thalamic nuclei of the rat. These terminals are large (average diameter: 2-3 μm), contain round vesicles and form asymmetrical contacts with dendritic protrusions, large dendrites, and more rarely, the soma. In line with these results, the present study was undertaken to analyze the spinal and trigeminal afferent terminal morphology in PB.

Injections of wheat-germ agglutinin conjugated to HRP were carried out in the spinal cord or in the trigeminal subnucleus caudalis (SNC) of 6 rats. Two days later, rats were perfused with mixed aldehydes and processed for the visualization of HRP using benzidine dihydrochloride as a chromogen (Peschanski, et al., Somatos, Res., 3, 1985) and embedded flat in Epon. The lateral and dorsal areas of PB containing labeled or degenerating terminals were trimmed out and thin-sectioned for ultrastructural studies.

Labeled spinal and trigeminal axons were large and myelinated. Labeled terminals made contacts with large and medium size dendrites, and rare axo-somatic contacs were observed. The synaptic contacts exhibited two different morphologies: (i) the majority of labeled terminals contained round synaptic vesicles (R type) and formed typical Gray type I (asymmetrical) synapses, (ii) the second type of terminal contained a pleomorphic population of synaptic vesicles (P type) and formed symmetrical contacts (Gray type II). The quantitative analysis carried out on 91 labeled contacts showed that the average diameter of the terminals ranged between 0.4 and 1.8 μm (mean: 0.98 ± 0.12 μm SD). The smallest diameters of the terminals on contacted dendrites varied widely, between 0.2 and 3.6 μm (mean: 1.13 ± 0.50 μm SD). No quantitative difference was observed between the R and P terminals.

These results indicate that spinal and trigeminal afferents to the PB exhibit morphological features which differ from those of the afferents originating from the same structures which terminate in the thalamus. The functional significance that can be assigned to these morphological results is presently a question beyond our reach, but it is reasonable to suggest, nevertheless, that the dissimilarities in synpatic arrangements may correspond to similarities in input-output relationships.

34.5 LAMINA I SPINOTHALAMIC TRACT (STT)/SPINOMESENCEPHALIC TRACT (SMT) PROJECTION NEURONS IN THE RAT ASCEND VIA THE DORSOLATERAL FUNICULI (DLF). R.L. Nahin, F. Anton* and J.L.K. Hylden. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies in our laboratory concerning the characterization of lamina I neurons with projections to the midbrain of the cat, have shown these cells to be nociceptive-specific, to project bilaterally through the DLF and to have collateral projections to the thalamus. The present study was aimed toward investigating the distribution and funicular projection of the analogous population of neurons in the rat and to examine the extent of collateralization to the thalamus by the use of retrograde labeling techniques.

Projection neurons were labeled following injection of WGA-HRP (0.1 µl, 25%, 72 h) into the midbrain (area surrounding the brachium conjunctivum at the pontine-mescencephalic junction). The distribution of retrogradely labeled cells was examined in transverse 50 µm sections of cervical and lumbar enlargements after reaction of the tissue sections with tetramethylbenzidine (TMB). Concentrations of labeled cells were observed bilaterally in lamina I, lateral lamina V and the lateral spinal nucleus, with more scattered cells occurring in deeper laminae and around the central canal. Bilateral lesions of the DLF at lower thoracic levels resulted in a profound decrease (62-91%) in the number of labeled lamina I cells caudal to the lesion.

Collateral projection of SMT/STT neurons were examined by combining large injections of the fluorescent dye fluorogold (FG; Fluorochrome Inc.; 0.3 µl, 3%, 14 d) into the thalamus with WGA-HRP midbrain injections. The combination of retrograde labeling by FG and WGA-HRP revealed that many lamina I STT cells had collaterals to the midbrain, but it was apparent that the very dense TMB reaction product obscured much of the FG fluorescence. Subsequent experiments combined FG labeling from the thalamus with labeling by rhodamine microspheres (Tracer Technology; 0.3 µl, 14 d) retrogradely transported from the ipsilateral midbrain. Frozen, parasaggital (25-50 µm) sections of cervical and lumbar enlargements were examined uncoverslipped using an epi-fluorescense microscope with appropriate filter combinations. In 2 rats with thalamic injections centered on VPL, 85-95% of labeled lamina I STT cells also contained the tracer from the ipsilateral midbrain, implying that the majority of lamina I STT cells have collateral branches to the midbrain. The converse was not true (i.e., many single-labeled SMT neurons were present). Double-labeled neurons were also noted in deeper laminae, but were not as likely to occur.

Our data indicate that there is a large population of lamina I neurons with projections through the DLF to the midbrain in the rat. A subset of this population also innervates the thalamus.

34.6 PHYSIOLOGICAL CHARACTERIZATION OF NOCICEPTIVE LAMINA I PROJECTION NEURONS IN HYPERALGESIC RATS. J.L.K. Hylden, R.L. Nahin and R. Dubner, NAB, NIDR, NIH, Bethesda, MD 20892.

Spinal lamina I neurons are excited by noxious stimuli, project to the brainstem and thalamus and are potentially involved in pain transmission. Since much of the characterization of these neurons has been done with acute noxious stimuli (pinch, heat) in normal animals, it is of interest to examine the behavior of such nociceptive neurons after tissue injury and inflammation. We have addressed this question by examining the responses of superficial lumbar dorsal horn neurons in rats 5 days after a s.c. injection of Complete Freund's Adjuvant (CFA) into the plantar surface of the ipsilateral hindpaw (0.15 ml, CFA:saline, 1:1). Rats treated in this way develop an inflammed hindlimb as evidenced by the presence of edema, erythema and hyperalgesia (decreased threshold to noxious stimuli) and demonstrate biochemical changes localized to the ipsilateral lumbar spinal cord (Iadarola & Naranjo, NS Abstr., 1986). Lamina I projection neurons were identified in chloralose-anesthetized rats by antidromic activation from upper cervical spinal cord.

Lamina I projection neurons (20 cells) in control rats had relatively small receptive fields (RF) with sharp borders (usually comprised of one or more toes) and responded exclusively to noxious cutaneous stimuli (pressure, pinch, heat). Heat thresholds (47° ± 1.8°C) and mechanical thresholds (4.9 ± 0.4 g) were consistent with classification of these cells as nociceptive-specific (NS). Five cells were noted as having spontaneous activity of less than 5 hz. In rats pretreated with CFA, lamina I projection neurons (37 cells) demonstrated heat thresholds similar to those of cells in untreated rats (47° ± 2.8°C) and only slightly lower mechanical thresholds (4.4 ± 0.8 g). However, the distribution of RF size for cells in CFA-treated rats included many larger RFs ($p < 0.05$, X^2), often including the entire surface of the ipsilateral hindpaw. In addition, 50% of these cells responded to movement of joints or deep tissue or had discontinuous RFs and were classified as NS/complex. Eighty-seven percent of cells in CFA rats demonstrated background firing rates of 2 to 30 Hz (10 ± 2) with some bursting-type activity. The spontaneous activity of a majority of cells tested was inhibited by electrical stimulation at C2 and/or the ipsilateral sciatic nerve.

The present data provide evidence that a population of lamina I nociceptive projection neurons demonstrates increased activity in a model of hyperalgesia. The increased responsiveness of these neurons, which are known to project to the midbrain and thalamus, may result in a range of pain sensations, including pain in the absence of stimulation of the affected part of the body and referred pain.

34.7 CHARACTERIZATION OF SOMATIC CONTACTS ON SPINOTHALAMIC NEURONS IN THE MONKEY. K.N. Westlund, and S.M. Carlton. Dept. of Anatomy & Neurosciences and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

The population of terminal and non-terminal elements contacting spinothalamic (STT) somata in the lumbar spinal cord of monkeys was examined by electron microscopy. Comparison was made between somata localized in laminae I and V. Monkeys were anesthetized and the hindlimb representation in the thalamus was located electrophysiologically. Animals received either a total of 3 µl of 25% HRP into the thalamus via a Hamilton syringe or physiologically characterized STT neurons in the cord were injected intracellularly with HRP. Following an appropriate survival time, animals were perfused with a brief saline rinse and fixative (2.5% paraformaldehyde, 0.2% glutaraldehyde). Spinal cord sections were cut on a vibratome (50 µm) and reacted for HRP using cobalt chloride and diaminobenzidine (Bowker et al., 1982). Tissue sections containing HRP labeled STT cells were immunostained for dopamine-β-hydroxylase (DBH), stained with 1% osmium and uranyl acetate, dehydrated and flat embedded in plastic. Labeled STT neurons were serially thin sectioned and sampled every 1-1.5 µm. Photographic montages of the perimeter of STT somata were constructed (x24,270) for analysis with the following results for one pair of neurons:

	LAMINA I (retrograde)		LAMINA V (intracellular)	
R	63.3%	(194)	69.0%	(376)
F	1.0	(2)	10.1	(55)
D1	12.1	(37)	12.8	(70)
D2	6.6	(20)	5.5	(30)
L1	12.1	(37)	1.5	(8)
L2	4.9	(15)	1.1	(6)
Total	100.0%	(305)	100.0%	(545)

Contacting neuronal terminals were classified as either containing round clear vesicles (R), round clear vesicles with >30% flat or oval vesicles (F), round clear vesicles with 2-4 dense core vesicles (DCV, 37-50 µm) (D1), round clear vesicles with large DCV (>50 µm) (L1), round clear vesicles with 5 or more small DCV (D2), or clear round vesicles with >5 large DCV. The population of terminals surrounding the cells in each lamina were similar in many respects. The predominant type of terminal observed contained round clear vesicles. Terminals containing flat or oval vesicles were a feature of the lamina V population, whereas the L1 classification was more abundant in lamina I. DBH terminals were observed contacting the somata of lamina I cells (4.4% or 17 terminals) but were not observed contacting lamina V somata to date. An additional 488 non-terminal elements (glia, dendrites, axons) were observed contacting a lamina I STT neuron compared to only 23 non-terminal elements contacting a lamina V neuron.

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34.8 A SPINO-DIENCEPHALIC RELAY THROUGH THE PARABRACHIAL NUCLEUS IN THE CAT. A. Blomqvist and K.J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306-1051.

The results of recent anatomical and electrophysiological studies have suggested that the parabrachial nucleus (PBN) serves as an important brainstem relay for ascending nociceptive information from neurons in lamina I of the spinal and medullary dorsal horns. Using single and double anterograde and retrograde tracing methods to compare the location of terminals in PBN of fibers from the spinal cord with the location of neurons projecting to the thalamus, it was recently found (Blomqvist and Berkley, 1986) that there is a dense non-topographically organized spinal input to the lateral parabrachial nucleus, part of which overlaps the location of thalamic-projecting PBN neurons (but fails to overlap the location of amygdala projecting neurons) and part of which overlaps neurons with unknown targets.

The present study extended these observations by comparing the spinal input to PBN with PBN's output to the hypothalamus. It was found that hypothalamic-projecting PBN neurons filled the region with previously unknown targets. Taken together, the results of these experiments indicate that a major portion of the spinal input to PBN appears to be relayed to the diencephalon, part to the thalamus and part to the hypothalamus via different populations of PBN neurons. The functional significance of this connective pattern awaits the results of physiological investigations.

Blomqvist, A. and Berkley, K.J., Soc. Neurosci. Abstr., Vol. 12, Part 1, p. 228, 1986.

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34.9 CONVERGENCE UPON AND EFFERENT CONNECTIONS OF A SPINAL CORD CENTER WHICH RECEIVES NOCICEPTOR INFORMATION FROM THE CRANIAL VASCULATURE. G.A.Lambert*, A.S.Zagami* and J.W.Lance* (SPON: D. Ferrington) Dept. of Medicine, University of New South Wales, NSW, 2031. Australia

We have recently reported on the occurrence in the upper cervical cord of an area (probably the lateral cervical nucleus-lcn) which receives input from the sagittal sinus. This area receives both A-delta and C-fibre input from the trigeminal system. In the experiments reported here, we have characterised the output connections of this area to the thalamus and examined the degree of convergence onto the area from several components of the cranial vasculature. Cats were anesthetized with urethane/chloralose and prepared for single unit recording in the upper cervical cord and the thalamus. A length of the dura and falx containing the superior sagittal sinus (SSS) was isolated from the the rest of the dura, placed on platinum hook electrodes, and stimulated electrically with supra-maximal shocks (40-120V, 250 usec, 1 per 5 sec). Additionally, segments of the middle meningeal artery (MMA) or the superficial temporal artery (STA) were similarly exposed and prepared for stimulation. Glass or glass-coated tungsten electrodes were used to record single unit activity. In the C2 region of the spinal cord cells having convergent input from three separate components of the cranial vasculature were found, as follows:

Numbers of convergent units				
SS	SS/STA	STA	SS/MMA	MMA
21	16	3	9	1

Stimulation of the SS or MMA evoked activities in the thalamus with both A-delta and C-fibre latencies as well as activity with very short latencies (2-3 msec). Responsive units were found in the thalamo-cortical projection (fibres) and a "shell region" of the vpm nucleus. Units only occasionally had receptive fields on the contralateral face and scalp. HRP injections into thalamic regions in which responsive cells were found labelled both the lcn and the sub-nucleus caudalis of the trigeminal nucleus. The degree of convergence from different vascular components is greater in the spinal cord than it is in the trigeminal sub-nuclei (Davis, K.A. & Dostrovsky,J.O., Soc Neurosci Abstr., 12, 230) but there is less convergent input from the facial and cranial skin. This indicates that the primary centre for processing of craniovascular sensation may be the cord and that referral of pain in migraine may be related more to convergence in the trigeminal nucleus. Occurrence of reactive units in and around the vpm nucleus is additional evidence for the processing of craniovascular information in the lcn, as well as in the trigeminal nucleus.

34.10 BRAINSTEM RELAYS MEDIATING STIMULATION-PRODUCED ANTINOCICEPTION FROM THE LATERAL HYPOTHALAMUS IN THE RAT. L.D. Aimone, C.A. Bauer* and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

Several lines of evidence have demonstrated a role for the lateral hypothalamus (LH) in an endogenous system of descending inhibition. The present study, in rats lightly anesthetized with pentobarbital, was undertaken to systematically examine neuronal pathways mediating descending inhibition of the nociceptive tail flick (TF) reflex produced by focal electrical stimulation in the LH. The local anesthetic, lidocaine, and the neurotoxin, ibotenic acid, were used to produce reversible, nonselective or irreversible, somata selective lesions, respectively, in the brainstem. Pharmacologic antagonists were employed in other experiments to determine the neurotransmitter(s) mediating the inhibition at the level of the medial medulla.

Rats were initially deeply anesthetized with pentobarbital (45 mg/kg) for craniotomy and cannulation of the femoral vein and artery. The rats were subsequently maintained in a lightly-anesthetized state (corneal and flexion reflexes present) with an i.v. infusion of pentobarbital (3-6 mg/kg/hr). Constant current stimulation (100 Hz, 100 µsec, 20-200 µA) with monopolar cathodal electrodes (0.15 mm dia.) was begun 10 sec prior to and continued during the application of heat to the tail.

The microinjection of lidocaine (4%, 0.5 µl) into the midbrain, dorsolateral pons or medial medulla resulted in significant increases in stimulation thresholds in the LH for inhibition of the TF reflex (89.1%, 67.4% and 73.6%, respectively). Selective lesions of cell bodies in these supraspinal sites, by ibotenic acid (10 µg, 0.5 µl), revealed relays in the periaqueductal gray and the nucleus raphe magnus (NRM) between the LH and the lumbar spinal cord.

The intracerebral administration of pharmacologic antagonists (5 µg, 0.5 µl) into the NRM revealed that the glutamate receptor antagonists gamma-D-glutamylglycine and 2-amino-5-phosphono-valerate produced the largest increases in stimulation thresholds in the LH for inhibition of the TF reflex (107.6% and 102.6%, respectively). Methysergide, a serotonin receptor antagonist, also produced a significant increase (81.5%) in the LH stimulation threshold; the opioid receptor antagonist naloxone, however, was without effect, producing only a 4.0% increase in the LH stimulation threshold for inhibition of the TF reflex. These results suggest that serotonin and/or an excitatory amino acid are transmitters at the bulbar relay in the medial medulla mediating descending inhibition of the TF reflex produced by focal electrical stimulation in the LH.

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34.11 CHARACTERIZATION OF T13-L2 SPINAL NEURONS RESPONSIVE TO NOXIOUS DISTENSION OF THE GUT IN SPINALIZED AND INTACT RATS. T.J. Ness and G.F. Gebhart, Dept. Pharm., U. Iowa, Iowa City, IA 52242

Distension of the descending colon and rectum is a noxious visceral stimulus since it evokes reports of pain in humans and aversive behavior in rats. In the present study, 132 neurons responsive to graded colorectal distension (20-100 mm Hg, 20s) were characterized in 35 spinalized and 7 deeply pentobarbital-anesthetized rats for long ascending projections (antidromic invasion from medulla), convergent cutaneous receptive fields (brush, pinch, heat, cold) and responses to the intraarterial administration of the algesic peptide bradykinin. Using the terminology of a previous study of L6-S1 spinal neurons (Ness and Gebhart, J. Neurophysiol. 57: June, 1987), neurons were grouped into four classes based upon responses to a standard 80 mm Hg, 20 s distending stimulus: [1] short latency-abrupt (SL-A; fast ONset of response, abrupt OFF-termination of response), [2] short latency-sustained (SL-S; fast ON, slow OFF), [3] long latency (LL; slow ON, slow OFF) and [4] inhibited (INHIB). The following table summarizes some of the data:

Neuronal Class	n (spinal:intact)	convergent cutaneous receptive field	long ascending projections	excited by bradykinin
SL-A	46 : 9	55/55	6/9	10/13
SL-S	31 : 11	42/42	9/11	12/12
LL	0 : 4	3/4	0/4	-
INHIB	24 : 7	31/31	3/8	1/4 3 (inhibited)

Neurons with convergent cutaneous input were mostly Class 2 and Class 3 (responsive to non-noxious and noxious or only noxious stimuli, respectively). Stimulus-response functions were linear, accelerating and monotonic. The threshold for response for SL-A neurons is near 0 mm Hg whereas SL-S neurons have a higher threshold (mean, 15 mm Hg for intact rats). Tonic inhibition or excitation in individual SL-A and SL-S neurons was demonstrated by spinal cold block of the cervical cord. LL neurons, not apparent in spinalized rats, 'converted' to SL-S neurons with cold blocks.

These data indicate that spinal neurons encoding for colorectal distension in the T13-L2 spinal cord of the rat are similar, if not identical, to neurons characterized in the L6-S1 spinal cord and likely are visceral nociceptors. Supported by DHHS awards NS 19912 and DA 02879; T.J.N. is supported by a Life and Health Insurance Medical Research Fellowship.

34.12 NOCICEPTIVE ACTIONS OF NMDA IN THE RAT SPINAL CORD L. M. Aanonsen* and G. L. Wilcox (SPON: L. Lichtblau) Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

An abundant literature now supports involvement of receptors for excitatory amino acids (EAA) in the mediation of synaptic transmission in the mammalian spinal cord. Recent studies have indicated involvement of glutamate in spinal nociceptive transmission (Schneider and Perl, Brain Res. *360* [1985]: 339; Willcockson *et al.*, J. Neurosci. *6* [1986]: 2509). While these experiments suggest that EAAs act as sensory neurotransmitters, few studies have been performed which systematically examine the role of the three EAA receptor subtypes in sensory pathways. The experiments presented here have compared excitation elicited by EAA receptor agonists (NMDA, Quis and KA) on identified spinal neurons in the rat and the effect of iontophoretically applied PCP on EAA-induced excitation.

Extracellular recordings were made from a total of 34 neurons in the lumbar spinal cord of 27 rats. We found that 65% of the cells tested were excited by at least one EAA. The modality of each neuron was classified as low threshold (LT), wide dynamic range (WDR) or high threshold (HT). NMDA-responsive cells were primarily identified as WDR or HT neurons:

Iontophoretic Agent	LT + Proprioceptive	WDR + HT	% Nociceptive
NMDA	3 (15)	12 (15)	80*
Quis	7 (16)	9 (16)	56
KA	5 (8)	3 (8)	38

* $p<0.05$ NMDA compared with KA, Chi-squared test

Cell firing induced by NMDA was inhibited in 100% of the cells tested with PCP. This is in contrast to Quis-induced firing in which 33% of Quis-excited cells were inhibited by PCP. PCP was also shown to enhance Quis-induced firing in 40% of the cells tested. KA-induced firing was not inhibited by PCP in any of the cells tested; however, firing induced by KA was enhanced by PCP in 43% of the cells tested. We concluded from these experiments that NMDA, more potently than Quis or KA, selectively excites neurons responsive to noxious stimuli and that the responses to NMDA are selectively blocked by PCP.

These results together with recent behavioral experiments (Aanonsen and Wilcox, JPET [accepted, pending revision], 1987) suggest that receptors for NMDA are selectively involved in nociceptive processing in the rodent spinal cord. (Supported by USPHS grants DA-01933, DA-04274 and DA-04090).

34.13 INTRACELLULARLY STAINED CELLS and AFFERENT FIBERS IN NUCLEUS CAUDALIS AND THE SPINAL TRIGEMINAL TRACT.
R.C. Shults and A.R. Light. Depts of Orthodontics and Physiology, UNC-Chapel Hill, NC 27514.
In anesthetized cats, primary afferent fibers and cells with nociceptive specific, multimodal (responding at higher frequencies to noxious pinch and noxious heat than to low threshold mechanical stimuli) and cooling specific response characteristics were recorded and then labeled by intracellular or intra-axonal injection of horseradish peroxidase. A cooling specific primary afferent fiber with a receptive field on the tip of the tongue was exceptionally well stained from rostral nucleus oralis (NO) through caudal nucleus caudalis (NC). The parent fiber gave off collaterals with terminal boutons in islands of neuropil termed the interstitial nucleus of the spinal trigeminal tract (ISVT) overlying NO and nucleus interpolaris (NI) rostral to obex. We have previously reported that these islands become apparent in the tract at the level when layers I and II break up and become separated from the underlying nucleus at the transition between NI and NC and continue in the tract rostrally as far as NO. Other collaterals from this same fiber terminated in layers I and II of NC. Therefore, collaterals from the same parent fiber terminated in ISVT, rostral to obex, and layers I and II of NC.
All but two labeled postsynaptic cells were distributed throughout the full width of layer IIo. Dendrites of cells with multimodal input traversed several laminae (layers I through IV). In contrast dendrites of cells with nociceptive specific responses were confined within layers I and II of NC. Two exceptional neurons were recorded; these cells had nociceptive response properties, were located in ISVT and had nociceptive receptive fields limited to midline perioral and oral structures.
These findings are consistant with the hypothesis that ISVT is homologous to NC layers I and II. Previous reports hypothesized this homology based on neuro-transmitters found in ISVT which are the same as those in NC layers I and II. However, the specificity of receptive fields to midline perioral and oral structures in ISVT is unique and thus ISVT may represent a nociceptive relay specific for these structures.
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34.14 Dental Surgery Induces Neck Muscle Inhibition in the Rat. V.A. Lewis, Department of Pharmacology, University of Texas, Dental Branch, Houston, Tx 77225.
Wall(1) has hypothesized that injury induces consecutive behavioral (pain related) states, identified as immediate, acute and chronic pain states. The effects of a dental extraction on identified sleep state indexes were studied to determine if Wall's post injury states could be measured. Sprague-Dawley rats were prepared with indwelling electrocorticographic (ECG), neck muscle electromyographic (EMG) electrodes and an indwelling intrajugular cathter and allowed to recover for 1 week. Under pentobarbital anesthesia (40 mg/Kg), the right three maxillary molars were extracted in 10 experimental rats. An equal number of control animals received all surgical drugs but no extraction. Recording of EEG and EMG activity were made at 3,9 and 27 hours following surgery. The recorded sample consisted of 60 2 second samples with a 30 second interval between recordings. Raw EEG and EMG recordings were converted to the following indexes for comparisons: integrated EEG zero crossing index(Z), EEG amplitude index(D), EEG theta/delta frequency ratio (F), integrated EMG(M), and integrated gross motor activity(T). Comparisons between the extraction and control were assessed using analysis of variance followed by a T test. Because the Z, M and T indices decrease with time, data for samples in minutes 4-8 and 16-20 were compared separately. Three hours following surgery integrated EMG was 21% of control in minutes 16-20, a significant reduction, $P=<0.05$. No other indices were significantly changed. Wall's hypothesis suggests that injury is followed by arousal and then depression. In this experiment the sleep state indexes were not altered by an injury that did depress neck muscle activity.
(1) Wall, P.D., Pain,6(1979)253-264.

34.15 RESPONSES OF TRIGEMINAL NUCLEUS CAUDALIS NEURONS TO MECHANICAL AND CHEMICAL STIMULATION OF CRANIAL BLOOD VESSELS.
A. Strassman, J. Pile-Spellman*, R. Oot*, P. Mason, M Moskowitz, R. Maciewicz. Neurology, Neurosurgery, and Radiology services, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114.
Stimulation of cranial blood vessels in humans during neurosurgical or angioplastic procedures typically evokes painful sensations that are referred to the ipsilateral forehead and eye. Previous work by this laboratory has shown that electrical stimulation of dural blood vessels activates a population of neurons in rostral trigeminal nucleus caudalis; many of these cells also have nociceptive cutaneous receptive fields in the ipsilateral first trigeminal division. The visceral and somatic convergence on single trigeminal neurons may explain the pattern of cutaneous referral of cranial vascular pain.
In order to further characterize the vascular responses of this population of caudalis neurons, extracellular unit activity was recorded during mechanical or chemical stimulation of cranial blood vessels in anesthetized cats. Neurons that responded to electrical stimulation of the superior sagittal sinus (SSS) were tested with mechanical stimulation by inflating an intraluminal balloon-tipped catheter in the internal maxillary artery (IMA), or by applying upward traction to the SSS. The neuronal response usually consisted of a short duration, high frequency burst of firing that did not outlast the stimulus. Chemical stimulation was carried out by perfusion of algesic substances either through a catheter positioned trans-femorally in the IMA at the origin of the middle meningeal artery, or through a needle advanced into the SSS through the dura. Approximately one-third of the neurons that responded to electrical stimulation of the SSS also responded to injection of 0.2-0.5 ml of bradykinin (10^{-4}M - 10^{-5}M) into the IMA or SSS. Neuronal responses to bradykinin injection were usually 30-90 seconds in duration. Responses were also found following injections of serotonin (200 ug/ml) and KCl (300mM), but not normal saline. Control injections into the inferior vena cava were similarly ineffective.
Systemic pretreatment with intravenous lidocaine in doses that relieve clinical vascular head pain in humans (2mg/kg) completely suppressed the neuronal response to bradykinin injection into SSS for a period of 15-20 minutes. Intravenous lidocaine had no effect on the cutaneous responses of brainstem trigeminal neurons, however, suggesting that this dose of lidocaine blocks the bradykinin response through a local effect on the blood vessel wall.
These results provide further evidence that brainstem trigeminal neurons with vascular responses are involved in mediating the painful sensations evoked by stimulation of cranial blood vessels; such neurons may also play an important role in the pathogenesis of clinical syndromes of vascular head pain.

35.1 **DOES CNS REMYELINATION RECAPITULATE CELLULAR AND MOLECULAR EVENTS OF MYELINATION?** C. A. Jordan,* V. L. Friedrich, Jr., F. de Ferra,* D. G. Weismiller,* N. K. Zeller,* K. V. Holmes* and M. Dubois-Dalcq. Laboratory of Molecular Genetics, NIH, Bethesda, MD 20892, The Wistar Institute, Philadelphia, PA 19104, and Department of Pathology, USUHS, Bethesda, MD 20854.

The major events characterizing CNS myelination in rodents consist of (1) the migration and proliferation of glial progenitor cells and their differentiation into oligodendrocytes expressing galactocerebroside, a myelin glycolipid, and (2) the subsequent expression by these oligodendrocytes of myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin associated glycoprotein (MAG), which will be incorporated into the myelin sheath. In the course of a demyelinating disease in mice induced by the A59 strain of mouse hepatitis virus, extensive remyelination occurs but it is not known whether the remyelinating cells regulate the synthesis of myelin gene mRNAs and proteins as developing oligodendrocytes do or whether these cells are derived from precursors identical to those present in the neonatal CNS. With the recent elucidation of surface molecules characteristic of progenitor cells and with the cloning and sequencing of myelin protein genes, it is now possible to approach these questions.

Dividing glioblasts were detected at 2 and 3 weeks post-inoculation (PI), when infected animals were pulsed with tritiated thymidine 2 hours before sacrifice. In order to correlate the presence and location of precursor cells and specific transcripts with proper myelin staining and inflammatory cell identification, we combined a series of techniques on frozen sections of optimally fixed spinal cord tissue of demyelinating animals. Demyelinating lesions, clearly delineated by Sudan black staining, contained inflammatory cells differentially stained by cresyl violet and the lesions also had increased immunostaining for glial fibrillary acidic protein. Adjacent sections were hybridized with single stranded DNA or RNA probes labeled with ^{35}S and specific for MAG, PLP or MBP transcripts. Binding of all probes was considerably decreased in the demyelinating lesions at 2 and 3 weeks PI, while transcripts appeared variably increased in the spinal cord tissue around the lesion at 3 weeks. There are four species of MBP transcripts which are differentially spliced during development. Exon 2 genetic information is characteristic of myelinating tissues during early development. Consequently, we performed Northern blot analysis on mRNA of spinal cord of demyelinating animals using an MBP exon 2 specific probe. Demyelinating animals showed a 50% increase in MBP exon 2 specific binding.

These observations suggest that demyelination and remyelination are associated with a series of proliferative and differentiational events which resemble, at least in part, the tightly regulated events of myelination during development. Topographic considerations suggest that factor(s) diffusing from lesioned areas to adjacent intact white matter may trigger these events.

35.2 COMPARATIVE FRACTURE FACE MORPHOLOGY OF MYELIN: IS PRIMATE MYELIN DIFFERENT FROM RABBIT AND RAT. R.G. Miller and L. Lalonde*. Dept. of Anatomy, The University of Calgary, Calgary Alberta Canada.

When myelin is prepared for freeze-fracture with the standard methods of glutaraldehyde fixation and glycerol impregnation, there is little difference between the P and E fracture faces. Major differences exist between these faces when tissue is frozen without fixation or glycerol impregnation. We have examined the face morphology of peripheral myelin obtained from the rat, rabbit, chicken and primate (Macaca nemestrina). Sciatic nerve was excised from anesthetized animal, briefly rinsed in phosphate buffered saline, cut into 1-2 mm pieces, mounted on gold Balzers specimen stubs, and frozen in partially solidified freon. Total time from excision to freezing was less than 2 min. Care was taken not to stretch the tissue.

In all species studied, lamellar myelin demonstrated two face morphologies. One face was smooth with few globular particles typic of other biological membranes. The other face contained a few globular particles but a high density of "string-shaped" particles which are packed so closely as to prohibit counting of the particles. By counting fracture planes from the Schwann cytoplasm and from the axon, the smooth face was determined to represent the P face while the face with stringy particles is the E face. The string-shaped particles were more distinct in the deeper lamellar myelin than in the juxta-cytoplasmic layer in all species.

The interperiod line of myelin represents the apposition of the extracellular surfaces of Schwann cell plasma membrane and has been shown to be a lanthanum penetrable gap. In fresh freezing of myelin, dendritic ice forms in aqueous compartments of the lamellar myelin. In the case of the rabbit, rat and chicken, this aqueous compartment containing dendritic ice was always found to be within the interperiod line. In contrast, in the monkey, dendritic ice was frequently found in the major dense line compartment which represents the apposition of the intracellular surfaces of the Schwann cell membrane.

Other species differences were noted in the axolemma and periaxonal Schwann cell membrane. In particular, clusters of bar shaped particles on the axolemma and hexagonal rosettes of large particles on the periaxonal Schwann cell membrane were present in the rat, but not in any other species studied. Clusters of small pleomorphic particles on the axolemmal P face seen in all other species studied may be analogous to the rosette seen in the rat. This work was supported by grants from NSERC and MRC.

35.3 DECREASED IRON LEVELS IN THE MYELIN-DEFICIENT RAT. J.R. Connor, B. Garber*, L. Potter*, and A. Oni*. Depts. of Physiology and Medicine, Geo. Washington Univ. Sch. of Med., Washington, DC 20037

Recent studies have demonstrated iron, transferrin (the iron mobilization protein) and a mRNA for transferrin in oligodendrocytes of the rat CNS. Furthermore, 80% of the iron content of the brain is reportedly in myelin. Also, iron uptake from plasma and the appearance of transferrin in the nervous system coincide with the onset of myelination. These observations strongly suggest a relationship between iron (mobilized by transferrin) and myelin formation and maintenance. The purpose of the study reported here is to determine the effect of dysmyelination on iron levels in the rat CNS. The animals used were a strain of Wistar rat with an X-linked recessive mutation which is characterized by dysmyelination (Csiza & deLahunta, Am. J. Pathol.,1979). These animals have ataxia and tremors and usually die within 3-4 weeks of birth. There is virtually no CNS myelin and most of the oligodendrocytes have ultrastructural abnormalities. Myelin-deficient (md) rats were matched with unaffected littermate controls and sacrificed between 25-30 days of age for either histological examination for iron (Perl's Reaction) or atomic absorption spectrophotometry (AAS). For the histology, animals were perfused with 10% neutral buffered formalin; followed by removal of the brain, cerebellum and pons. Sections were cut at 50 µm thickness and placed into Tris buffered saline. The Perl's reaction consisted of incubating tissue in 2% HCl and 2% Potassium Ferrocyanide (1:1) for 30 minutes followed by 15 minutes in 3-3'diaminobenzidine (DAB) to enhance the iron reaction product. Sections incubated in DAB only were used as control tissue. For AAS, the animals were perfused with saline, the tissue removed and frozen on dry ice and then stored at -20°C until time for assay. The tissue was prepared for assay by weighing it, lyophilzation and then digested with hydrogen peroxide. Iron samples were read on a Varian-Techtron Atomic Absorption Spectrophotometer. Only cells in the white matter of both the control and md rats contained iron reaction product. In the normal animals, the iron-positive cells had a round soma and short, sinewy processes. In the md rat, the somata were amorphous or rectangular and some of the processes had a broad initial segment. The cells in both cases are presumably oligodendrocytes, but ultrastructural confirmation is necessary. Quantitative analysis with AAS revealed a decrease of iron (meq/gm wet wt) in the brain (91.8% difference); pons and cerebellum (100%); liver (75.8%) and heart (33.3%) of the md rat compared to normal. The quantitative data demonstrate a severe lack of iron in the md rat which is not confined to the CNS. This latter observation suggests that uptake of iron may be effected in the md rat perhaps at the level of the transferrin receptor. JRC is supported by NS 22671 and 25 07 RR05359-23 (GWU).

35.4 OLIGODENDROCYTES IN DEVELOPING JIMPY MUTANT MICE: A QUANTITATIVE IMMUNOCYTOCHEMICAL ANALYSIS. M.S. Ghandour*, P.E. Knapp and R.P. Skoff (SPON: D. Michael). Dept. of Anat. and Cell Biol., Wayne State Univ. School of Med., Detroit, MI 48201.

The processes leading to the abnormalities in the CNS of jimpy mutant mice and the defects in cell differentiation and/or maturation are still not elucidated. To determine if either the oligodendrocyte differentiation or maturation is affected we analyzed the number of oligodendrocytes at different ages in tissue sections labeled for oligodendrocyte markers, and in primary cultures at light and scanning electron microscopic levels. We examined the cerebellum and corpus callosum of jimpy mice at 6, 10 and 15 days postnatally using the immunogold labelling techniques for galactocerebroside (GC). The cell surface labelled with gold particles was examined at scanning electron microscope level for GC. Brains from 6, 10 and 15 day old normal and jimpy mice were removed after perfusion with 4% paraformaldehyde in PBS with or without 0.1% glutaraldehyde, and then postfixed for 72 hrs at 4°C. 100 um vibratome thick sections were used for immunohistological study. Sections were incubated with monoclonal antibody to GC for 1hr at room temperature. Afterwards the sections were incubated for 2 hrs at room temperature with colloidal gold and peroxidase conjugated goat anti-mouse IgG. Living cells in culture from 5 day old cerebella and from newborn cerebral hemisphere were stained for GC and analysed at light and scanning electron microscope levels after labelling with gold particles and silver enhancement. The number of oligodendrocytes (GC+) in cerebellum and corpus callosum per section in normal animals at 6d of age are 75±18 and 80±22 respectively. This number increases to 122±20 and 137±16 at day day 10 and to 126±24 and 160±18 at 15 days respectively. No significant differences in the numbers of stained cells in jimpy cerebellum and corpus callosum at the same ages was observed. For jimpy cerebellum the number of cells is 65±18, 111±15 and 130±16 at 6, 10 and 15 days of ages respectively, while in corpus callosum the number of stained cells is 77±22, 126±16 and 154±24 at 6, 10 and 15 days respectively. Our data suggests that the differentiation of oligodendrocytes in vivo in the jimpy mutant occurs according to the normal developmental clock. The cells are able to express the different markers (MBP, CAII, and CNP). These differentiated oligodendrocytes are unable to reach maturation.

35.5 JIMPY MYELIN LACKS PLP AND HAS A DEFECT IN THE INTRAPERIOD LINE. I.D. Duncan, J.P. Hammang* and K.F. Jackson*. Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

The jimpy mouse, an X-linked myelin mutant, has been the subject of considerable recent molecular biological investigation. The localization of the proteolipid protein (PLP) gene to the X chromosome, and the subsequent finding of a mutation in this gene with the production of a PLP message which has a 70-74 base pair deletion, has focused interest on this major CNS myelin protein. The present study follows similar work which we have done with another X-linked myelin mutant, the myelin deficient rat, where we showed that the myelin produced was myelin basic protein (MBP) positive, yet PLP negative, with an abnormally compacted intraperiod line (Duncan, et al. PNAS, submitted). To compare these findings with jimpy, 10 jimpy mice at 16 days of age, and age matched controls were perfused with a modified Karnovsky's fixative (3) or mercuric chloride (2), or 0.1% glutaraldehyde (2), or immersion fixed in formalin (3), and the C_1-C_2 and L_1-L_2 spinal cord segments processed for embedding in epon, paraffin or vibratome sectioning. Plastic sections were stained with toluidine blue or etched and reacted with antisera to either MBP or PLP, and stained with the PAP technique. Paraffin and vibratome sections were similarly stained. The plastic sections were further trimmed for thin sectioning . Numerous myelinated fibers were seen throughout the spinal cord white matter. Immunocytochemistry showed that these fibers stained strongly for MBP yet were negative for PLP. This was consistent for tissue fixed and processed in the different ways noted above. EM examination showed that many fibers had thick, compacted myelin sheaths (up to 23 lamellae). However, higher power examination revealed an abnormal intraperiod line, with rare evidence of a double leaflet. This was in marked contrast to controls. These results add further weight to the role of PLP in the pathogenesis of the mutation but question the role that this protein plays in the formation and compaction of CNS myelin.

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35.6 DEMYELINATION IN A TISSUE CULTURE MODEL OF PERIPHERAL NERVE BY SERUM FROM PATIENTS WITH GUILLIAN-BARRE SYNDROME (GBS). C.L. Koski*, M.B. Clark, and S. Mane* Depts. of Neurology and Anatomy, Univ. of Maryland, Sch. of Med., Baltimore, MD 21201.

Using an assay that detects antibody (Ab) by fixation of the first component of complement this laboratory has previously shown that titers of Ab to peripheral nerve myelin (anti-PNM Ab) are elevated 6-56 times over controls in the serum of acute phase GBS patients, that anti-PNM Ab titers fell dramatically during the first 2 to 3 weeks following hospitalization, and this decline in anti-PNM Ab titer correlated with clinical improvement (Koski et. al. PNAS 82:905 1985). It has been suggested that the primary demyelination of peripheral nerve associated with GBS is immunologically mediated, although no specific mechanism of myelin destruction has been demonstrated. To study a possible role for anti-PNM Ab in demyelination of peripheral nerve, we compared serums from acute-phase GBS patients with high anti-PNM Ab titers (100-220 units/ml) to serums of the same patients during the recovery-phase (0-14 units/ml) and to serums of normal controls without disease (0-10 units/ml)for their ability to mediate complement dependent demyelination in a tissue culture system in which rat Schwann cells have myelinated dorsal root ganglion neurons.

Groups of well myelinated cultures were treated with heat inactivated (HI) serums diluted 10 fold in tissue culture media which contained ascorbic acid (50 ug/ml). Factor B depleted normal human serum (Bd-NHS), 20%, was used as a source of fresh complement in order to prevent activation of the complement cascade by the alternative pathway in the absence of specific Ab. Cultures, maintained in serum free media for 48 hrs, were incubated with experimental serums and observed after 6,12,24 and 48 hours. Myelin integrity was assessed by light microscopic observations of living and aldehyde fixed, Sudan black stained cultures. Only HI-serums from acute-phase GBS patients with high anti-PNM Ab titers in the presence of Bd-NHS caused demyelination in the cultures. Myelin breakdown occurred progessively. Swellings appeared along myelin internodes followed by segmentation of the internode into ovoids and smaller balls of myelin.Demyelination was obvious as early as 6 hrs in some cases and pronounced by 24 hrs. Nerve cells were not obviously affected by GBS serum. Neither HI - control or low titer GBS serum with Bd-NHS, GBS HI-serum alone or Bd-NHS alone caused demyelination or Schwann cell death. These studies, using a tissue culture model in which the effects of other cellular agents can be eliminated suggest that anti-PNM Ab in the serum of GBS patients can mediate demyelination of peripheral nerve and that in this in vitro system, the effect is dependent on the classical pathway of complement. (Supported by NIH-NINCDS grant# 2 P01 N 220022-04.

35.7 SUPPRESSION OF SCHWANN CELL PROLIFERATION IN CULTURE BY ETHANOL. F.A. Mithen, M.M. Wessels* and R. Birchem*. Dept. of Neurology, St. Louis Univ., St. Louis, MO 63110 and John Cochran Veterans Administration Medical Center, St. Louis, MO 63106

It is possible to treat dissociated embryonic rat dorsal root ganglia in culture to inhibit proliferation of all non-neuronal cells except Schwann cells. Neurons have been shown to elaborate a mitogenic stimulus to Schwann cells under these conditions. Groups of sibling cultures (10-15 cultures per group) were exposed to various concentrations of ethanol (0, 43, 86, or 172 mM) for 4 weeks. Cultures were assessed weekly in a blind fashion for evidence of Schwann cell proliferation by light microscopy. There was a 94% reduction in Schwann cell proliferation in the presence of 172 mM ethanol (P less than .001 by Fisher's one-tailed t-test with four separate trials), but not in lower concentrations. No obvious differences in neuronal morphology were observed among the various groups of cultures by light or electron microscopy.

These observations suggest that ethanol might interfere with Schwann cell proliferation in culture by one or both of the following means: (1) inhibit neuronal production of Schwann cell mitogens or (2) impede Schwann cell response to neuronally produced mitogens. Investigation of these possibilities in culture may provide insight into neuropathological mechanisms operative in the fetal alcohol syndrome or alcohol associated peripheral neuropathy in humans.

(Work supported by Veterans Administration)

35.8 TRANSFECTION OF SCHWANN CELLS WITH SV40 LARGE T ANTIGEN GENE UNDER THE CONTROL OF WILD TYPE AND SYNTHETIC METALLOTHIONEIN PROMOTER (MT-1). G. Tennekoon* and K. W. C. Peden* (SPON: G. McKhann). Department of Neurology and Department of Genetics and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The availability of immortal Schwann cell lines with properties identical to those of secondary Schwann cells would have obvious advantages for studying Schwann cell-axon interaction. Neonatal rat Schwann cells were transfected with SV40 large T antigen gene under the control of the wild type MT-1 promoter. The transfection efficiency was 0.01% and the cells that had integrated the foreign DNA were selected with G418. After single cell cloning, it was apparent that the cells expressed SV40 large T antigen, but the expression of this protein was not stringently regulated by the activity of the MT-1 promoter (by changing zinc concentration in the medium). Although large T antigen decreased by about 50% when zinc was eliminated from the medium, the transfected cells continued to express proteins and enzyme activities characteristic of untransfected cells, albeit at a reduced level. Moreover, the rapid doubling time (20 hr) also was maintained. Since it was likely that the continued expression of the large T antigen was due to basal activity of the MT-1 promoter, we attempted to provide more stringent regulation by using synthetic metallothionein promoters. The synthetic promoter contained either 4 or 5 metal regulatory elements (MRE) and a TATA box, but none of the sequences responsible for basal activity. Each synthetic promoter was ligated to either chloramphenicol acetyltransferase (CAT) or SV40 large T antigen gene. The wild type MT-1 promoter was much stronger than the synthetic promoter. After transfection, selection with G418, and single cell cloning, stably integrated lines were examined for large T expression. With both synthetic promoters in the presence of zinc, cells divided rapidly but they were contact-inhibited and had none of the features of a transformed cell. One clone containing the 4 MRE promoter demonstrated tight regulation of large T antigen expression as examined by indirect immunofluorescence and Western blot and lysis. Moreover, when zinc was eliminated from the medium these cells divided more slowly. Currently other cell lines are being examined for large T expression.

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35.9 MODIFICATIONS OF MORPHOLOGY AND ANTIGENIC EXPRESSION BY CULTURED, TRANSFECTED SCHWANN CELLS. T.J.Neuberger, J.Yoshino *+, G.H. DeVries° and C.J. Cornbrooks+. Dept. of Anat. & Neurobiol., UVM, Burlington, VT.; +Dept. of Psych., Colgate Coll., Hamilton, N.Y. and °Dept. of Biochem., VCU, Richmond, VA.

Differentiation of Schwann cells (Sc) during development or regeneration occurs in a sequentially ordered series of biochemical stages which normally proceed with exquisite precision. These stages are directly influenced by environmental factors and include interactions with soluble molecules as well as extracellular matrix (ECM) and other cells. One approach toward characterizing the molecules which regulate the progression of Schwann cell differentiation is to examine transfected cells. Accordingly, we have begun to study a transfected Schwann cell line established by utilizing a plasmid containing the SV40 large T oncogene regulated by the mouse metallothionein (MT-1) promoter (Trans. Am. Soc. for Neurochem.(1986) 17, 114). Transfected Schwann cells (tSc) were established on three substrates: glass, type 1 collagen and Matrigel, a mixture of ECM molecules. The cells were assessed for alterations in their morphology, rate of proliferation and the expression of known Schwann cell antigens. Indirect immunohistochemical methods were used to describe the localization of actin, vimentin, glial fibrillary acidic protein (GFAP), plasminogen activator and laminin or heparan sulfate proteoglycan and C4, two antigens expressed by Sc after neuronal contact.

Unlike pure populations of normal Sc which only maintain a bipolar to round morphology in vitro, tSc also assumed a multipolar or fibroblast-like shape on the three substrata. Moreover, tSc usually formed aggregates and fascicles which radiated from the cellular masses. The transfected cells continued to proliferate on the three types of substrates although those seeded on Matrigel divided at a reduced rate. While the cytoskeletal proteins, actin and vimentin, were both expressed by normal and tSc, the filamentous pattern was more apparent in the pleomorphic cells. Further, GFAP expression was prominent in the cell line but minimal in normal Sc. In comparison to normal Sc, laminin immunoreactivity was diminished and plasminogen activator immunoreactivity was increased in or on the transfected cells. Surprisingly, both the proteoglycan and the C4 antigen were expressed by aggregated tSc; whereas, normal Sc require axonal contact before expressing the same staining characteristics.

These observations demonstrate that tSc, which are suspended in an early stage (proliferation) of differentiation, assume a morphological shape and a partial antigenic repetoire which hallmark the more mature stages of Sc differentiation.

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35.10 cDNA CLONING OF SPECIFIC TRANSCRIPTS FROM A MOUSE CEREBELLAR ASTROGLIAL PERMANENT CELL LINE. +T. Rhyner *, ++ E. Lecain *, + J. Mallet *, and ++B. Pessac (SPON:B. Zalc). +Centre de Génétique Moléculaire, CNRS, 91190 Gif-Sur-Yvette, France. ++Centre de Biologie Cellulaire, CNRS, 94200 Ivry-Sur-Seine, France.

We have previously reported the derivation from 8 day post natal mouse cerebella of two permanent clonal cell lines with astroglial properties which might be the in vitro counterparts of the velate protoplasmic astrocytes (clone D19) and of the Golgi Bergmann epithelial cells (clone C8S) (Alliot, F. and Pessac, B. Brain Res. 306, 283-291, 1984). These two clonal cell lines originated from the same culture and have been spontaneously immortalized. These two astroglial clones provide for an excellent survival of embryonic cerebellar neuroblasts and furthermore clone D19 triggers the differentiation of the majority of these neuroblasts into granule cells (Pessac, B. and Alliot, F., Soc. Neurosci. Abstr., vol 12, Part 1, p. 397, 1986).

We then decided to use the techniques of molecular genetics, described by Rhyner T. et al. (J. Neurosci. Res., 16, p. 167-181, 1986), in an effort to isolate mRNAs that are specifically expressed in D19 as compared to C8S cells.

A subtracted cDNA library was constructed from cytoplasmic poly(A)$^+$ RNA of D19 cells. Single stranded cDNA was hybridized in liquid medium with an excess of C8S poly(A)$^+$ RNA. The unhybridized cDNA was then purified by hydroxylapatite chromatography, transcribed into a double-stranded form inserted into a plasmid and cloned in E. Coli.

This library was enriched about 12-fold for sequences selectively expressed in D19 cells, as 92% by weight of the cDNAs were eliminated by hybridization. The average size of the inserted sequences was about 200 bp.

4000 cDNA-clones were differentially screened with subtracted cDNA probes derived from D19 and C8S cells. The D19 cDNA-probe subtraction was carried out as above and the C8S cDNA-probe subtraction was performed with C8S poly(A)$^+$ mRNA in order to enrich this probe for low abundance sequences. The clones which only reacted positively with the subtracted D19 cDNA-probe were further analyzed by Northern-blot using poly(A)$^+$ RNA from D19 and C8S cells. 5 clones hybridized to transcripts selectively expressed in D19 relative to C8S cells while two clones were overexpressed in D19 cells.

The distribution of these mRNAs was studied by Northern-Blot analysis in various mouse tissues and at least one of them was found specifically expressed in the CNS.

35.11 **MOLECULAR CLONING OF A RAT BRAIN S100 ß cDNA.** D.C.Hilt[1] and D.Kligman[2]. ([1]Lab of Biochem. Genetics, NHLBI, [2]Lab of Cell Biology, NIMH, NIH, Bethesda Md. 20892).

We have previously shown that a Neurite Extension Factor (NEF) purified from bovine brain (PNAS, 82,7136-9) shows virtual protein sequence homology with a glial cell protein-S100 ß. NEF is a disufide bonded form of S100ß that stimulates neurite extension from dissociated chick embryo cerebral cortical neurons and murine neuroblastoma cells (Neuro-2A cells) in defined medium. In order to characterize the expression of NEF and S100 ß at the molecular level, we undertook the cloning of a cDNA for S100 ß/NEF. A 36 base oligonucleotide probe corresponding to a homologous segment near the C-terminal region of S100 ß and NEF was synthesized. This probe was used to screen a rat brain lambda gt11 cDNA library. Ten positive clones were plaque purified and further characterized. Clone 9.3 had the longest cDNA insert which was 1.7kb in length. This cDNA was mapped with restriction enzyme digestion and restriction fragments were subcloned into M13 and completely sequenced in both directions. The cDNA was also subcloned into a SP6/T7 promoter vector to generate single stranded probes which were used for Northern and Southern blot analyses. Northern blot analysis of rat brain, C6 glioma, and murine NS20Y neuroblastoma RNAs was performed. A broad single 1.7kb mRNA hybridizing species was detected in rat brain and C6 RNA, but not in NS20Y RNA. Southern blot analysis of rat genomic DNA after digestion with BamHI and EcoRI with this probe showed multiple hybridizing species. In order to determine if related but nonhomologous RNA species are present, RNAse protection studies are presently being performed. This full length cDNA probe is currently being used to study the regulation of S100 ß expression in C6 glioma cells.

35.12 MORPHOLOGICAL ASSESSMENT OF INTACT "PROTOPLASMIC" ASTROCYTES ISOLATED FROM RAT CEREBRAL CORTEX. A. K. Salm and K. D. McCarthy*. Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina, 27514.

The technique of bulk-isolating intact astrocytes (Farooq and Norton, 1978) is attractive to investigators as it allows biochemical and morphological analysis of individual cells. In such preparations we have consistently (18+ isolations) observed two morphologic extremes of glial fibrillary acidic protein immunoreactive (GFAP+) astrocytes which correspond to previous descriptions of "fibrous" and "protoplasmic" astrocytes. Intermediate forms are also often seen. Here we describe the protoplasmic phenotype observed in these preparations. We have isolated these cells from adult (110+ days) rat cerebral cortices and examined them with a number of corrolative approaches including fluorescence and peroxidase immunocytochemistry (ICC), phase contrast microscopy, stereo scanning electron microscopy (SEM), computer-assisted morphometry, and enzymatic variation during the isolation procedure. Unlike the morphological variety exhibited by other isolated astrocytes, the protoplasmic cells exhibit marked structural consistency both within and between preparations. This subtype is seen to have a spherical form with symmetrically projecting GFAP+ processes radiating from a central nuclear area. These processes branch extensively until subbranches are no longer discernable with fluorescence microscopy. Superimposed upon this infrastructure is velate material which, comparatively, immunostains only slightly with anti-GFAP. A number of processes often project beyond the spherical bounderies of these cells and terminate in endfoot-like profiles. When viewed with phase contrast microscopy the surface topography of these cells appears rough and highly convoluted. At high magnification with stereo SEM this is seen to be due to the presence of innumerable membraneous protrusions on the cell surface. Occasional thin processes are visible coursing over the cell surface. Double immunostaining of these preparations with antibodies to the 200K neurofilament (NF) protein, although resulting in immunopositive processes elsewhere in the cell fractions, show only sporadic NF+ fibers associated with the GFAP+ protoplasmic cells. Computer-assisted morphometry of 67 of these cells determined the average area to be $\sim 1709 \pm 500\ \mu m^2$ ($\bar{X}$ + S.D.). Assuming a sphere-shape, volume estimates indicate that processes of these cells distribute within a volume of neuropil of $\sim 54{,}882 \pm 24{,}822\ \mu m^3$ cell. We have altered the enzymatic conditions of the isolation procedure and have observed a persistent appearance of these cells in preparations obtained after dissociation with trypsin, dispase II, or no enzyme. From these assessments we conclude that we are able to obtain isolated protoplasmic astrocytes in these preparations which resemble those seen with in situ HRP and Lucifer Yellow injections, Golgi impregnation and GFAP ICC of tissue sections. These preparations will prove useful in future studies of protoplasmic astrocyte structure and function. (Supported by NIH grant #NS20212).

35.13 STRUCTURAL CHANGES OF ASTROGLIAL MEMBRANES IN VITRO. J. H. Tao-Cheng, J. Bressler and M. W. Brightman*.
Lab. of Neurobiology, NINCDS, NIH, Bethesda, MD 20892.

Astrocytes have characteristic orthogonal arrays of particle assemblies in the P fracture face of their plasma membranes. These assemblies are distributed heterogeneously in vivo, with distinctly localized high concentrations (300-500/μ^2) in the astroglial membranes facing the blood vessels and the pia. To test whether the apposing blood vessels or the pia membranes have an effect on the concentration of the astroglial assemblies, astrocytes from 2-3 day old rats were cultured alone or co-cultured with bovine brain endothelial or rat meningeal cells and examined with freeze-fracture electron microscopy. The possible effect of the secondary messenger, cyclic-adenosine monophosphate (cAMP), on the astroglial assemblies was also studied.

Secondary, enriched astrocyte cultures (McCarthy and de Vellis 1980) have much lower concentrations of assemblies (1-10/μ^2) than the primary cultures (5-60/μ^2), and therefore, serve as a better baseline for monotoring any increase of assemblies brought about by various factors. The assembly concentration in solo, secondary astroglial cultures increased gradually over time up to 23 days in culture, the longest time the cultures were maintained. Co-culturing the secondary astrocytes with brain endothelium or meningeal cells consistently increased the assembly concentration at least 3-6 fold at all time points between 6-17 days. Bovine pulmonary artery, aortic endothelium and a tracheal derived cell line did not increase assembly numbers in astrocytes. However, fibroblasts from subcutaneous tissue of 2-3 day old rat did increase the astroglial assembly concentration ~ 5 fold. This result is not surprising because (a) fibroblasts are present in the adventitia of blood vessels larger than capillaries and (b) meningeal cells are a form of specialized fibroblasts. Thus, brain endothelial cells, meningeal cells and fibroblasts consistently induce an increase in astroglial assembly concentration.

cAMP may be an important second messenger for the increase in assemblies. Forskolin, which at 50 µM increases cellular cAMP levels in astroglia, augmented assembly concentration in solo astroglial cultures by 3-4 fold after 18-24 hours of incubation, and by ~15 fold after 3 days of incubation. In addition, the beta-agonist isoproterenol (10 µM) which also raises cAMP levels in astrocytes, induced an increase in assembly concentration and this induction was augmented if 3-isobutyl-1-methyl-xanthine, which inhibits cAMP degradation, was included. These results suggest that cAMP may be linked to the presence of assemblies in the astroglial plasma membrane. The consistent and substantial increase in astroglial assembly concentrations by these agents may be useful in isolating the assembly protein in order to ascertain its function.

35.14 **ROLLER TUBE EXPLANT CULTURES FOR STUDIES OF CNS PATTERN FORMATION.** **Dennis A. Steindler and Kristine L. Harrington*. Dept. of Anatomy and Neurobiology, Univ. Tenn. Memphis, 38163.**

One of the shortcomings of in vivo studies of nervous system pattern formation is establishing causality in the sequence of events leading to the development of functional circuitries. Attempting to uncover conditions responsible for the sorting of a given population of CNS neurons into a geometrically ordered functional unit requires examination of a myriad of extrinsic factors (e.g. afferents) which might provide cues to pre-aligned neurons. We have set out to use the roller tube explant system, originally described by Gähwiler (Neurosci., 11:751, 1984) for studies of the anatomy and physiology of identified neurons in slices, to determine intrinsic versus extrinsic factors involved in CNS pattern formation. Our initial focus is on the maturation of glial elements in slice cultures of neonatal mouse CNS. Previous studies performed in vivo (Cooper, N.G.F. & D.A. Steindler, Brain Res. 380:341, 1986; Steindler and Cooper, Dev. Brain Res. in press) implicate glia and associated glycoconjugates during pattern formation in numerous CNS areas. Brains from neonatal mice were rapidly sectioned at 400 um in the parasagittal or tangential planes. Slices, collected in Geys balanced salt solution (BSS), were then transferred to a plasma clot on a cover slip and cultured in test tubes in a medium made up of horse serum (25%), basal Eagle medium (50%), and Hank's BSS (25%) supplemented with glucose. The test tubes were rotated in a roller-drum (Bellco) rotated at 10 rev/hr in an incubator for up to 12 days. Parasagittal slices containing numerous, synaptically-related brainstem, midbrain, and forebrain structures were viable throughout the culturing period. Fixed slices processed for immunocytochemistry using antibodies to glial fibrillary acidic protein (GFAP) or vimentin show a differentiation of glial elements within explants that mimicked that observed in vivo. Slices cultured on postnatal day 2 and fixed 9 days later reveal immunoreactive cortical astrocytes with laminar organization that resembled that observed in vivo on postnatal day 11. In addition to an apparent normal maturation process of glial elements and cortical laminar arrangements of neurons in these explants, numerous glial boundaries were observed between various structures as seen during postnatal development in situ. These boundaries are made up of bundles of glial elements including radial fibers and astrocytes. The findings indicate that slice cultures can be used to study the development of CNS patterns with little or no compromising of normal morphogenetic processes.

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35.15 ASTROCYTE-INDUCED DETACHMENT OF CULTURED NEURONS. PREVENTION AND LOCALIZATION OF THE ACTIVITY. G.M. Gilad, V.H. Gilad*, D. Dahl and A. Bignami. Department of Pathology, Harvard Medical School, Spinal Cord Injury Research, Veterans Administration Medical Center, West Roxbury, MA 02132 and The Center for Neurosciences, The Weizmann Institute of Science, Rehovot, Israel.

We have recently observed that detachment of neurons in primary embryonic CNS cultures is caused by the growing co-cultured astrocytes. Neurons degenerate secondary to their detachment from the substratum and this is probably caused by an activity associated with the astrocyte membrane. The present study demonstrates that in such primary culture systems, peripheral sensory neurons and motoneurons which project outside the CNS, as opposed to intrinsic neurons of the CNS, remain attached and closely associated with the growing astrocytes. Detachment of central neurons can be prevented by a variety of drugs which prevent the proliferation of astrocytes. Several types of proteinase inhibitors did not affect the process of detachment, while among several types of glycosaminoglycans only heparan sulfate could reversibly inhibit neuron detachment. Antiserum to membranes of cultured astrocytes that can block the astrocyte-induced detachment of central neurons, was localized immunocytochemically on surface membranes of astrocytes. These in vitro findings imply that in the CNS astrocytes may express a membrane-associated activity which can disturb the contacts of growing or regenerating central axons with their growth substrata.

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36.1 DENDRITIC TRANSPORT OF RNA: AN ENERGY DEPENDENT PROCESS. L. Davis, C. Dotti*, G. Banker, and O. Steward. Department of Neuroscience, University of Virginia, Charlottesville, VA 22908, and Department of Anatomy, Albany Medical College, Albany, NY, 12208.

We have recently described a dendritic transport system for the selective delivery of RNA into dendrites using hippocampal neurons grown in culture (Davis, et al., Soc. Nesc. Abst. 12;1572, 1986). Dendritic transport of RNA occurs at a rate of around 0.5 mm/day, and the rate is linear over time suggesting an active transport system. To further investigate whether dendritic transport is an active energy-dependent process we evaluated whether transport was blocked by metabolic poisons in the same way that axonal transport is.

Hippocampal neurons were prepared as described previously and plated onto poly-lysine coated glass coverslips (Bartlett & Banker, J. Neurosci. 4;1944-1953, 1984). Cultures were incubated with 3H-uridine for 1 hour, rinsed in unlabelled medium to remove radioactivity and then transferred into either a medium containing an excess of cold uridine, or a medium containing azide (10mM) or DNP (10mM). After 6 hours, the cultures were fixed with paraformaldehyde and processed for autoradiography. In both azide and DNP-treated cultures as well as in control preparations (no metabolic poisons) the bulk of the labelling was found over the nucleus and cell body. In control preparations, the dendrites were labelled, indicating transport of RNA. In contrast, cultures that had been treated with azide or DNP exhibited only a small amount of labelling over proximal dendrites.

Some cultures were pulsed with 3H-uridine, incubated with azide for six hours, and then stained with fluorescein diacetate, a marker for viable cells. Fluorescein diacetate stained cells also showed very little transport of labelling out of the cell body. To assess viability in a different way, cultures were incubated with azide followed by a 30 minute pulse application of 3H-leucine; these cultures showed incorporation of label over the cell bodies and dendrites. These results indicate that cells were viable following treatment with metabolic poisons, yet the transport of RNA was inhibited. Taken together, these results suggests that the selective dendritic transport of RNA is an energy-dependent process, similar to that of fast axonal transport. Supported by NIH NS23094 to G.B., O.S., and C.D. and predoctoral fellowship NIH NS07199 to L.D.

36.2 TRANSSYNAPTIC TRANSFER OF THE BINDING FRAGMENT OF TETANUS TOXIN. P. S. Fishman, J. M. Savitt, and D. R. Carrigan (SPON: S. R. Max). VA Research Labs, Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Both tetanus toxin and its 45 kD binding fragment (C-fragment) are transferred extensively from retrogradely labeled motorneurons to presynaptic terminals in the spinal cord. We have used immunocytochemistry and enzyme histochemistry on both the light microscopic and ultrastructural level to elucidate the cellular basis for this unique property. C-fragment (CALBIOCHEM) was injected intramuscularly into the forearm or tongue of mice. Animals were sacrificed at intervals from 1 to 21 days, and tissue processed for immunocytochemistry using antisera raised in rabbits against purified C-fragment. Within two days following injection, there is extensive movement of label from motorneurons to the adjacent neuropil. Over the next several days, there is a slow expansion of the region of labeled neuropil around the originally labeled motorneurons. The neuropil is uniformly labeled without distinct labeled neurons seen with other transsynaptic labels such as WGA-HRP. Supraspinal neurons were rarely labeled. A second series of animals were injected with C-fragment conjugated to horseradish peroxidase. Spread of C-fragment-HRP through the neuropil after intramuscular injection, as demonstrated by enzyme histochemistry, is identical to that of native C-fragment detected with immunocytochemistry. C-fragment-HRP was also localized on a subcellular basis. Motorneurons had many labeled vesicles characteristic of uptake by endocytosis. Virtually all axosomatic synapses onto motorneurons were labeled within two days after injection. Synaptic clefts were densely labeled, as well as both post and presynaptic membranes. Synaptic vesicles of presynaptic terminals also were frequently labeled, regardless of whether terminals contained round or eliptical vesicles. Non-synaptic vesicles of presynaptic terminals were only infrequently labeled. Membrane not adjacent to synaptic terminals rarely showed HRP reaction product. It appears that C-fragment accumulated at the neuromuscular junction is selectively directed to synaptic regions of the motorneuron. It is intercalated into the postsynaptic membrane where it is able to pass into the synaptic cleft and to membrane of the presynaptic terminal. C-fragment remains in the presynaptic terminal where it is incorporated into synaptic vesicles. Little C-fragment in the synaptic regions appears available for further retrograde transport to the cell bodies of presynaptic neurons. C-fragment, like tetanus toxin, has a high affinity for its receptor, which is concentrated at synapses. The transfer of C-fragment from post to presynaptic structures may refect the transfer of a normal synaptic component.

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36.3 LOSS OF MATERIAL FROM THE RETROGRADE AXONAL TRANSPORT SYSTEM. R.E. Snyder* (SPON:R.S. Smith). Dept. of Appl. Sci. in Med., Univ. of Alberta, Edmonton, Canada T6G 2G3.

It is well-known that protein is lost (or deposited) from the rapid anterograde transport system. Loss from the retrograde system has as yet not been investigated. Knowledge of whether or not loss occurs is important to our understanding of the purpose of retrograde transport, however, is difficult to obtain using conventional approaches such as ligature techniques. We have developed a method for the continuous monitoring of a pulse of anterogradely moving radiolabelled protein and, following reversal at a lesion, the retrograde movement of a second pulse of radiolabel (Snyder, R.E., J. Neurobiol., 17,637, 1986). We report here upon the loss of radiolabel from the retrogradely moving pulse.

A pulse of ^{35}S-methionine labelled material was created in a 50-55 mm length of amphibian sciatic nerve maintained *in vitro* at 23.0±0.2°C. Continuous observations were made for 18-22 h of the anterograde movement of the pulse, and, following reversal at a ligature, the retrograde movement of a fraction of the anterograde pulse by placing the nerve in contact with a position-sensitive detector of ionizing radiation. By monitoring the progress of both the anterograde and retrograde pulses it was possible to determine their transport rates and, using these rates, estimate the ratio of the retrogradely to anterogradely moving label which traversed each segment of the nerve. This ratio should increase by a factor of $1/[(1-a)(1-r)]$ for each segment of nerve moved in the distal direction where a(r) is the fraction of label lost (deposited) to each segment from the anterograde (retrograde) pulse. By comparison of the plateau of activity in each segment following the passage of the anterograde pulse to the activity in the pulse it was possible to determine an upper limit to the fraction a.

Seven nerves were studied. The retrogradely to anterogradely moving label which traversed 4-6 contiguous 3.18-mm segments of nerve which contained no terminating axons was estimated, as well as the fraction of label lost from the anterograde pulse. It was found that $a \leq 0.056 \pm 0.003$(±SEM) and $r \geq 0.049 \pm 0.014$, indicating that endogenous protein is lost from the retrograde transport system.

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36.4 SLOW TRANSPORT OF SCa AS SOLUBLE COMPONENTS SHOWN BY BEADING PARTITIONING OF NERVE FIBERS. Sidney Ochs and Ralph Jersild Jr. Dept. of Physiology/Biophysics and Dept. of Anatomy, Indiana Univ. School of Med., Indianapolis, IN 46223.

The slow transport of tubulin and neurofilament triplet proteins in nerve fibers at the SCa rate of 0.25-2.0 mm/day has been proposed in the Structural Hypothesis to represent an outward movement of microtubules and neurofilaments in assembled form following the incorporation of their subunits into these cytoskeletal organelles in the cell bodies. Alternatively, in the Unitary Hypothesis, the tubulin and neurofilament triplet subunits are considered to be transported down into the fibers as such, and to turn-over in their respective organelles within the fibers.

To differentiate between the two possibilities, beading was used to partition the filamentous cytoskeleton from the soluble components in the fibers of the cat L7 dorsal roots at times from 7-30 days after injection of the precursor ^{3}H-leucine into the L7 dorsal root ganglia. After slow transport had carried labeled proteins out into the roots at rates corresponding to SCa, the roots were removed, lightly stretched to bead them, fast-frozen and freeze-substituted to hold the beading in place, and the tissue prepared for autoradiography. Beading is seen as a series of constrictions interspersed between relatively normally expanded regions along the nerve fibers. In the constrictions the microtubules and neurofilaments are found to be closely packed into a cross-sectional axonal area as small as 5% that of the normal myelinated fiber (Neuroscience, in press).

In the autoradiographs of the beaded fibers the density of radioactive grains per unit area over the constrictions was very small while over the nonconstricted regions the density was high at times corresponding to SCa rates. This is in accord with the labeled proteins carried down by SCa being present mainly as soluble proteins able to move with the axoplasmic fluid as it and other soluble components are squeezed away from the cytoskeleton left remaining in the beading constrictions.

A small increase in the grains over the constrictions in the beaded fibers was seen later at 3-4 weeks, times too long for the SCa proteins to be carried down within the cytoskeleton at that rate. This later increase is indicative of a slow uptake of the labeled precursors into the microtubules and neurofilaments.

The findings indicate that the carrier which accounts for fast transport could serve also for slow transport if it allows for an early drop-off of tubulin and neurofilament subunits to provide for their turn-over in the stationary cytoskeletal organelles.

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36.5 THE OCCURRENCE OF mRNA AND BIOLOGICALLY ACTIVE POLYRIBOSOMES IN THE SQUID GIANT AXON A. Giuditta, C. Perrone Capano, E. Menichini[1], E. Castigli[1], A. Gioio[2], R. Martin[3], and B.B. Kaplan[2] Dept. Gen. & Environ. Physiol., Univ. of Naples (Italy); [1]Inst. Cell Biol., Univ. of Perugia (Italy); [2]Dept. Psychiatry, Univ. Pittsburgh (USA); [3]Sec. Elec. Microsc., Univ. Ulm (FRG)

Axons and nerve terminals are unique subcellular structures of nervous tissue that are widely believed to lack an endogenous protein synthetic capacity. In accord with this view, the proteins synthesized in the isolated squid giant axon are believed to derive entirely from ada-axonal glia cells. We have previously reported, however, that axoplasm extruded from isolated squid giant axons contains an RNA fraction that can program protein synthesis in a rabbit reticulocyte cell-free translation system (Giuditta et al., Neurochem. Intern. 8:435, 1986). Here we report that the squid giant axon contains a diverse population of poly(A)$^+$mRNAs and biologically active polyribosomes.

Total axoplasmic RNA obtained from the giant axon of the squid, Loligo paelii, and was used as a template for cDNA synthesis in standard reactions containing AMV reverse transcriptase and oligo(dT) as primer. The cDNA synthesized was heterogeneous in size with most fragments larger than 450 nucleotides (nt). Results of a RNA-cDNA hybridization analysis indicate that the sequence complexity of axoplasmic RNA is approximately 3.1 x 10^5 nt, a value sufficient to code for 200 poly(A)$^+$mRNAs averaging 1500 nt in length. The presence of axoplasmic poly(A)$^+$RNA was also determined by in situ hybridization histochemistry. In these experiments, glutaraldehyde-fixed, paraffin-embedded tissue sections of stellate ganglia and nerves were hybridized to [^{3}H]poly(U). Upon autoradiography, a significant number of silver grains were visualized in the giant axon. This signal was decreased substantially by pretreatment of the tissue with RNase A. In control experiments, little hybridization was observed when [^{3}H]poly(A) was used as a probe. Additionally, isolated giant axons were incubated in filtered sea water containing [^{35}S] methionine and polyribosomes isolated from detergent-treated axoplasm by sedimentation through 2.0 M sucrose. The resulting pellets were displayed on linear sucrose gradients, and the alkali-resistant, acid-precipitable radioactivity of each gradient fraction was determined. Importantly, radioactive polysome profiles were consistently obtained from axoplasm. The radiolabeled amino acid associated with axoplasmic polyribosomes was sensitive to disruption by treatment with both EDTA and RNase. Consistent with these findings, preliminary results of an electron microscopic study, reveal dense, osomophilic structures resembling polyribosomes in axons located in both the giant fibre lobe and stellate ganglion. These images appear especially abundant beneath the neurolemma and postsynaptic elements of axon-axonic synapses.

36.6 IMMUNOCYTOCHEMICAL LOCALIZATION OF KINESIN IN THE SQUID GIANT AXON. T.P.O. Cheng, B. J. Schnapp, M.P. Sheetz, and T.S. Reese, Laboratory of Neurobiology, NINCDS, NIH, at MBL, Woods Hole, MA 02543.

We have examined the distribution of kinesin in the squid giant axon and stellate ganglion by post-embedding immunocytochemistry. Araldite sections of either fixed (aldehyde/OsO_4) or quick-frozen and freeze-substituted (OsO_4/acetone) axons or stellate ganglia were etched with potassium ethoxide and sodium meta-periodate for 5 min before incubation with various dilutions of serum from rabbits immunized with affinity-purified kinesin or a monoclonal antibody against the 110 kD subunit of kinesin; distributions of antibody were then visualized by indirect immunofluorescent or immunogold labeling. Fluorescent label in the chemically fixed specimens was in numerous discrete small spots near or at the surfaces of small cytoplasmic organelles, apparently inside of some other larger organelles, and in numerous discrete patches in the cytoplasm. The spotty labeling near or at the surfaces of organelles did not coincide with parallel labeling done with an antibody to tubulin. In contrast, the distribution of fluorescent label in the freeze-substituted axoplasm was uniform and diffuse, and labeling near organelles was not apparent. However, at the ultrastructural level, anti-kinesin immunogold in the freeze-substituted axoplasm was concentrated in small spots at the surfaces of small cytoplasmic organelles, and near or at axoplasmic subdomains where small organelles cluster in the vicinity of microtubules. The immunogold was also seen, though only occasionally, in the narrow gaps between organelles and microtubules. Examination of the distribution of immunofluorscence or immunogold in continuous serial sections suggested that kinesin is distributed in discrete spots at the surfaces of organelles instead of enveloping them in a continuous layer. Control plastic sections treated with normal serum or antiserum pre-absorbed with kinesin were unlabeled by immunofluoresce and immunogold. Our findings indicate that some of the kinesin in the squid axoplasm is associated with organelles, an interpretion which is consistent with previous biochemical evidence that kinesin binds to organelles. Our findings also provide further evidence that there are two pools of kinesin in the axon: a cytoplasmic pool diffusely distributed throughout the organelle-rich domains of axoplasm as well as the discrete fraction which is bound to organelles.

36.7 DIFFERENT NEUROFILAMENT DENSITIES SHOW IDENTICAL RANDOM DISTRIBUTIONS IN SIDE-BY-SIDE AXONS.
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We have studied the space-filling properties of neurofilaments (NFs) in two different types of axons that each have a large NF content -- somatic motor axons and parasympathetic axons of the oculomotor system. These axons originate in the oculomotor nucleus, and they course together (side-by-side) through the oculomotor nerve; nonetheless, the packing density of the NFs in the parasympthetic axons is 30% greater than it is in the somatic motor axons. Visual inspection of electron micrographs of parasympathetic and somatic motor axons suggested that the NFs are uniformly distributed in both types of axons. To test this possibility, we quantified the distributions of the NFs, and then we compared those distributions with ideal randomly generated (Poisson) NF distributions. Axons in 28-day-old chickens were fixed by perfusion with 4% paraformaldehyde, 2% glutaraldehyde, 0.005% CaCl2, in 0.1M cacodylate buffer, pH=7.4, and post-fixed for 1hr in 1% OsO4, and then dehydrated and embedded in Polybed 812. Areas of cross-sectioned axons (from more than 250 axons of each type) were measured with a Vanguard Digitizer and NF densities were determined by placing an acetate sheet with 72 hexagonal areas (0.93 cm2, spaced 1.3 cm apart) over 58000x prints. (Areas containing more than 10% membranous organelles or in which NFs were not cut in cross section were excluded from the analyses.)

NFs are more densely packed in the parasympathetic axons (average density X=10.8NF/hexagon, n=2877NF) than in the somatic motor axons (X=8.6NF/hex, n=3512NF). Histograms of the NF distributions for the two types of axons were compared with ideal Poisson distributions. The Poisson curves closely matched the actual distributions of NF densities in both the parasympathetic axons (p>0.99, chi-square test) and somatic motor axons (p>0.95, chi-square test). This indicates that, although the average densities differ, NFs are similarly and homogeneously distributed in the cross-sectional planes of both types of axons. A homogeneous Poisson distribution can be produced by intracellular mechanisms that distribute NFs via simple and essentially random forces within the axonal cross-section. Thus, in these axons, the NF polymers behave as if they are independent "molecules," organized by simple and non-specific stochastic forces. On this basis, we propose that more constraining forces, such as interactions between NF sidearms, are relatively weak and that in vivo the NFs are not extensively crosslinked irto a rigid network.

36.8 SUBCELLULAR LOCALIZATION OF AXONALLY TRANSPORTED MACROMOLECULES BY HIGH RESOLUTION IMMUNOELECTRONMICROSCOPY. M. Ellisman, T. Deerinck*, P. Taylor¶. Lab. Neurocytology, Dept. Neurosciences and ¶Dept. Pharmacology, UCSD, La Jolla, CA 92093.

Axoplasmic membrane systems have been examined with respect to their movement in axonal transport (Ellisman & Lindsey, J. Neurocytol. 12:393, 1983). Distinctions are made between vesicles, vesiculo-tubular structures, the axoplasmic reticulum, dense cisternae, and multilamellar and multivesicular bodies. To further define the roles of each, the precise locations of specific macromolecules are being determined. At issue is whether separation of different transported molecules is maintained in the axon. Since antigen concentration is expected to be low and the experiments require precise localizations we have employed the most sensitive and discerning immunolabeling techniques available. Our progress toward both localization of the NaK ATPase and acetylcholinesterase, representing macromolecules components transported at different rates, as well as an adaptation of ultrathin-frozen section labeling technologies will be described. A battery of polyclonal antibodies with demonstrated specificities for NaK ATPase and acetylcholinesterase have been used. NaK ATPase antibodies (which recognize alpha and alpha+ forms and the beta subunit [439-2, Ariyasu et al., J. Neurosci. 5:2581, 1985]), localizes the antigen to discrete vesicles and vesiculo-tubular structures in axons from rat trigeminal nerve. No staining of the axoplasmic reticulum, dense cisternae, multilamellar or multivesicular bodies was observed with this antibody. A subpopulation of vesicles and vesiculo-tubular structures within 50μ of the nodes of Ranvier exhibit a high level of staining. Antibodies to guinea pig NaK ATPase that reacts predominantly with the alpha+ form of NaK ATPase (GP-17, McDonough & Schmitt, Am. J. Physiol. 248:247, 1985; Ariyasu & Ellisman, in press) stains similarly. Antibodies to acetylcholinesterase (Lee et al., J. Biol. Chem. 257:12283, 1982) that recognize both the hydrophobic dimer and the asymetric form of the enzyme (and thus both rapidly and slowly transported varieties) labels the axoplasmic reticulum. To enable this level of localization modifications of ultrathin cryosectioning and labeling procedures were required. Technical refinements include: Cryoprotection and freezing of paraformaldehyde fixed tissue to minimize the formation and size of ice crystals. After immunolabeling a glutaraldehyde secondary fixation and OsO_4 incubation is followed by UA staining and re-embedded in a thin matrix of LR white. This results in intra-axonal membrane systems that are preserved, delineated with great detail, and specifically labeled. Thus with technical enhancements and specific antibodies we have determined that these different axonally transported macromolecules are located in differing membrane systems.

36.9 VISUALIZATION OF MITOCHONDRIA IN AXOPLASMIC TRANSPORT.
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Visualization of mitochondria during axonal transport was accomplished using specific fluorescent labelling. We stained cultured cells from dorsal root ganglia of mice with such vital staining dyes as pinacyanol, neutral red and rhodamine. Rhodamine 123 was especially useful for specific fluorescent labelling of mitotochondria without cytotoxicity or phototoxicity. Cultured cells grown on coverslips were incubated with rhodamine 123 (10 ug/ml) for 5 min in a 5% CO_2 incubator at 37°C. The coverslips were rinsed twice and the cells were observed under a fluorescence microscope. Overall movements of mitochondria vary from the axon to the axon. The flow rate of the mitochondria was on the average one mitochondrion per some ten seconds. At any time, more than half of the mitochondria (density ≈ 1 mitochondrion per 1 um length of the axon) were observed to move in both anterograde and retrograde directions in a jerky, stop-and-go fashion.

The instantaneous velocity of the transport was 0.55 ± 0.11 um/sec in the anterograde direction, and 0.60 ± 0.10 um/sec in the retrograde direction. These velocities were compared with velocity histograms obtained with a non-specific light microscope technique: contrast enhancement Nomarsky optics. The histograms of both the anterograde and retrograde directions appeared to be composed of slow and fast components. A single slow component for both directions corresponded to the transport of mitochondria. In contrast, the fast component of the anterograde transport corresponded possibly to the transport of storage vesicles or smooth membranes, while the fast component of the retrograde transport corresponded possibly to the transport of prelysosomal organelles.

This new technique appears to offer a reliable method of studying both the normal and pathological aspects of mitochondrial transport in the axon.

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36.10 EFFECT OF PARATHYROID HORMONE (PTH) ON FAST AXONAL TRANSPORT: SPECIFICITY OF BIO-ACTIVE FRAGMENTS ON MYELINATED AND UNMYELINATED AXONS.
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Parathyroid hormone (PTH) has been demonstrated to increase fast axonal transport speed of organelles in both the anterograde (antero) and retrograde (retro) directions as observed by differential interference contrast optics and computer image processing techniques [Breuer AC and Atkinson MB: Neurology 37 (Suppl 1): 187, 1987]. The increases occur with topical application of PTH to mammalian axons in in vitro experiments. A similar response is seen in rats following continuous infusion of PTH with Alzet mini-pumps. Response to the application of PTH by axons in vitro occurs within minutes. To evaluate the species specificity of the PTH effect on myelinated and unmyelinated axons, either human PTH (synthetic fragment, 1-34) or rat PTH (synthetic fragment, 1-34) was applied to excised rat sciatic nerve axons at a concentration of 0.025 µg/ml. All other variables (pH, buffer, temperature, etc.) were kept constant. Resulting speed distributions were compared using the Kolmogorov-Smirnov test. Control mean speeds for myelinated axons were antero = 1.49 ± 0.04 µm/sec (n = 88) and retro = 1.81 ± 0.03 µm/sec (n = 317). Control mean speeds for unmyelinated axons were antero = 1.44 ± 0.03 µm/sec (n = 60) and retro = 1.85 ± 0.02 µm/sec (n = 303). Application of either rat or human PTH to myelinated axons induced anterograde increases of 24.2% (1.85 ± 0.04 µm/sec, n = 156, $p < 0.01$) and 17.4% (1.75 ± 0.07 µm/sec, n = 48, NS) and retrograde increases of 23.2% (2.23 ± 0.02 µm/sec, n = 454, $p < 0.001$), and 13.2% (2.05 ± 0.03 µm/sec, n = 225, $p < 0.001$), respectively. Application of either rat or human PTH to unmyelinated axons induced no significant changes in the anterograde transport [1.47 ± 0.03 µm/sec (n = 104) and 1.52 ± 0.04 µm/sec (n = 70) respectively] or in the retrograde transport [1.94 ± 0.02 µm/sec (n = 300) and 1.75 ± 0.02 µm/sec (n = 369) respectively]. Thus axonal transport in myelinated axons is responsive to the application of either rat or human PTH whereas unmyelinated axons are unaffected. This PTH-induced alteration of fast axonal transport suggests the presence of PTH receptors on myelinated axons (which may alter transport directly or indirectly) and the absence of such receptors on unmyelinated axons. These results imply the existance of an axonal transport modulator selective for functionally different axon types. (Supported in part by a grant from the Whitaker Foundation).

36.11 EFFECT OF PARATHYROID HORMONE (PTH) ON FAST AXONAL TRANSPORT: TIME COURSE OF RETURN TO NORMAL FUNCTION.
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Previous results from this laboratory have demonstrated an increase of mean axonal transport speed after treatment with parathyroid hormone (PTH, Bovine Fragment 1-34, Synthetic) on both anterograde (Antero) and retrograde (Retro) transport as analyzed by differential interference contrast optics and computer image analysis techniques. The PTH effect was found in both in vitro [Breuer AC and Atkinson MB: Neurology 37 (Suppl 1): 187, 1987] and in vivo (unpublished results) experiments on rat sciatic nerve. These changes could result from long-term structural (anatomic) changes in the axonal transport machinery or short-term, functional (physiologic) changes by either direct action of the PTH or indirect action through an intermediary (Ca++, cAMP, etc) as suggested by the immediate effect of the in vitro experiments. To assess the mode of action, Alzet mini-osmotic pumps (Model 2001) were implanted in rats with a dosage of PTH known to induce speed increases (2.5 units PTH/hour). The pumps were removed after one week, the animals sacrificed and transport speeds in the sciatic nerve analyzed at four time periods. Resulting speed distributions were compared using the Kolmogorov-Smirnov test. The speed found immediately after pump removal in the antero transport was increased from 1.47 ± 0.03 µm/sec (n = 148) in controls to 2.14 ± 0.04 µm/sec (n = 122) with PTH ($p < 0.001$). Retro transport was increased from 1.82 ± 0.02 µm/sec (n = 620) in controls to 2.71 ± 0.03 µm/sec (n = 321) with PTH ($p < 0.001$). At 6 hrs the antero speed increase had been reduced by 41.8% (1.86 ± 0.03 µm/sec, n = 66, $p < 0.001$ vs. controls) and the retro by 64.0% (2.14 ± 0.02 µm/sec, n = 220, $p < 0.001$ vs. controls). At 24 hrs the antero speed increase had been reduced by 79.1% (1.61 ± 0.03 µm/sec, n = 85, $p < 0.01$ vs. controls) and the retro by 88.8% (1.92 ± 0.02 µm/sec, n = 316, $p < 0.01$ vs. controls). There were no significant differences between the experimental group [antero = 1.59 ± 0.05 µm/sec, n = 101 and retro = 1.91 ± 0.02 µm/sec (n = 349)] and the control group at 72 hrs. The relatively rapid reduction of speeds following removal of the PTH source in these in vivo experiments along with the in vitro results suggest a physiological modulatory effect of PTH on the regulation of fast axonal transport. (Supported in part by the Whitaker Foundation).

36.12 N-CAM ANTIBODIES CAN HALT INTRAAXONAL MOTILITY FOLLOWING DISPERSION OF LFA BINDING SITES.
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Ganglion cell axons of goldfish retinal explants have varicosities and intervening phase-dense inclusions (IPDIs) that undergo bidirectional movements representing a bulk form of transport [Koenig, et al., 1985, J.Neurosci., 3:715]. Recent work indicates that Limax Flavus agglutinin (LFA), a lectin specific for sialic acid residues, binds preferentially to the surfaces of varicosities, IPDIs and growth cones and causes a complete arrest of all transport that is irreversible [Edmonds & Koenig; submitted]. Succinylated WGA (sWGA), which is specific for N-acetylglucosamine (GlcNAc), does not arrest transport but causes a dispersion and redistribution of the contents of most but not all varicosities [Edmonds & Koenig, unpublished]. The LFA receptors that are largely associated with varicosities and IPDIs undergo a dispersion into intervening axonal segments pari passu with the dispersion of the contents of varicosities.

As neural cell adhesion molecules (N-CAM) are polysialated membrane glycoproteins in developing brain [Hoffman et al., 1982, J. Biol. Chem., 257:7720], experiments were conducted to test for an effect on transport. Based on immunocytochemistry of axonal fields, antibodies raised against N-CAM from either rat brain or chick brain, kindly supplied by Dr. Joshua Sanes, show generally a preferential distribution that is similar to that of LFA. When antibodies against either rat or chick brain N-CAMs are introduced into the viewing chamber while organelle transport is being monitored by video microscopy, most axons appear normal. Although organelle transport activity seems largely unaltered, occasional axons appear hyperdense with phase-contrast or DIC optics, wherein it is not possible to discern organelle movement activity. If axonal fields are first treated with sWGA, and the sWGA is then removed by treatment with GlcNAc, exposure to N-CAM antibodies now causes an arrest of virtually all transport within 5 minutes, and the development of arrest is accompanied by structural changes involving a reduction in axonal girth and proliferation of lateral processes.

The significance of the pretreatment and subsequent reversal of sWGA for inducing an arrest of transport by N-CAM antibodies is unclear. Whether it is related to a possible redistribution of putative N-CAM in response to sWGA is uncertain; nevertheless, these preliminary results suggest a potential for transmembrane modulation of the axonal cytoskeleton. Supported by grant NS21843 from the NINCDS.

36.13 SELECTIVE NEURONAL INVOLVEMENT IN MOTOR NEURON DISEASE (WOBBLER MOUSE): UNIMPAIRED SLOW AXONAL TRANSPORT IN THE UNAFFECTED HINDLIMBS. K. KURAHASHI*, H. MITSUMOTO, G. BUNGE*. Cleveland Clinic Foundation, Cleveland, OH 44106.

Since motor neuron disease in the wobbler mouse predominantly affects the forelimb system but not the hindlimb, this animal model offers an unique opportunity to study why certain groups of motor neurons are selectively affected while others are not in motor neuron disease. The rate of slow axonal transport was impaired and the relative amount of transported neurofilaments was reduced in the affected forelimb system (Ann Neurol 19:36, 1986). We investigated slow axonal transport in the hindlimb system to study whether impaired slow axonal transport actually corresponds to the disease process in this model. All mice were studied at three months of age. All experimental procedures were done under halothane anesthesia. ^{35}S-methionine (1280Ci/mmol, adjusted to 250 uCi/ul, Amersham) was stereotaxically injected into five locations of the right ventral horns at the L5 and L6 spinal levels. At 1, 2, or 3 weeks after injections, the mice were perfused with saline through an intracardiac catheter. The nerves were dissected from the spinal cord, including the corresponding ventral roots down to the ankle level. The nerves were cut into 2 mm segments. The radioactivity of each nerve segment was determined in an aliquot, and the remainder was processed for one dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After superimposing the fluorogram, major bands were cut from dried gels and their radioactivity was determined.

The rate of the transport, based on the distance at which the cumulative 50th percentile transported radioactivity was reached, was 0.9 mm/day in controls (normal littermates) and 0.8 mm/day in wobbler mice. There were no differences in the polypeptide composition. Individual polypeptides, such as actin, tubulin, and neurofilament (145KD and 68KD) were also conveyed in the same fashion in both groups of animals, and the ratios of neurofilament proteins to actin and tubulin were the same.

In conclusion, slow axonal transport in the unaffected hindlimb system was unimpaired in contrast to the affected forelimb system. Thus, abnormal slow axonal transport seems to closely correspond to the disease process, suggesting an underlying pathogenic significance in this motor neuron disease. (Grant support by NIH, NS-21742)

36.14 PROLONGED ALTERATIONS IN RETROGRADE AXONAL TRANSPORT TO THE CELL BODY AFTER SCIATIC NERVE TRANSECTION. D. Fink, D. Purkiss*, and M. Mata. Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105.

In order to better define the nature of the signal from axon to cell body of irreversible nerve injury, we undertook this study of retrograde axonal transport after nerve cut injury using ^{3}H NSP labeling.

Male Sprague Dawley rats were anesthetized with chloral hydrate, and the sciatic nerve cut at the knee. A 5 mm segment was removed and the proximal stump ligated with suture to prevent regeneration. At 1,3,5,7 days and 2,3 or 5 weeks after cut the animal was reanesthetized and the nerve injected with 125 microCuries of ^{3}H NSP about 1 cm proximal to the cut end, using a 31 gauge needle attached to an infusion pump. One day or 7 days after ^{3}H NSP injection the animals were sacrificed and the amount of TCA precipitable radioactivity in the L4, L5 and L6 dorsal root ganglia and in the ventral horn of spinal cord determined.

In a parallel set of experiments, after identical operations and injections the animals were sacrificed by intracardiac perfusion and the distribution of radioactivity determined by light microscopic autoradiography.

Transport to the cell bodies in the DRG and in ventral horn of spinal cord measured 1 day after ^{3}H NSP labeling was not significantly altered by nerve transection, although there was a tendency towards increased values beginning in animals injected 5 days after cut in 1 day transport to DRG, and beginning 7 days after cut in 1 day transport to the spinal cord. This was more obvious in the cell body/nerve ratios.

7 day accumulation in the cell bodies showed a complex pattern. There was a significant increase in transport to the spinal cord in animals injected 5 and 7 days after nerve transection, which fell to lower than normal values which persisted to 3 and 5 weeks. Similarly in the DRG neurons a transient increase in transport was seen in animals injected 5 days or 7 days after transection, followed by a decline to less than normal values. By 5 weeks however those values had begun to return towards normal.

Autoradiography confirmed the intracellular localization of the labeled proteins and in general the amount of grains followed the pattern of total counts. Grains were found both in cell bodies which appeared relatively normal, as well as in those with eccentric nuclei suggesting central chromatolysis.

The similarities and differences between the pattern of changes after nerve transection and those which we have previously observed during regeneration after nerve crush, and the implications of these changes, will be discussed.

Supported by VA Merit Review Grants to Dr. Fink and Dr. Mata.

36.15 ALTERED AXONAL TRANSPORT IN RAT SCIATIC NERVE FOLLOWING ACUTE AND CHRONIC ETHANOL EXPOSURE. J.A. McLane and J. Gomez*. Veterans Administration Hospital, Hines, IL 60141 and Department of Biochemistry, Loyola University Stritch School of Medicine, Maywood, IL 60153.

The axonal transport system supplies essential proteins and other cellular components to the distal portions of peripheral nerve axons. Interference with this process may lead to peripheral neuropathies associated with metabolic disorders or neurotoxin exposure. We have carried out studies to test whether ethanol has detrimental effects upon axonal transport in peripheral nerves, which may contribute to the development of peripheral neuropathy in chronic alcoholism. Rat dorsal root ganglia-sciatic nerve preparations were exposed acutely to ethanol by incubating 18 hr in vitro. The ganglia were exposed to (^{35}S)-methionine to radiolabel newly synthesized protein and the nerves were exposed to 79, 198 or 395 mg % ethanol in Krebs-Ringer solution. Transport was evaluated by measuring the amount of (^{35}S)-methionine-labeled protein accumulated at a distal sciatic nerve ligation at the end of the incubation period. Exposure of the nerves to 395 mg % ethanol significantly reduced the accumulation of radiolabeled protein by 70%. Chronic ethanol exposure was accomplished by pair-feeding an ethanol or isocaloric control diet to rats for 9, 16 or 28 weeks. In vitro axonal transport experiments were again carried out, but without the acute ethanol exposure. Transport was found to be unchanged in nerves of rats fed the ethanol diet for 9 weeks, but was significantly reduced 44% after 16 weeks and 47% after 28 weeks of ethanol feeding. There was no significant difference in the level of incorporation of radiolabeled precursor into the dorsal root ganglia in either study. Retrograde axonal transport is also being examined in chronically ethanol-fed rats by injecting (2,3-^{3}H)N-succinimidyl propionate into the midthigh region of the sciatic nerve and measuring the accumulation of label in the dorsal root ganglia 7 days later. Preliminary data from these experiments suggest that movement of protein by this mode of transport is also decreased in chronic ethanol-fed rats. These combined results suggest that both fast anterograde and retrograde axonal transport are being affected through a single mechanism. One possibility is that microtubule stability is being altered. Microtubules have been shown to be a necessary component of the fast transport machinery. In liver it has recently been shown that acetaldehyde formed from ethanol metabolism forms covalent adducts with tubulin protein and thereby interferes with its polymerization into microtubules. Similarly, acetaldehyde-tubulin adduct formation in nerves may be altering axonal transport.

36.16 p-XYLENE INHALATION DEPRESSES AXONAL TRANSPORT OF [^{3}H]LABELED GLYCOPROTEINS AND [^{35}S]LABELED PROTEINS IN THE RAT RETINAL GANGLION CELLS. S. Padilla* and D. Lyerly*[1]. (Sponsor: W. Boyes). US Environmental Protection Agency and Northrop Services[1], Research Triangle Park, North Carolina, 27711.

Xylene, a solvent used extensively in industry and agriculture, is suspected of producing nervous system malfunctions in animals and humans. Little is known, however, about the neurochemical consequences of xylene inhalation. The intent of this study was to determine the effect of p-xylene exposure (0 or 800 ppm for 6 hrs/ day, 5 days/week for 1.5 weeks) on the synthesis and axonal transport of glycoproteins and proteins within the retinofugal tract of rats (male, Long Evans hooded, 60 days old).

Immediately after removal from the inhalation chambers each animal received an intraocular injection of 5.0μCi [^{3}H]fucose and 2.5μCi [^{35}S]methionine to measure glycoprotein and protein synthesis. After sacrifice at 0.25, 0.5, 1, 2, 3, or 4 hours postisotope injection, the retinas were removed, and the distribution of soluble and precipitable (ppt) radioactivity (10% trichloroacetic acid/ 0.5% phosphotungstic acid: TCA/PTA) was determined by liquid scintillation counting (LSC). p-Xylene treatment did not alter either the rate or the amount of radiolabel incorporated into either TCA/PTA soluble or ppt material, but by 4 hours post-injection, the [^{3}H]/[^{35}S]ratio of the retinal TCA/PTA ppt material was significantly increased in the p-xylene-exposed animals. In a separate but similar experiment, animals were exposed to the same p-xylene inhalation regimen and axonal transport was measured by intraocular injection of [^{3}H]fucose and [^{35}S]methionine followed by sacrifice 20 hours later. At this time all areas of the retinotectal tract were removed, and the amount of TCA/PTA ppt radiolabel determined by LSC. The [^{3}H]/[^{35}S] ratio was increased in the retinofugal tract of the p-xylene-treated animals; this apparent increase was due to a decrease in transport of both [^{3}H] and [^{35}S]labeled material with a relatively larger decrease in the transport of [^{35}S]labeled material. Specifically, the p-xylene treated animals (n=6) had a significant reduction in the axonal transport of both [^{3}H]glycoproteins and [^{35}S]proteins to the optic tract and of [^{35}S]proteins to the superior colliculus.

These data indicate that although p-xylene inhalation did not disrupt the initial rate or amount of synthesis of retinal [^{3}H] glycoproteins or [^{35}S] proteins, it did decrease the amount of newly synthesized glycoproteins and proteins supplied to the projections of the rat retinal ganglion cells. This submaximal availability of necessary materials to the axon and nerve ending regions may play a role in the nervous system dysfunction noted in solvent-exposed animals and humans.

37.1 EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON NEUROTRANSMITTERS AND THEIR METABOLITES IN VARIOUS BRAIN REGIONS OF THE RABBIT. C.S. Kim* and G.R. Breese (Spon: C.G. Lineberry). Biol. Sci. Res. Ctr. and Dept. of Psychiatry, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514

2,4-D is known to produce a variety of neurotoxic effects in man and experimental animals such as myotonia, lethargy, stupor and coma prior to death (IARC Monogr. 15:111-138; 273-300, 1977). During 2,4-D intoxication, its level is increased in various brain regions. The brain stem and cerebellum accumulate more 2,4-D than other brain areas. The present in vivo study was undertaken to determine the changes in the level of neurotransmitters and their metabolites after 2,4-D administration. Albino rabbits (New Zealand) were injected i.p. with either saline or 2,4-D (80 or 160 mg/kg). Two hours later, rabbits were sacrificed by decapitation and their brains were rapidly removed. Various brain regions (caudate nucleus, pon, medulla and hypothalamus) were dissected, weighed and immediately frozen on dry ice. Levels of dopamine (DA), serotonin (5-HT) and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) were determined by high performance liquid chromatography (HPLC). Injection of 2,4-D (160 mg/kg) increased the levels of both 5-HT and 5-HIAA by 32% ($P < 0.01$) in the medulla and by 25% and 27% ($P < 0.05$), respectively, in the pons compared to saline-treated rabbits. However, the contents of DA and DOPAC were unchanged. The concentration of HVA was increased by 17% and 25% ($P < 0.05$, $P < 0.01$) in the caudate nucleus and hypothalamus, respectively. The level of 5-HT was increased by 44% ($P < 0.01$) in the hypothalamus when 80 mg/kg of 2,4,-D was injected. However, these injections produced no change in the concentration of 5-HIAA or DA in the hypothalamus. These results indicate that the induced-increase of both 5-HT and 5-HIAA by 2,4-D primarily affects the brain stem suggesting that 2,4-D interacts with the serotonergic mechanism in this area. Supported in part by NIH grants: HD-03110 and ES-03458.

37.2 ATTENUATION OF INCREASES IN CEREBRAL BLOOD FLOW AND OXYGENATION ARE CORRELATED DURING REPETITIVE SEIZURES. B. L. Brizzee* and N. R. Kreisman. Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, LA 70112.

Cerebral oxygenation normally increases during individual seizures because cerebral blood flow (CBF) and O_2 delivery increase more than oxygen consumption ($CMRO_2$). After 1-2 hrs of status epilepticus, however, CBF diminishes, despite maintained $CMRO_2$. Meldrum and Nilsson (Brain 99:523-542, 1976) proposed that brain oxygen supply may be insufficient to meet demand under these conditions. In order to test this hypothesis, we measured cerebral oxygenation and blood flow in 16 anesthetized, paralyzed rats in which generalized seizures were induced repetitively (at 5-10 min intervals) with pentylenetetrazol.

Cerebral tissue Po_2 was measured polarographically with microelectrodes and relative changes in the oxidation/reduction state of cytochrome aa_3 were measured with a dual wavelength reflection spectrophotometer. Local CBF was measured intermittently by the method of H_2 washout, and cerebrovascular resistance (CVR) was estimated by dividing the mean arterial blood pressure by CBF. Cerebral oxygenation increased phasically during early seizures in all rats as indicated by increased tissue Po_2 and greater oxidation of cytochrome aa_3. The increases in cerebral oxygenation gradually attenuated in all 16 rats. By 1.5 to 2.5 hrs of seizures, cerebral oxygenation either failed to increase at all or decreased, despite normal systemic oxygenation (n=8). These changes in cerebral oxygenation are in agreement with those reported previously (Kreisman et al Brain Res. 218:175-188, 1981). During early seizures, CBF increased 200-400% and CVR decreased to about 50% of pre-seizure values. Both the 2- to 4-fold increase in CBF and the fall in CVR were sustained in animals that maintained phasic increases in cerebral oxygenation during periods of increased seizure activity. In rats that developed decreased cerebral oxygenation, the increase in CBF tapered off to less than 160% of pre-seizure values, and CVR returned to control levels. The data suggest that there is a critical level of CBF, about 160% of control, below which cerebral oxygenation is compromised. Attenuation of the increase in CBF suggests there is maladjustment in cerebrovascular tone and reactivity during repetitive seizures. Derangements in cerebrovascular reactivity and cerebral oxygenation may contribute to tissue damage during status epilepticus.

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37.3 SPATIAL STATISTICAL MAPPING: A THREE DIMENSIONAL ANALYSIS OF CEREBRAL METABOLIC ACTIVITY PATTERNS IN RATS WITH AND WITHOUT NIGRAL STIMULATION. H.H.HOLCOMB, H. LOATS, M. KADEKARO, P. GROSS, E MATTHEW and A. PERT. Lab. of Cerebral Metabolism, NIMH, Bethesda, MD, and Loats Assoc., Inc., Westminster, Md. 221157.

Movement is partially regulated by neurons projecting from the motor cortex (MCTX) to the dorsal caudate-putamen. Unilateral electrical stimulation of the substantia nigra results in ipsilateral elevations in local cerebral glucose utilization (rCMRglu) and turning behavior. Following 20 minutes of electrical stimulation to the substantia nigra reticulata(SNr), some ipsilateral structures exhibit unequivocal, robust metabolic increases (globus pallidus, entopeduncular n., subthalamic n., superior colliculus). Others increase consistently, but only for restricted, highly localized regions (MCTX). In an effort to provide a three dimensional analysis of activated regions we have quantified the location, area, projection and volume of regions exhibiting elevated rCMRgl in the cortex, caudate, globus pallidus and entopeduncular nuclei of electrically stimulated (unilateral SNr, 750 microAmps) rats and sham controls. Serial autoradiographs were obtained and processed by the methods described by Sokoloff and colleagues (J. Neurochem 1977). The three dimensional, registered images were statistically transformed to reflect volumetric, isoactivity curves. Area projection and centroid location measurements demonstrate the rostro-caudal trajectory of cortico-striate activity. Three dimensional maps of statistically transformed metabolic data are especially useful devices for reducing and simplifying complex image data. Stereotaxic organization of histologically matched brain sections further enhances the interpretation of these data rich studies.

37.4 PARIETAL CORTEX STIMULATION IN THE RAT INHIBITS CORTICAL AND THALAMIC GLUCOSE METABOLISM. J.W. Sharp*, M.F. Gonzalez, M.T. Morton*, and F.R. Sharp, Dept. Anatomy, U.C. Davis, and Dept. Neurology, UCSF and VAMC, S.F., CA 94121.(SPON:R. Kitchell)

Cortical and thalamic glucose metabolism were measured in six adult rats using the (14C) 2-deoxyglucose method. Bipolar electrodes were implanted into either the right hindlimb motor and sensory cortex or into the right face parietal cortex. Hindlimb cortical stimulation produced repetitive left hindlimb movements.

Electrical stimulation increased the glucose metabolic rate of cortex around the stimulating electrode in all subjects. Local cerebral glucose utilization (LCGU) decreased in immediately adjacent areas of cortex compared to control regions in far left lateral cortex. LCGU increased in the contralateral (left) cortex in broad as well as narrow columns. Alternating with these columns were columns of low LCGU which were less than the control areas of cortex. These contralateral cortical columns of increased and decreased LCGU were abolished by sectioning the corpus callosum. Lesioning cortex did not lead to contralateral columns of high or low LCGU, implying that columns of decreased LCGU produced by stimulating cortex were due to activation of inhibitory pathways rather than being due to decreased excitation.

Hindlimb and face parietal cortical stimulation also increased LCGU in portions of the ventrobasal (VB) and posteriomedial (POm) nuclei of the ipsilateral thalamus. However, surrounding areas of both VB and POm showed decreased LCGU. Similarly, ipsilateral lateral posterior and lateral dorsal nuclei had decreased LCGU compared to the contralateral unstimulated side of the thalamus. It is proposed that since the reticular nuclei perikarya increased their LCGU and were therefore activiated by parietal cortical stimulation that their GABA inhibitory terminals in thalamus led to the inhibition of thalamic glucose metabolism. Alternative explanations for the decreases of thalamic metabolism include corticothalamic inhibition, and basal ganglia inhibition of thalamus. It is suggested that the metabolically activiated areas of thalamus represented a sum of the reticular inhibiton and direct cortical excitation. The inhibition of cortical and thalamic LCGU produced by parietal cortical stimulation is relevant to selective sensory attention and gating as well as to the greater epileptogenicity of motor compared to sensory cortex.

37.5 EFFECTS OF APAMIN ON LOCAL GLUCOSE UTILIZATION IN THE BRAIN AND IN THE SPINAL CORD IN RATS. S. Cohen*, M. Kadekaro, M.L. Terrell*, H. Gary, Jr.* and H.M. Eisenberg. (SPON: B. Moore). Div. of Neurosurgery, Univ. of Tex. Medical Branch, Galveston, Tex. 77550.

Apamin is an 18 residue polypeptide component of bee venom. It possesses powerful neurotoxic properties derived from its ability to block one class of Ca^{2+}-dependent K+ channels, which is responsible for the long-lasting after potential hyperpolarization. Systemic administration of apamin in rats and mice elicits, in a dose-dependent manner, gross, uncoordinated movement of the whole body, with eventual lethal respiratory insufficiency. The symptoms are mediated by the actions of apamin on the central nervous system, although this neurotoxin poorly crosses the blood-brain barrier.

The objective of the present study was to identify with the quantitative autoradiographic [^{14}C]deoxyglucose method neural structures involved in the production of symptoms of apamin neurotoxicity in rats.

Male Spraque-Dawley rats (280-320g) were anesthetized with halothane and polyethylene catheters were implanted in one femoral artery and vein. The animals were allowed to recover from the effects of anesthesia for at least two hours before administration of apamin (2 mg/kg, ip) into experimental animals (n=7) or saline into control animals (n=8). After the administration of apamin the animals lost exploratory behavior, remaining completely immobile, with the four limbs overextended, unresponsive to auditory stimuli. The symptoms started to occur 45-60 min after injection of apamin. Arterial blood pH, PCO_2, PO_2, arterial blood pressure, hematocrit and plasma glucose concentration were not significantly affected by apamin. The deoxyglucose experiments started 75 min after injection of apamin.

Glucose utilization was significantly decreased in nine out of 25 neural structures examined. These structures included the cochlear nucleus, superior olivary nucleus, inferior colliculus, medial geniculate body, fastigial nucleus, globus pallidus, caudate-putamen, nucleus accumbens and the lumbar ventral horn of the spinal cord. These results imply that the effects of apamin on functional activity of the brain is not generalized but rather specific to the auditory and motor structures.

37.6 TAURINE MAY REGULATE CELLULAR HYDRATION AND CELL VOLUME IN THE BRAIN. J.V. Wade[1]*, J.P. Olson[2], S.R. Nelson[3], F.E. Samson[3] and T.L. Pazdernik[1] (SPON: E.J. Walaszek). Department of Pharmacology, Toxicology and Ther.[1], Department of Anatomy[2], and Ralph L. Smith Research Center[3], University of Kansas Medical Center, Kansas City, Kansas 66103, USA.

Changes in free extracellular amino acids within the rat brain were measured during intracranial microdialysis with hypo-osmotic media or during systemic water intoxication. Dialysis fibers implanted in the piriform cortex were perfused (1 ul/min) with Krebs-Ringer bicarbonate (KRB, 305 mOsm) for a 1 hr baseline period and then switched, in steps, to media of decreased osmolarity (KRB with adjusted NaCl content) and then stepwise back to standard KRB as follows: 295/285/295; 280/255/280; and 205/105/205 mOsm (n=3 each group). Effluent perfusate samples were collected at 30 min intervals in the awake, freely moving rat and analyzed for amino acids using HPLC. In order to examine the effect of a generalized increase in brain water content, an additional group (n=4) was perfused with standard KRB, anesthetized, and then given a 20% body weight dose of distilled water, I.P. Perfusate samples were collected at 30 min intervals for 3 hr, at which time rats were sacrificed and brains removed. The specific gravity of piriform cortex contralateral and ipsilateral to the dialysis fiber was measured with a bromobenzene density gradient column and percent tissue volume increase calculated. Measurements were also made in rats without dialysis fibers, receiving the same treatment, to establish the time course of tissue volume increase. Extracellular taurine (Tau) increased markedly during hypo-osmotic perfusion, mean levels reaching 2, 5, and 15-fold baseline, dependent upon the degree of osmotic change. In all cases, Tau levels returned to normal upon perfusion with standard KRB. No significant changes were noted in aspartate, glutamate, asparagine, serine or glutamine. Systemic water intoxication produced a 12.5% volume increase in the piriform cortex on both the fiber and contralateral sides. Extracellular Tau increased within the first 60 min, reaching over 6-fold baseline levels at 150 min. Again, other extracellular amino acids were not affected. Taurine is one of the most abundant free amino acids in the brain. These findings suggest that Tau may play an important role in the regulation of cellular hydration and cell volume within the CNS. Supported in part by U.S. Army DAMD 17-86-G-6038.

37.7 AMILORIDE REVERSAL OF ALKALINE INTRACELLULAR pH IN HIPPOCAMPAL SLICES. J.C. LaManna, H. Assaf*, T.J. Sick, and T.S. Whittingham. Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The transport of intracellular protons to the extracellular space by exchange with extracellular sodium ions through the action of an amiloride-sensitive H^+/Na^+ antiporter has been described for a number of tissues. The presence of the antiporter has yet to be shown in intact, electrophysiologically functional mammalian central nervous system tissue.

Blood-free hippocampal slices (400-500 μ) were made from brains removed from ether-anesthetized rats after thoracotomy and perfusion through the ascending aorta by cold buffer solution. Slices were kept in oxygenated holding containers at room temperature until use. Half of the slices were placed in a 50 μM solution of the pH indicator dye, Neutral Red (NR) in standard buffer, for 30 minutes, then removed, washed twice and placed in a separate holding container at room temperature until used. The spectrum of light passing through an unstained slice was determined in a modified recording chamber with a scanning spectrophotometer. This spectrum was used as a reference spectrum for slices which were stained with NR. NR absorption curves were obtained as the log of the ratio of spectra from slices containing NR to that of the reference slice. Use of the reference spectrum removed all non-specific absorption, leaving only the NR absorption spectrum. Calibration curves were constructed from homogenized brain tissue. The ratio of absorbance at the maximal wavelengths of absorption for the acid and base forms of the dye is linearly related to the pH over the range 6-8 and is independent of, and insensitive to, changes in dye concentration.

Under resting conditions (125 mM NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 10 mM glucose; equilibrated with 95% O_2/5% CO_2, pH = 7.4 at 37 °C), the intracellular pH of these hippocampal slices averaged 7.64 ± 0.02 (by the NR method; n = 12) and 7.68 ± .05 (by the creatine kinase equilibrium method; n = 8). Thus, pHi of normally prepared hippocampal slices is not only more alkaline than found in vivo, but is more alkaline than the external medium. Switching the bathing solution to contain 1 mM amiloride resulted in an intracellular acidification to near neutrality within 1 hour (pHi = 7.08 ± .05; NR method, n = 12), and which was maintained for at least another hour. This pHi is closer to the expected in vivo pHi and represents a restored proton gradient. The acidification due to amiloride suggests that the H^+/Na^+ antiporter is abnormally activated in the hippocampal slice preparation.

37.8 Intracellular pH of Astrocytes Rises Rapidly with Cortical Stimulation. M. Chesler and R.P. Kraig. Department of Neurology, Cornell University Medical College, New York, New York 10021

Glial cells play an important role in brain homeostasis, and accordingly, are thought to increase their metabolic rate in response to neuronal activity. This response may be elicited by elevations in external K^+ (Orkand et al., Brain Res. 55:467), but whether other ion shifts are implicated is unknown. Small changes in intracellular pH (pH_i) can profoundly alter glycolytic rate (Fidelman et al., Am. J. Physiol. 242:C87) and gap junctional conductance (Spray et al., Science 211:712), and thus, could significantly modulate glial function. Interstitial pH (pH_o) transients accompany brain stimulation (Kraig et al., J. Neurophysiol. 49:831), but how electrical activity impacts on glial pH_i is unknown. We have therefore monitored pH_i of mammalian cortical astrocytes in vivo during evoked activity and cortical spreading depression (CSD).

Wistar rats were anesthetized with halothane, immobilized and artificially ventilated. Double-barrel, pH sensitive microelectrodes were inserted into frontal cortex bathed with warm (35-37°C) Ringer gassed with 5% CO_2 in O_2. Glia were identified by a high membrane potential and lack of injury or synaptic discharge. Their resting pH_i was 7.04 ± 0.02 with a membrane potential of 73 ± 1 mV (mean ± SEM; n=43).

In 44 records from 37 glial cells, bipolar stimulation of the cortical surface produced a rapid glial depolarization, attributable to a rise in external K^+, accompanied by an intracellular alkaline shift (IAS). The size of the IAS correlated with the degree of depolarization and ranged from 0.05-0.40 pH. With prolonged stimulation pH_i reached a peak, slowly became less alkaline, and after stimulation, rebounded to a more acid level than baseline. Stimulation in the presence of 1-2 mM Ba^{2+} caused a hyperpolarizing response (Ballanyi et al., J. Physiol. 382:159) and completely blocked the IAS. Similar, but generally larger pH_i responses occurred with CSD, with an IAS of 0.11-0.78. After withdrawal from cells, the predominant interstitial response to stimulation and CSD was a slow acidification.

The mechanism of the IAS remains to be elucidated but available data indicate that it requires an active acid extrusion mechanism or internal consumption of protons. Its sensitivity to Ba^{2+} suggests that glial depolarization is necessary to elicit the response. The observed lability of glial acid-base status suggests that pH_i shifts may serve to rapidly modify the functional state of glial cells in response to demands imposed by surrounding neurons.

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37.9 pH SHIFTS IN THE CA1 LAYER OF THE HIPPOCAMPAL SLICE. W. Walz. Dept. of Physiology, College of Medicine, University of Saskatchewan, Saskatoon S7N 0W0, Canada.

Changes in the pH of the extracellular space in the pyramidal cell layer (CA1) of slices (500 um thick) from rat hippocampus were monitored with the aid of microelectrodes. The electrodes were double-barreled, thick septum microelectrodes manufactured according to a method introduced by Ransom and co-workers (Borrelli et al., J. Neurosci. Methods 15 (1985): 141). One channel tip was filled with the Fluka proton cocktail for measuring the pH signal and the other one contained a hepes-buffered salt solution for measuring the field potentials. The pH response was calculated from the difference of both signals. The salt solution bathing the slice was a bicarbonate buffered saline with a pH of 7.35. During stepwise impalement of the slice with the double-barreled microelectrode towards the site with the highest field potential amplitude during stimulation of the Schaffer's collaterals the pH decreased to 7.1. Hence, there is a pH gradient within the slice with more acidic values at areas which have higher activity and are in the center of the slice. This gradient was present also without stimulation of the Schaffer's collaterals. Stimulation showed two frequency-dependent features: A transient alkaline shift at the onset of stimulation was followed by a sustained acidic shift. The transient alkaline shift was apparent at 20 Hz stimulation (0.04), had a maximum at 50 Hz (0.07) and was reduced at 100 Hz. The sustained acidic shift reached values of 0.15 (5 Hz), 0.2 (20 Hz), 0.26 (50 Hz) and 0.17 (100 Hz). After the end of the stimulation the pH slowly returned to the resting level with a time course of 0.03 pH units per min. A one minute stimulation with 50 Hz was selected for drug analysis of the phenomenon. The Na^+/H^+ exchange blocker amiloride (1mM) did not influence the resting pH level. During stimulation the transient alkaline shift was not influenced. However, the amplitude of the acidic shift was reduced by one third. Acetazolamide (1 mM) a blocker of the carbonic anhydrase, which is probably a glial specific enzyme, elicited a slow acid shift of the resting pH level (0.05 pH units per min). Stimulation during the presence of acetazolamide caused a three times increased transient alkaline shift (0.2 pH units) which lasted almost one min, but did not influence the acid shift. When acetazolamide and amiloride were added together, the acid shift almost disappeared and the most prominent feature was the trained feature was the transient 0.2 alkaline shift. It is concluded that the alkaline and the acid shift are features of two different processes involved in neurotransmission, which overlap each other. The glial specific enzyme carbonic anhydrase plays a major role in one of these processes.

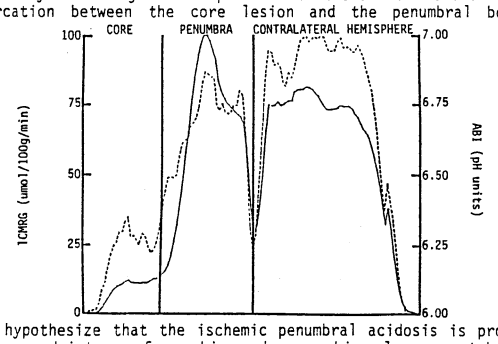
The author is a scholar of the Medical Research Council.

37.10 COMPENSATORY CHANGES THAT PROMOTE SURVIVAL OF TURTLE BRAIN DURING ANOXIA. Zi-Cai Feng. Myron Rosenthal and Thomas Sick, Department of Neurology, Univ of Miami Medical School, Miami, FL 33101

Previous studies demonstrated that extracellular potassium ion activity (K^+o) rose only 3-5 mM during 24 hours of anoxia in turtle brain and recovered within a few minutes following restoration of normoxia. This is in contrast to rat brain in which K^+o rose to over 70 mM after only a few minutes of N_2 inspiration. Recent studies indicate that ion transport in anoxic turtle brain was maintained through energy from anaerobic glycolysis since transmembrane gradients were lost during ischemia (1 hr) or during anoxia with glycolytic inhibition (superfusion of brain with iodoacetate). The question remains whether other compensation processes, such as those that may decrease brain energy demand, also contribute to ion homeostasis during prolonged anoxia in turtle brain. In these studies, K^+o was recorded with ion-selective microelectrodes in intact brains of turtles anesthetized with pentobarbital. Turtle brains were stimulated to increased activity by two methods: a) application of electrical pulses to the brain surface (2 sec trains, 20 Hz, 0.5msec/pulse) to record amplitudes of K^+o efflux provoked by this stimulation and rates of K^+o reaccumulation at the stimulus site; and b) application of electrical pulses to the olfactory nerve for recording evoked potential activity in the olfactory bulb. Amplitudes of evoked potentials declined within 30 minutes of N_2 inspiration and decreased to approximately 40% of normoxic levels at 2 hours. Magnitudes of K^+ efflux were also decreased and rates of reaccumulation of K^+o were slowed. These data demonstrate that synaptic transmission was inhibited by anoxia as was ion transport that occured in response to neuronal activation, suggesting that turtle brain compensates for anoxia by decreasing excitability and also by decreasing the rate of K^+o-stimulated transmembrane cation transport. The slowing of K^+o reaccumulation is in apparent contrast with findings that K^+o reaccumulation is unchanged by hypoxia in rat brain. In turtle brain, K^+o recovery rates were most retarded at low K^+o values suggesting that the presence of high K^+o "loads" either overcomes the apparent compensatory decrease in ion transport rate or that an addition transport mechanism is activated to promote rapid reaccumulation when stimulus-provoked K^+o increments are high. This secondary process may not be present in mammalian brain and it may protect turtle brain against anoxic depolarization. It remains to be determined whether protective mechanisms of turtle are novel to that species, or whether thay can be adapted or encouraged in mammals. However, it is clear that turtle provides an extraordinary model for studies of brain survival since it demonstrates basic features of vulnerability and also of adaptation which may be present in mammal but are not easily available for investigation because of the rapidity of degenerative events. (Supported by PHS grants NS14325 and HL38657 and the National Parkinson Foundation through the Evenor Armington Fund).

37.11 ACIDOSIS CORRESPONDS WITH GLUCOSE UTILIZATION IN FOCAL ISCHEMIA. K.E. Peek, M. Izumiyama*, A.H. Lockwood,* E.W.H. Yap*, J.Y. Labove*. Univ. of Tex. Med. Sch., Houston, TX 77025.

Post-ischemic recovery potential may be compromised by tissue acidosis. Focal cerebral ischemia was produced in anesthetized male Wistar rats by insertion of a silicone-tipped 4-0 nylon suture via the internal carotid to the origin of the middle cerebral artery (Koizumi, Jpn J Stroke 8:1-8, 1986). [^{14}C]-dimethyloxazolidine dione (DMO, 175 µCi/kg) was injected iv followed 1 h later by [^{14}C]-deoxyglucose (DG, 100 µCi/kg). After 45 min of timed arterial blood sampling, the animal was killed and 20µbrain sections were autoradiographed. After DMO sublimation a second autoradiograph was prepared. Following computer-controlled digitization, image subtraction produced DMO and DG images from which local cerebral metabolic rate for glucose (lCMRG) and acid-base index (ABI) were computed. Two hours of tamponade-induced focal ischemia reduced lCMRG and ABI in frontal to occipital cortex, lateral caudate-putamen (CPU) and thalamus, amygdala, and internal capsule. In the penumbra of the reduced lCMRG zone, regions of increased lCMRG were observed affecting medial CPU, hippocampus, cortical columns, and more medial thalamic nuclei. Less severe acidosis was seen in regions of abnormally high lCMRG. The graph shows a topographic profile of calculated lCMRG (solid line) and ABI (dotted line) values along a line transversely bisecting a frontoparietal section. Note the sharp demarcation between the core lesion and the penumbral border.



We hypothesize that the ischemic penumbral acidosis is produced by an admixture of aerobic and anaerobic glucose metabolism. Tissue survivability may be enhanced by improving tissue oxygenation, titrating metabolic acids, and limiting the availability of excess glucose.

37.12 THE DIFFERENTIAL EFFECTS OF HYPOXIA ON NEUROTRANSMITTER RELEASE MECHANISMS. G.B. Freeman, T. Manger and G.E. Gibson. Cornell Univ. Med. Coll. at the Burke Rehab. Ctr., White Plains, NY 10605.

Diminished oxygen availability differentially alters the K^+-stimulated release of acetylcholine (ACh), glutamate and dopamine (DA). In mouse striatal slices, anoxia (100% N_2) under depolarizing K^+ concentrations reduces media levels of ACh and increases those of DA and glutamate (Freeman et al., 1987). In order to determine if the presynaptic terminal is the origin of the excess extracellular DA and glutamate, the effects of anoxia on release were measured in partially purified striatal presynaptic terminals (i.e. synaptosomes; Leslie et al., 1983). Chemical hypoxia was induced by the addition of KCN (0.5 mM). Anoxic-induced changes in total media concentrations of the neurotransmitters differed with the concentration of Ca^{2+} in the incubation media. In low Ca^{2+} media (0.1 mM), increases were evident for ACh (5.7%), DA (168%) and glutamate (318%) whereas, in 2.3 mM- Ca^{2+}, ACh decreased 63% but DA and glutamate were elevated 30% and 112%, respectively. Anoxic-induced alterations of ACh and glutamate release also occurred with a purified rat striatal synaptosomal preparation (Hajos, 1975). Total concentrations during anoxia with 0.1 mM-Ca^{2+} were reduced 50% for ACh and increased 170% for glutamate. With 2.3 mM-Ca^{2+}, ACh concentrations declined 40% with anoxia while glutamate increased 79%. The results suggest that the increased extracellular concentrations of DA and glutamate that accompany anoxia and ischemia in vivo and in vitro are due to alterations in presynaptic release mechanisms that are related to changes in calcium homeostasis.

37.13 SENSITIVITY OF SYNAPTIC ACTIVITY IN THE CA1 REGION OF THE HIPPOCAMPAL SLICE PREPARATION TO COMBINED HYPOXIA AND HYPOGLYCEMIA: A MEDIAN-EFFECT ANALYSIS.
K. H. Reid, C. B. Shields, W-Q Dong*, and A. Schurr. Laboratory for Cellular Neuroscience, Departments of Physiology, Surgery and Anesthesiology, School of Medicine, University of Louisville, Louisville KY 40292.

Hippocampal slices from 200-300 g Sprague-Dawley rats , cut 400 um thick, were maintained in an interface chamber at 34°C and subjected to various combinations of hypoglycemia and hypoxia. Synaptic responsiveness was evaluated by stimulating Schaffer collaterals once/min at 2x threshold, and recording the CA1 population spike. Slices were considered unresponsive if this stimulation did not evoke a response > 1 mv in amplitude. The typical prehypoxic response to this stimulus intensity was a population response 10-20 mV in amplitude.

The exposure to low glucose (hypoglycemia) lasted 60 minutes. During the last 10 minutes of this period, oxygen was replaced partially or completely by nitrogen, to impose a hypoxic stress. Baseline conditions were restored for 30 minutes post-stress before testing for recovery of synaptic function.

A total of 1063 slices were tested under 16 different combinations of hypoglycemic and hypoxic stresses. Median-effect analysis was used to establish ED50 loci for 2 conditions: constant oxygen tension with varying glucose concentration, and constant glucose concentration with varying oxygen tension. The two loci agreed within 5%. This analytic approach was also found effective in a previous study of the interactions of ACSF K^+ and temperature (Soc. Neurosci. Abstr 16:1526, 1986). We conclude that median-effect analysis is an appropriate tool for the analysis of the effects of a variety of interacting environmental stresses on brain tissue, and may prove useful in evaluating both the relative importance of different mechanisms contributing to brain damage and the value of putative protective agents.

37.14 MODERATE INCREASES OF BLOOD FLOW TO CAT WHITE MATTER IS INDICATIVE OF AN INCREASED LATENCY OF THE CORTICAL COMPONENT OF THE SOMATOSENSORY EVOKED POTENTIAL AND A PERIOD OF ELECTRICAL RECOVERY FOLLOWING SYSTEMIC HYPOXIA. P.E. Coyer and F.A. Simeone* . Department of Neurosurgery, The Pennsylvania Hospital, Philadelphia, PA 19107.

We have reported the finding of a linear increase in the latency of the cortical component of the somatosensory evoked response (SEP) during contralateral forepaw stimulation in response to systemic hypoxia in cats (Coyer, Michele, Simeone; Neurosci. Abstr 12: 1130, 1986). The dependency of the cortical wave latency on oxygen concentration seemed to be a function of the rate and degree to which hypoxemia was achieved as suggested by comparing the responses of two groups of 12 cats each ventilated under a different oxygen/nitrous oxide regimen.

In this paper we report the observations of several other parameters related to these studies. First, oxygen availability to the brain tissue remained relatively constant over ventilation concentration of 9-18%. This observation probably reflects the maintenance of blood flow to the gray matter and the significant rise in blood flow to the white matter especially at 7% O_2. Additionally, the frequency of spontaneously-occurring activity increased during decreases in oxygen availability although initially there was a transient depression in observed multi-unit action potential generation. Thus, these experiments indicate that hyperperfusion of the white matter is associated with maintenance of the somatosensory evoked potential although the latency is extended due to a slowing of conduction associated with a constant metabolic rate and accumulation of lactate (Lesnick et al., Stroke 17: 1247-120, 1986).

In several animals whose electroencephalographic (EEG) activity recovered following exposure to hypoxia and a return to normal oxygen atmosphere, there was still an extension of the cortical component of the somatosensory evoked response. This could indicate that white matter is dependent upon hyperperfusion,which was observed,and its recovery upon a period of time to restore normal electrical conditions.

The results of these experiments suggest that the SEP cortical wave latency is a sensitive indicator of hypoxemia during hyperperfusion of the white matter conduction pathways. Perhaps, oxygen availability in the cortex remains relatively undisturbed in the gray matter due to maintenance of blood flow and accounts for the continued excitability of neurons there. On the other hand, white matter pathways are blocked and the SEP cortical component's appearance is extended. Resumption of EEG activity without restoration of the normal SEP cortical wave latency may suggest that a period of time is necessary for recovery of metabolism following hypoxic insults in white matter.

37.15 FOCAL CORTICAL PERFUSION, BRAIN pH AND EEG DURING PROLONGED HYPOCAPNIA: HALOTHANE VS. ISOFLURANE. M.A. Morón*, R.E. Anderson*, T.L. Yaksh* and T.M. Sundt, Jr.* (SPON: D.W. Klass). Departments of Pharmacology and Neurologic Surgery, Mayo Clinic, Rochester, MN 55905.

Though both halothane (HAL) and isoflurane (ISO) are halogenated anesthetics, they have different effects on the regulation of cerebral blood flow (CBF), particularly in response to changes in arterial $PaCO_2$. The present studies were designed to examine the effects of equi-anesthetic concentrations (1 MAC) of these two anesthetics on focal cortical and regional CBF (fCBF and rCBF), intracellular brain pH (pHi) and EEG in response to changes in $PaCO_2$, and also to determine any changes in fCBF and rCBF during prolonged hypocarbia. fCBF and rCBF were estimated in cats maintained at 1 MAC of HAL or ISO by measuring the clearance of the molecular form of umbelliferone (UMB), and xenon-133. UMB is a lipid soluble intracellular pH indicator (Brain Res. 186:355, 1980). For each cat a CO_2 response curve was generated using $PaCO_2$ values of 40, 60, 30 and 20 measured at 15-min intervals (feline normocapnia: 34-38). Subsequently, each cat was maintained at a $PaCO_2$ of 20 for 3 hr. In a third group of animals under ISO, a similar protocol was followed, but the $PaCO_2$ was maintained at normocapnia for 3 hr. EEG and BP/HR were monitored throughout the experiment.

CBF-CO_2 response: The slope (ml/100 g/min/torr) of the HAL and ISO CO_2 response curves were: 0.658 ± 0.133 and 0.463 ± 0.120 with UMB; 1.520 ± 0.224 and 1.218 ± 0.144 with xenon-133 for HAL and ISO, respectively. CBF over time: For the HAL-hypocapnic groups CBF had returned to normocapnic flow levels by 1 hr while arterial $PaCO_2$ remained low. The ISO-hypocapnic group showed a progressive CBF reduction of 23%, 37% and 35% at 1, 2 and 3 hr, respectively ($P < 0.05$). The normocapnic ISO group showed no change in flow over time. Brain pH: Normocapnic intracellular pH was 7.03 ± 0.1 for all groups. After 1 hr of hypocapnia the pH was 7.12 ± 0.04 and 7.14 ± 0.03 for HAL and ISO, respectively. At 2 hr the HAL group displayed a normal pH while for the ISO group the pH was 7.18 ± 0.03 ($P < 0.05$). The pH of the ISO-normocapnic group remained stable throughout the period of normocapnia. EEG: HAL EEG activity was normal throughout the experiment. A burst suppression pattern was seen in five of the seven cats in the ISO-normocapnic and all the cats in the ISO group. This pattern was more severe in the latter group.

This investigation shows that for prolonged hypocapnic ISO in contrast to HAL anesthesia that CBF remains reduced and a significant intracellular alkalosis ensues. EEG activity for both ISO groups demonstrated a burst suppression pattern which was exaggerated during hypocarbia. (Supported by NIH grant NS-24329, TLY.)

37.16 NADH FLUORESCENCE CHANGES IN RESPONSE TO SPREADING DEPRESSION IN RAT BRAIN CORTEX AND HIPPOCAMPUS. J. Ott, S. Brewster*, C. H. Barlow* and J. J. Kelly*. Humphrey Laboratory, The Evergreen State College, Olympia, WA 98505.

The brain's metabolic response to spreading depression results in changes in the redox state of NAD^+/NADH. These changes may be followed by imaging NADH fluorescence. Using this approach we have compared the metabolic responses to spreading depression of the cortex with the response in the hippocampus in rat brain.

Pentobarbital anesthetized Sprague-Dawley rats were used without artificial ventilation. The skull was exposed and a 1mm hole was drilled through the skull 2mm caudal to and 2mm to the right of the bregma. Spreading depression was initiated by insertion and removal of a 25 gauge needle to the appropriate depth. The brain was then funnel frozen at specified times following initiation of spreading depression. Photographs recorded the NADH fluorescence from polished surfaces of the frozen brain.

When spreading depression was induced in cortex only, a narrow zone of oxidized NAD^+ immediately followed by a wide zone of reduced NADH moved across the cortex at 2-3mm/minute. The metabolic response did not spread to the hippocampus. Simultaneous stimulation of cortex and hippocampus resulted in the same cortical response, however in the hippocampus the entire ipsolateral side became reduced within approximately 6 seconds with no evidence of the metabolic response on the contralateral side. After 3 minutes the hippocampus had recovered (disappearance of NADH fluorescence), while the cortex was still reduced and did not show oxidized NAD until 20 minutes.

Clearly different nervous structures have differential responses to the onset and recovery of spreading depression. The fact that spreading depression rapidly affects the entire hippocampus may be related to the structural organization of the hippocampus as compared with the cortex.

Allowing the animal to breath 95% O_2/5% CO_2 for 10 minutes prior to the elicitation of spreading depression prevented the metabolic response to spreading depression in both the cortex and hippocampus. These studies suggest that the metabolic rate of the affected cells is accelerated during spreading depression and drives the tissue into anoxia. This anoxic state can be overcome with adequate prior oxygenation of the tissue.

The rapidly reversible nature of the metabolic response to spreading depression in the hippocampus suggests the possibility of employing spreading depression as a temporary inhibitor of hippocampal function in studies of spatial behavior and memory.

Supported by Professional Development Grant from The Evergreen State College.

37.17 PROTON MAGNETIC RESONANCE SPECTROSCOPY IN A RAT MODEL OF CEREBRAL HYPOXIA AND RECOVERY. E.A. Crisostomo, J.A. White*, C.M. Gasparovic*, R. Griffey*, and G.A. Rosenberg. Dept. of Neurology and Center for Non-Invasive Diagnosis, University of New Mexico, Albuquerque, New Mexico 87131.

Oxygen deprivation in the brain leads to a series of biochemical events which include augmentation of glycolysis, accumulation of polyenoic free fatty acids and inhibition of mitochondrial respiratory enzymes. ^{1}H magnetic resonance (MR) spectroscopy allows the detection of energy metabolites and membrane phospholipids which are sensitive to cerebral O_2 perturbation. We applied this technique to a rat model of hypoxia with subsequent recovery to study changes in the spectral pattern of intact brain.

Male Sprague-Dawley rats, with free access to food and water, were anesthetized with pentobarbital and ventilated (70% N_2O/30% O_2). The paralyzed animal was fitted into a probe with the head positioned directly over a two-turn proton surface coil. ^{1}H MR spectra were obtained using a 9-cm bore 7-T superconducting magnet operating at a proton frequency of 300 MHz. Brain localization was achieved by means of a modified depth selective pulse sequence. An initial 30-min period of normoxia was followed by 30 min of hypoxia (15% O_2). Subsequently, recovery was attempted for 1-2 hr with restoration of O_2 to 30%. Arterial blood gases and mean arterial blood pressures were monitored during the course of each experiment.

Proton spectra collected during baseline normoxic periods contained well-resolved resonances at 2.0 ppm and 1.5-1.7 ppm. We tentatively assigned these resonances to the methyl group of N-acetylaspartate and to lipids, respectively, although we could not exclude the possible contributions of other metabolites. Spectral peaks of amino acids at 1.9 and 2.1 ppm, and of lactate β-CH_3 at 1.3 were less consistently identified. Hypoxia induced a prominent and rapid reduction of the 2.0 ppm peak. Peak levels of resonances assigned to lipids were simultaneously increased in some animals. A late but persistent increase in the signal corresponding to β-CH_3 resonance of lactate was invariably associated with lack of recovery. A return to normoxic level quickly restored the signal at 2.0 ppm. Failure of this peak to recover to baseline level, as well as substantial increases in the signals at 1.3, 1.5 and 1.7 ppm usually correlated with the animal's death.

These results suggest that ^{1}H MR spectroscopy is a useful tool for characterizing specific metabolic changes during hypoxia and recovery. The availability of this system to study animal models of hypoxia and ischemia has potential utility in screening drugs that will facilitate recovery.

37.18 ANTIHYPOXIC EFFECT OF FLUNARIZINE ON NEURONAL TISSUE IN VITRO. A. Schurr, C.A. West*, M.T. Tseng*, W.-Q. Dong* and B. M. Rigor. Departments of Anesthesiology and Anatomy, University of Louisville School of Medicine, Louisville, KY 40292.

It has been suggested that flunarizine, a fluorinated derivative of cinnarizine (Jenssen Pharmaceutica), possesses antimigraine properties. The possibility that the antimigraine action stems from flunarizine's calcium blocking capability has been refuted. Nevertheless, flunarizine's action against hypoxic damage has been investigated using different ischemic/hypoxic models, where it has been shown to have such activity. In a recent study we failed to demonstrate flunarizine's antihypoxic effect using the rat hippocampal slice preparation and attributed it to the drug's poor solubility (<1 μM) in the artificial CSF (ACSF). In the present study we used the same in vitro system while supplying flunarizine as a liposomal suspension. Rat hippocampal slices (400 μm) were maintained in a dual interface chamber, supplied with humidified 95% O_2/5% CO_2 and ACSF. Evoked field responses from the CA1 pyramidal cell layer were recorded (once/min) from one slice in each compartment of the chamber, following orthodromic stimulation of the Schaffer collaterals. Additional slices (10-15/compartment) were checked for the presence of such response at the beginning and at the end of the experiment. After 15 min of baseline recordings, slices in one compartment were perfused with phosphatidyl-choline (PC) : dicetylphosphate (DP) (10 : 0.6 μmole) liposomal suspension in ACSF (control), while the slices in the other compartment were perfused with liposomal suspension made of PC : flunarizine : DP (10 : 10 : 0.6 μmole). Each suspension (25 ml) was recirculated for 45 min. Thirty min following the application of liposomes, hypoxia was produced in both compartments (95% N_2/5% CO_2) for 15 min, after which time O_2 atmosphere was restored for 30 min.

Of 30 control slices only 5 exhibited recovery of synaptic function (evoked response>3 mV) following 15 min hypoxia. In contrast, 16 of 30 experimental slices showed such recovery following the insult ($P<0.01$). Flunarizine's lipophilicity limits its use in aqueous solutions. However, when applied as a liposomal suspension, it is readily available to the target tissue and may exert its effect in a dose-dependent fashion. Using this approach we were able to demonstrate flunarizine's antihypoxic effect on neuronal tissue in vitro.

37.19 INCREASED BRAIN AMINOPEPTIDASE A ACTIVITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT. M. J. Sullivan and A. K. Johnson. Departments of Psychology and Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242

The spontaneously hypertensive (SH) rat exhibits an increase in the half-life of angiogensin II (ANG II) and III (ANG III) introduced into the lateral ventricle compared to its normotensive counterpart, the Wistar-Kyoto (WKY) rat (Wright et al., J. Neurochem, in press). Angiotensin-induced pressor responses in the SH rat are greater in magnitude and duration than those observed in the WKY. An increase in sensitivity to other peptides has also been noted in the SH rat. These findings suggest aberrations in peptidase metabolism in the SH rat.

Aminopeptidases (APs) play a primary role in the degradation of angiotensins and other peptides in the central nervous system. In the periphery, Ang II is hydrolyzed to Ang III by membrane bound AP-A (angiotensinase). Central degradation of Ang II follows a similar pattern (Abhold and Harding, submitted). Recent data suggest that Ang III may be the biologically active form of angiotensin in the central nervous system (Fink and Brunner, 1985, Am. J. Physiol. 249; Harding and Felix, Brain Res. in press). One explanation of the sensitivity to Ang II displayed by the SH rat is an increase in Ang III production. In this study we investigated the possibility of increased central AP-A activity in the SH rat.

Adult, male SH and WKY rats were anesthetized and perfused with cold, buffered saline to remove contaminating blood peptidase activity. The whole brain was homogenized and an enriched synaptosomal fraction prepared. Colorometric assays employing L-glutamic acid -(4-methoxy- naphthylamide) as a substrate were used to quantify AP-A activity.

Brain tissue from the SH rat exhibited an increase in the metabolism of the substrate compared to tissue from the WKY. The activity was enhanced by calcium and inhibited by amastatin. Bestatin, did not significantly inhibit degradation of the substrate.

The results suggest an increase in the capacity of the SH rat to metabolize peptides such as Ang II in the central nervous system while half-life studies indicate a decrease in metabolism. These data suggest that the rate limiting step in metabolism of Ang II in the ventricles of the rat may occur following the production of Ang III. This could result in increased amounts of Ang III available to bind the angiotensin receptors and an increase in subsequent pressor responses in the SH rat. (Supported by NIH Grant HL 14388)

38.1 AMNIOTES AND ANAMNIOTES MAY POSSESS HOMOPLASTIC RETINOPETAL PROJECTIONS FROM THE ISTHMIC TEGMENTUM. T. F. Schilling* and R. G. Northcutt . (SPON: C. Gans). Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109 and SIO Neurobiology Unit and Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

Retinopetal projections that arise in the isthmus have been reported in lampreys (Vesselkin et al. 1980) and a boney fish, Polypterus (Meyer et al. 1983), as well as reptiles and birds, but do not appear to exist in cartilaginous fishes, amphibians or mammals. In the process of examining the retinofugal projections of the northern pike, Esox lucius, we discovered an isthmal nucleus with retinopetal projections, suggesting that this cell group may be widely distributed among boney fishes. Retrogradely labeled cells were observed bilaterally in a cell column in the dorsal tegmentum of the isthmus following application of HRP by either of two methods.

Gel foam pledgets saturated with a 20% HRP solution were inserted into one orbit following eye removal in each of 4 fish. After 4-6 day survival periods the animals were perfused with 0.1 M phosphate buffer followed by 4% gluteraldehyde in phosphate buffer. Transverse frozen sections cut at 35um were reacted by the method of Hanker-Yates ('77) or with diaminobenzidine as the chromagen. Fusiform cells, heavily labeled in the contralateral isthmus, occupied a position ventrolateral to labeled cells of the trochlear nucleus. These same cells were lightly labeled ipsilaterally.

In two additional fish, following eye removal, the proximal stump of the optic nerve was sucked into a small piece of polyethylene tubing and the HRP solution was injected into the tube. Brains were processed as above. In these animals the isthmic tegmental cells were labeled while trochlear cells were not. Retrogradely labeled axons of these cells left the isthmus in a compact bundle coursing through the midbrain and diencephalon to enter the medial optic tract. Therefore we are sure that they project through the optic nerve and not to extrinsic eye muscles.

It seems unlikely that this retinopetally projecting cell group in pike is homologous to the isthmo-optic nucleus of sauropsids. While the isthmo-optic nucleus receives an input from the optic tectum, unilateral HRP injections into the tectum of six pike failed to reveal a projection to this isthmic nucleus. Instead, several cell bodies were labeled ipsilaterally and apparently project to the tectum. Therefore, given the differences in phyletic distribution as well as connectivity it seems most parsimonious to conclude that isthmic nuclei with retinopetal projections have evolved independently in amniotes and anamniotes. (Supported in part by NIH grants NS11006 and EY02485.)

38.2 DOES NUCLEUS ELECTROSENSORIUS OF GYMNOTOIDS HAVE A HOMOLOGUE IN THE DIENCEPHALON OF NON-ELECTRORECEPTIVE TELEOSTS? Georg F. Striedter* (SPON: M.S. Northcutt). SIO Neurobiol. Unit and Dept. Neurosci's., UCSD, La Jolla, CA 92093.

As part of an effort to arrive at rules governing the central neural changes associated with the evolution of electroreception in teleosts, where this sensory modality has evolved at least twice independently, I have asked whether nucleus electrosensorius in the diencephalon of gymnotoids can be homologized to nuclei in the diencephalon of closely related, non-electroreceptive teleosts.

Nucleus electrosensorius (NE), which receives a massive input from the torus semicircularis, could be homologous to either a preglomerular, a pretectal, or a dorsal thalamic nucleus, or it may not have a strict homologue in non-electroreceptive teleosts and thus be a de novo structure. In cyprinids, the sister group of gymnotoids and catfishes, both the preglomerular complex (PG) and the central posterior nucleus (CP) of the dorsal thalamus receive toral input, but only the former has been shown to have connections with the telencephalon. The pretectal nuclei, in contrast, are not known to receive a toral input, but are typically interconnected with the retina and/or the optic tectum. To discriminate among the four hypotheses I injected HRP into the telencephalon and tectum of Apteronotus leptorhynchus (6 and 4 cases, respectively) and, after survival times of up to 14 days, examined the brains for label according to the method of Hanker-Yates. Although I find labelled fibers passing near NE after both tectal and telencephalic injections, I can observe neither labelled cells nor terminal fields in NE. However, after telencephalic injections both cells and terminals are clearly labelled in the preglomerular area just ventral to NE. The pretectal area of gymnotoids has several derived features, including a hypertophied nucleus paracommissuralis and a hypertrohpied dorsal periventricular nucleus. NE, however, receives neither tectal nor retinal (Sas and Maler, '86) input, and is therefore probably not a pretectal nucleus.

Thus, using strict connectional criteria, NE is not homologous to any pretectal or preglomerular nucleus in cyprinids. NE may be homologous to CP of the dorsal thalamus in cyprinids, but additional information on the connections of CP is needed to test this hypothesis. Alternatively, NE may be a de novo structure without a strict homologue in non-electroreceptive teleosts. Supported by NIH grants NS24869 and NS24669 to R. G. Northcutt.

38.3 TELENCEPHALIC AND RETINAL PROJECTIONS ALLOW REINTERPRETATION OF THE DIENCEPHALON IN MORMYRIDS. M. F. Wullimann and R. G. Northcutt. SIO Neurobiol. Unit and Dept. Neurosci's., UCSD, La Jolla, CA 92093.

African mormyrid fishes are one teleost group that has evolved electroreception. The related neuroanatomical changes obscure a comparative morphological interpretation of certain brain subdivisions in mormyrids. One example is the diencephalon, which contains relay centers of ascending and descending sensory systems. Most teleosts exhibit - from dorsal to ventral - four retinofugal diencephalic nuclei, which project in turn to the cerebellum: dorsal periventricular pretectal (PPd), central pretectal (CPN), dorsal (DAON) and ventral accessory optic nucleus (VAON)(Northcutt and Wullimann, '86). Recently, it was demonstrated that in the mormyrid Gnathonemus petersii three diencephalic nuclei project to the corpus cerebelli: nucleus of the posterior commissure (NPC), nucleus glomerulosus (NG) and dorsal anterior pretectal nucleus (DAP)(Meek et al., '86). Only NPC, however, receives retinal input (Lazar et al., '84) and might therefore be homologous to PPd of other teleosts. Two explanations for this discrepancy seem possible: 1) NG and DAP have lost the retinal input due to a reduction in vision in mormyrids, and the nuclei are indeed homolcgues of CPN and DAON in other teleosts; 2) alternatively, the cerebellopetal pattern in mormyrids is derived among teleosts, and the nuclei under consideration are not homologous to CPN and DAON. To evaluate these two hypotheses we used horseradish peroxidase (HRP, Sigma VI) to examine retinal projections and telencephalic connections of the diencephalon in Gnathonemus petersii. After 2 - 7 days survival times at 27^{0}C the animals were perfused with 1% Heparin in teleost ringers, followed by 4% glutaraldehyde in phosphate buffer (0.1M, pH 7.4). Transverse brain sections (35u) were reacted according to a Hanker-Yates or Adams (heavy metal intensification of DAB) protocol. Our results indicate that NG may be homologous to CPN, as its lateral portion receives retinal input. No DAON or VAON can be identified, however, based on a retinal terminal field. Although DAP is devoid of optic fibers, it does receive extensive projections from the telencephalon and, in addition, gets an input from the torus semicircularis (Finger et al. '81), all of which is typical of nucleus preglomerulosus in other teleosts (Northcutt and Wullimann, '86) but not of DAON. We therefore suggest, that DAP is a subdivision of the preglomerular nucleus, related to electrosensory information, which has evolved a new cerebellar projection rather than being a homologue of DAON. Supported in part by the Swiss National Science Foundation (MFW) and NIH grants EY02485, NS24869, and NS24669 (RGN).

38.4 DEVELOPMENT OF THE MECHANO- AND ELECTROSENSORY SYSTEMS IN EIGENMANNIA VIRESCENS (Gymnotiformes, Teleostei) H.A. Vischer, W. Heiligenberg and R.G. Northcutt
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South American weakly electric fish of the species Eigenmannia virescens were induced to spawn by simulating the conditions of the rainy season. Developmental stages were determined by age and mean standard length (MSL). Mechanosensory neuromasts and electrosensory ampullary and tuberous organs were studied by means of Scanning Electron Microscopy (SEM), whole-mount skin preparations, serial sections, and vital staining with 0.015% Methylene Blue, so that details of individual neuromast receptor cells could be recognized in live embryos.

The first neuromasts appear on the head (3.5 days, 5.9 mm MSL), while the lateral line primordium of the trunk begins to migrate ventro-caudally. At day 4 (6.3 mm MSL) the neuromasts on the head begin to form supra- and infraorbital and mandibular lines. The neuromasts on the trunk begin to give rise to a ventral and then a median line at day 6 (7.1 mm MSL). Those of the preopercular and otic line arise at day 5 (6.6 mm MSL). After day 10 (8.3 mm MSL) the total number of neuromasts remains fairly stable, with small variations among the different areas. The neuromasts develop roughly in four stages. First the neuromast receptor cells form an apical cavity which is situated just below the surface; subsequently the overlying epidermal layer subsides and fuses with the cavity, and the receptor rises to the surface. At day 8 (8.0 mm MSL) the first cupulae of the neuromasts begin to elongate in an axis perpendicular to the major axis of the later canals. The head canals start to close on day 17 (13 mm MSL), while the trunk canal begins to form on day 22 (16 mm MSL).

Tuberous organs can first be recognized on day 6 (7.0 mm MSL), while ampullary organs are identifiable from day 7 (7.4 mm MSL) on. On the head, both types develop in rows parallel to the neuromast lines. On the trunk, ampullary organs appear rostrally to caudally in three parallel lines.The first ampullary organs occur among the neuromasts of the median trunk line. Subsequently more dorsal and ventral accessory lines develop adjacent to the dorsal midline of the trunk and on the anal fin. Tuberous organs develop along the myosepta.

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38.5 DEVELOPMENT OF THE LATERAL LINE SYSTEM OF THE CHANNEL CATFISH. R. Glenn Northcutt. SIO Neurobiol. Unit and Dept. Neurosci's., UCSD, La Jolla, CA 92093

Silurid teleosts, like most nonteleost fishes, possess electroreceptive and mechanoreceptive lateral line organs. However, the phylogenetic distribution of teleost electroreceptors, as well as their anatomy and physiology, suggest that they are homoplastic to the ampullary organs of nonteleost fishes. Although there is considerable information on the origin and development of mechanoreceptive neuromasts from ectodermal placodes in amphibians and fishes, there is little information on the development of silurid electroreceptors (Sato, '56, Annot. Zool. Jap. 29:207-212).

Developing embryos and larvae of Ictalurus punctatus were staged following Armstrong and Child (Stages in the Development of Ictalurus nebulosus, Syracuse Univ. Press, 1962) and fixed in 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Head and trunk ectoderm were removed by microdissection and stained with 1% methylene green to reveal differentiating lateral line organs. Whole embryos were postfixed in 1% osmium tetroxide and prepared for scanning electron microscopy. Finally, embryos and larvae were embedded in methacrylate and transverse sections (3µm) cut to compare with the ectodermal preparations. At 22-24°C embryos hatch at stage 43 (day 8). By this stage the lateral line placodes of both the head and trunk have completed migration, and primordia of the neuromasts can be recognized. Neuromast primordia of the head differentiate and erupt to the surface by stage 47 (days 10 and 11), and canal formation begins by stage 49 (day 12). Formation of the trunk canal begins much later (approximately 40 days post-fertilization). The first primordia of external taste buds can be recognized at stage 43 (day 8), and the primordia of electroreceptors can be definitely recognized by stage 48 (day 11). Taste bud primordia erupt by stage 48 (day 11) and electroreceptor primordia by stage 50 (day 13). Similarities in the early development of taste buds and electroreceptors complicate the interpretation of the embryonic origin of the electroreceptors. However, the first recognizable electroreceptors develop adjacent to the tissues forming canal neuromasts, which is consistent with a hypothesis that both electroreceptors and neuromasts arise from the same placodes.

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38.6 TYROSINE HYDROXYLASE AND SEROTONIN IMMUNOREACTIVITY IN THE BRAIN OF AN ELASMOBRANCH, PLATYRHINOIDIS TRISERIATA. W. L. R. Cruce, S. L. Stuesse, and R. G. Northcutt. Neurobiology Dept. N.E. Ohio College of Medicine, Rootstown, OH 44272; and Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CA 92093.

Elasmobranchs exhibit a wide range of brain to body weight ratios, overlapping those of bony fishes, as well as birds and mammals. We determined the distribution of immunoreactivity to tyrosine-hydroxylase (catecholaminergic cell groups) and 5-hydroxytryptamine (5HT, serotonergic) in the brain of the thornback guitarfish, an elasmobranch with a low brain:body ratio. 5HT and TH cell groups have been well characterized in rats, thus the presence or absence of various nuclei in this elasmobranch could be compared with a mammalian brain. Thirteen P. triseriata were collected and kept in the laboratory. Nine were given injections of colchicine to block axonal transport; 4 were untreated. The guitarfish were anesthesized, perfused, and 20-40 um frozen transverse serial sections were made of the brain. The sections were incubated with rabbit antibodies to either TH or 5HT. Antibody location was determined by a goat anti-rabbit secondary antibody conjugated to fluorescein or by a biotinylated secondary antibody and avidin-biotin peroxidase histochemistry.

TH positive cells were found throughout the dorsal and lateral pallium. Numerous TH positive cells were found in the diencephalon (preoptic, suprachiasmatic, ventromedial and ventrolateral thalamic nuclei, and the posterior tuberculum). In the mesencephalon, TH cell groups were found in raphe linearis, the ventral tegmental area, and substantia nigra. The rhombencephalon contained TH positive cells in the locus coeruleus, subcoeruleus and possibly an A7 group. Probable A2/C2 and A1/C1 groups were also located, but no group which corresponded to A5 was found. Many serotonergic cell groups were found in the paraventricular hypothalamic organ and extended into the pituitary. In the mesencephalon, 5HT cells were found in raphe linearis and in the nucleus reticularis pedunculopontinis pars compacta. Cells in metencephalon were found in reticularis pontis oralis lateralis and medialis, the reticulo-tegmental nucleus, nucleus centralis superior, reticularis magnocellularis, and reticularis pontis caudalis. In the mylencephalon additional 5HT cells were found in raphe pallidus, reticularis paragigantocellularis lateralis, and reticularis ventralis pars alpha. The cell morphologies of these reticular groups were strikingly similar to those of rat. Thus this guitarfish has most of the 5HT and TH cell groups found in rats with the major exception that no 5HT cells were found in a nucleus which might correspond to raphe dorsalis. (Supported by grants from the Ohio Board of Regents and the National Institutes of Health.)

38.7 **PROJECTIONS OF GIANT FIBERS IN THE BRAIN OF THE HATCHETFISH (GASTEROPELECUS).** M.A. Barry and M.V.L. Bennett. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Giant fibers (GFs) are large axons which receive excitatory input at axo-axonic synapses from both Mauthner cells and in turn form excitatory synapses on pectoral fin motoneurons. GFs are responsible for the bilateral components of the Mauthner cell-mediated startle response. The present study revealed extensive GF projections to central targets other than the pectoral fin motoneurons.

Physiologically identified giant fibers were filled intracellularly with Lucifer yellow. The GF cell bodies are located in a column adjacent to the IVth ventricle just dorsal and lateral to the medial longitudinal fasciculus (MLF). The dendrites are numerous and very fine. The single axonal process decussates at the level of the cell body, and bifurcates to form ascending and descending branches within the MLF. The descending process and its projections to the pectoral fin motoneurons have been described previously (Gilat et al., Brain Res. 365: 96, 1986). However some collaterals project into the dorsal columns at the level of the rostral spinal cord. The ascending branch has numerous collaterals which appear to terminate in the trigeminal, facial, and oculomotor nuclei, but probably not the abducens nucleus. Collaterals also terminate on small cells adjacent to the Mauthner cell ventral dendrite, which are probably part of the Mauthner inhibitory network (PHP cells, Korn and Faber, J. Neurophysiol. 38: 452, 1975). There are no direct GF projections to the Mauthner cell. Other collaterals at many levels of the rhombencephalon terminate in medial parts of the reticular formation. These projections may participate in later components of the startle response or may constitute part of a general arousal pathway.

The morphology and projections of GFs in adult hatchetfish are virtually identical to those of T-reticular neurons described in zebrafish larvae (Kimmel et al., J. Comp. Neurol., 233: 365, 1985); thus these cells can be considered as homologs. The cranial relay neurons described in goldfish (Hackett and Faber, Neurosci. 8: 317, 1983) have rostral projections similar to those of GFs and T-reticular cells, but lack a descending process and projections to the reticular formation.

Fishes possess a startle reflex system not requiring Mauthner cell activation (Eaton et al., J. Comp. Physiol. 145: 485, 1982). The presence of numerous synapses on GF somata (Model et al., Brain Res. 45: 288, 1972) and of an extensive dendritic field suggests that GFs are capable of operating independently of the Mauthner cell. Thus, they may participate in non-Mauthner as well as Mauthner mediated startle reflexes.

38.8 AFFERENT CONNECTIONS OF THE VENTRAL TELENCEPHALON OF THE GOLDFISH. H.E. Sloan and L.S. Demski. School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Neurons of the telencephalon and diencephalon were labeled following injections of 60-80 nl of 4% horseradish peroxidase into the pars ventralis (Vv) and pars supracommissuralis (Vs) of the ventral telencephalon. In the telencephalon labeled cells occurred bilaterally in the central area of the dorsal telencephalon (Dc). In the following areas a preponderance of ipsilateral positive cells were observed in: 1) the central area of the ventral telencephalon (Vc) ; 2) the dorsal nucleus of the ventral telencephalon (Vd); 3) Dc, Vd, Vv, Vs and the anterior portion of the nucleus preopticus parvocellularis (PPa) at the level of injection; 3) Vd, Vs, PPa caudal to the injection site at the level of the anterior commissure. Filled cells were found only on the ipsilateral side in: 1) an area ventrolateral to Vd and the postcommissural nucleus of the ventral telencephalon (Vp); 2) the intermediate nucleus and the medial portion of nucleus taenia of the caudal telencephalon. Labeled fibers of an unknown origin were found in the ipsilateral lateral zone of the dorsal telencephalon. Other filled fibers (mostly ipsilateral) were observed in the olfactory tracts, in Vd, in the medial forebrain bundle and the laterally extending tracts from the anterior commissure.

Bilaterally in the diencephalon filled cells were present in the nucleus dorsalis posterior of the thalamus at the level of the posterior commissure (PC). Labeled neurons were also found in the nucleus tuberis posterior and the periventricular nucleus of the posterior tuberculum also at the level of PC (mostly ipsilateral). Positive cells, only observed on the side of the injection, were found: 1) in the lateral preoptic are situated between the entopeduncular nucleus and PPa; 2) at the lateral perimeter of the nucleus preopticus magnocellularis, pars magnocellularis; 3) in the dorsal hypothalamic zone at the level of the habenular nuclei; 4) dorsal to the caudal hypothalamic zone. Fibers of an unknown origin were observed in the medial forebrain bundle, lateral to the dorsal hypothalamic zone at the level of the habenular nuclei, and in the area dorsal to the caudal hypothalamic zone, at the level of and caudal to the posterior commissure. Most of these are ipsilateral.

Larger Vv-Vs injections labeled terminal nerve ganglion cells, olfactory mitral cells and somata in the locus coeruleus. Supported in part by a Biomedical Sciences Research Support Grant RR 07114.

38.9 **AN IMMUNOCYTOCHEMICAL ANALYSIS OF THE DISTRIBUTION OF CATECHOLAMINE-SYNTHESIZING ENZYMES IN THE GOLDFISH BRAIN.** P.J. Hornby* and D.T. Piekut. Neuroendocrine Unit, University of Rochester, Rochester, NY 14642.

The distribution of presumptive-dopaminergic and noradrenergic neurons is elucidated in the goldfish brain for the first time by use of immunocytochemical techniques and antibodies to the catecholamine-synthesizing enzymes, tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), and phenylethanolamine-N-methyltransferase (PNMT). The use of these antibodies provides a means for specifically marking each neuronal system, i.e., dopaminergic structures are TH-immunoreactive (-ir) only, noradrenergic structures are TH-and DBH-ir, and adrenergic neurons are PNMT-ir.

In the hindbrain, presumptive-dopaminergic and noradrenergic neurons are codistributed in three anatomically distinct and separate regions which are; 1) the dorsal surface of the postobecular region, 2) the central medullary tegmentum from the level of the greatest expansion of the vagal lobe to the medullospinal transition, and 3) the isthmal tegmentum dorsolateral to the medial longitudinal fasciculus in the locus ceruleus. DBH-ir neurons are not observed rostral to the hindbrain; however, a dense DBH innervation of the diencephalon is observed in periventricular regions, basal hypothalamus, and inferior lobe. Presumptive-dopaminergic neurons are observed following the diencephalic regions; 1) the posterior tuberal nucleus, 2) the paraventricular organ, 3) the ventromedial thalamic nucleus, 4) the nucleus pretectalis periventricularis, pars ventralis, 5) the suprachiasmatic nucleus, and 6) the preoptic nucleus. In the telencephalon, TH-ir neurons are observed in the area dorsalis telencephali, pars centralis, the area ventralis telencephali, and the olfactory bulb. Controls for the specificity of immunocytochemical staining included the preadsorption of both antisera with their respective enzymes prior to incubation of tissue sections, in which cases the immunoreaction product is completely eliminated. PNMT-ir structures are not observed in the goldfish brain.

These data provide an anatomical basis for the functional role of catecholamines in the fish brain, for example, the localization of dopaminergic neurons which modulate the release of pituitary hormones. In addition, this study has provided evidence for fundamental similarities in the organization of hindbrain catecholamine systems in vertebrates, for example, TH- and DBH-ir neurons are localized in the medullary tegmentum and locus ceruleus in both fish and mammals. Finally, certain features of the catecholamine system may be unique to teleosts, for example, the presence of presumptive-dopamine neurons in the telencephalon.

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38.10 CHOLINERGIC STRUCTURES IN THE BRAIN OF A SONIC ("VOCALIZING") FISH LOCATED WITH A MONOCLONAL ANTIBODY TO CHOLINE ACETYLTRANSFERASE. R. K. Brantley and A. H. Bass. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

A well-characterized monoclonal antibody to choline acetyltransferase (ChAT) has allowed localization of putative cholinergic structures (ChAT-like immunoreactive, ChAT-IR) in the brain of a teleost fish, the midshipman, Porichthys notatus (Batrachoididea, toadfishes). ChAT-IR cells were found in: cranial motor and sensory nuclei, the reticular formation, mesencephalic tectum, and the telencephalon. Ten male midshipmen were used in the study. Brains were sectioned and free floating sections reacted with primary antibody (gift of Dr. Bruce Wainer, University of Chicago) and then visualized with the peroxidase anti-peroxidase method. Visualization of terminals and fibers was enhanced through osmium tetroxide treatment.

Cranial motor nuclei III, IV, V, VI, VII, IX, and X are robustly immunoreactive. The sonic motor nucleus, which is located at the junction of the spinal cord and medulla, and which innervates swimbladder "drumming" muscles, also labels intensely. ChAT-IR cells are sparsely distributed within three cranial sensory nuclei: nucleus solitarius, the trigeminal descending nucleus, and the trigeminal principal nucleus.

Superior, medial, and inferior divisions of the reticular formation contain subpopulations of ChAT-IR cells, which often send long processes ventrolaterally. The cerebellum also contains a few labelled cells in the granule cell layer.

Other ChAT-IR brainstem sites include nucleus isthmi, the periventricular layer of the tectum, and the octavolateralis efferent nuclei which project to peripheral lateral line, acoustic, and vestibular end organs.

In the telencephalon, ChAT-IR cells are found in the ventral nucleus of area ventralis (Vv), an area that has been compared to the medial septal nucleus of tetrapods (Northcutt and Braford, in: Comp. Neurol. of the Telencephalon, Ebbesson, ed., Plenum, 1983), a location of cholinergic neurons in mammals. ChAT-IR fibers and dense patches of punctate structures suggestive of terminals are found in restricted portions of the central (Dc), dorsal (Dd), and medial (Dm) zones of area dorsalis. Dd has been compared to the dorsal pallium, and portions of Dc and Dm to the striatum of tetrapods. Among mammals, these zones are also sites of cholinergic terminals. The source of cholinergic inputs to the pallium of Porichthys is not clear, although it does not appear to be the ChAT-IR cells found in Vv.

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38.11 LONG ASCENDING FIBERS IN THE WEAKLY ELECTRIC TELEOST GNATHONEMUS PETERSII. T. Szabo, S. Libouban°, Dept. Neurophysiol. Sensorielle, Lab. Physiol. Nerveuse, C.N.R.S., 91190 Gif sur Yvette, France.

The existence of long descending pathways has been demonstrated in teleost fishes with tracing techniques (Kimmel et al., JCN 205: 113, 1982, Hlavacek et al., J.Hirnforsch. 25:603, 1984); in contrast, similar information about long ascending fibers in this category of vertebrates is still lacking.

Experiments with the Fink-Heimer degeneration method and HRP axonal transport were conducted in order to identify long ascending fibers in the spinal cord of the mormyrid G.petersii. Sections of the spinal cord or injections of 30% HRP were performed at the 30-32nd segments (total number of 42). After a survival time of 2-3 weeks the animals were fixed either by immersion in 10% formaldehyde or by transcardial perfusion with a 2% glutaraldehyde and 2% formaldehyde mixture. In HRP injected animals the brain sections were treated according to Hanker et al. (1977).

Observations of degenerated as well as labeled fibers permitted detection of three ascending fiber systems in the spinal cord of G.petersii. The largest is the dorsal column which receives direct fibers from the periphery entering via dorsal roots at each segment into the spinal cord. The fibers course up to the medulla where they end in the posterior lateral funicular nuclei (Fl2). The latter do not correspond to a lemniscal system of higher vertebrates since they project to the cerebellum and not to the thalamus (Libouban and Szabo, Neurosci.Lett.6:115, 1977). A second ascending fascicle is revealed in the ventral marginal sector of the lateral column. It originates from large cells in the ventral horn and runs towards the medulla giving off numerous collaterals to the lateral reticular nucleus. Coursing further rostrally it passes anteriorly round the lateral lobe and enters into the caudal lobe where it terminates after crossing the midline. This pathway may correspond to the ventral spinocerebellar tract of higher vertebrates. A third long fiber fascicle is detected in the dorsal part of the lateral column next to the dorsal gray. The fine fibers which constitute this ascending bundle extend to the medulla where they likely terminate in the anterior region of the descending trigeminal nucleus. Their origin could not be identified.

The earliest phylogenetic appearance of long ascending fibers previously demonstrated has been in the amphibian dorsal column (Szabo, Arch.Sc.Physiol. 9:27, 1955). The results show that long ascending fibers occur in fish, although their existence may be a peculiarity of the family Mormyridae, as is the hypertrophied cerebellum.

38.12 CALCITONIN-GENE-RELATED-PEPTIDE-LIKE (CGRP) AND CHOLECYSTOKININ-LIKE (CCK) IMMUNOREACTIVITIES ASSOCIATED WITH BRAINSTEM VISCERAL AND GUSTATORY NUCLEI IN THE GOLDFISH. T. E. Finger and C.E. Adams, Dept. Cellular & Structural Biol., U. Colo. Hlth. Sci. Ctr., Denver, CO 80262.

In the brainstem of goldfish, the general visceral sensory and gustatory sensory functions are processed in separate primary sensory nuclei. The primary gustatory nuclei are themselves divisible into vagal, glossopharyngeal and facial lobes, each of which deals with gustatory afferentation from a single cranial nerve. Further, the visceral motor nuclei are clearly divisible into branchiomotor and general visceral subnuclei. In our continuing series of studies on these nuclei, the distribution of two neuropeptides, CCK and CGRP, was studied in relation to the known visceral and gustatory centers. CCK-like and CGRP-like immunoreactivities were studied by means of the peroxidase-anti-peroxidase technique as applied to frozen free-floating sections prepared from goldfish, Carassius auratus, which had been perfused with 4% paraformaldehyde in 0.1 M phosphate buffer.

Both CCK and CGRP immunoreactive (ir) fibers are present in layer ii in the superficial part of the vagal lobe. The CGRPir fibers enter the vagal lobe with the superficial roots of the vagus nerve to fan out across the surface of the vagal lobe, as has been described previously for substance P-ir fibers (Finger, 1984). The CCKir plexus is coincident with that of the CGRPir plexus, but CCKir fibers could not be seen entering with the vagal sensory fibers. CGRPir but not CCKir is present in low levels within somata of the motor neurons situated deep in the vagal lobe. In contrast, a plexus of CCKir fibers and terminals is present in the outer, neuropil layer (layer xiii) of the motor layer of the vagal lobe. The CCKir terminals in this layer arise from a CCKir fiber bundle which courses along the ventrolateral margin portion of the pontine and medullary tegmentum. This CCKir fiber bundle also gives rise to a terminal plexus in the vicinity of the proximal dendrites and somata of some vagal general visceral efferent neurons (= dorsal motor nuc. of vagus).

Populations of CGRPir somata exist within the tegmentum of the pons and caudal medulla. The strongly immunoreactive CGRPir neurons of the pons lie dorsal to the lateral lemniscus at approximately the level of the locus coeruleus. A less immunoreactive, but more numerous population of CGRPir neurons extends from the rostral pons into the caudal midbrain. These neurons are situated at the ventral margin of the secondary gustatory and secondary general visceral nuclei. A heavy plexus of CGRPir fibers occurs around the nuclei surrounding the lateral recess of the third ventricle within the inferior lobes.

38.13 LUNGFISH POSSESS A NERVUS PRAEOPTICUS (PINKUS' NERVE) AND A NERVUS TERMINALIS. D.L. Meyer* and C.S. von Bartheld* (SPON: D. Bonke). Dept. of Neuroanatomy, University of Göttingen, Federal Republic of Germany.

Pinkus (1894, 1895) described a new cranial nerve in the lungfish. This nerve was later named the "Nervus praeopticus" (Sewertzoff 1902). However, Locy (1905) identified Pinkus' nerve as the homologue of the Nervus terminalis, a cranial nerve that had been described in several other vertebrates. This interpretation has to the present time been accepted. Our study provides evidence that Pinkus actually described a cranial nerve other than the terminal nerve.

Our data are obtained from the African lungfish (Protopterus dolloi) which received injections of horseradish peroxidase (HRP) into the olfactory epithelium. Brain sections were cut in a cryostat and processed for HRP activity. In addition to the primary olfactory projection, a fasciculated fiber tract, consisting of four bundles, was labeled in the brain. This pathway courses with the olfactory nerve and bypasses the glomerular layer of the olfactory bulb on its caudal course through the dorsomedial telencephalon (archipallium). One fascicle branches off ventrally, passes through the archipallium, and reaches the neuropil adjacent to the septum. The dorsal fascicles partly terminate in the archipallium, and partly turn ventrally towards the septal areas just rostral to the anterior commissure. No other structures in the brain were labeled. Therefore, we discard the possibility of false positive results by transneuronal (secondary olfactory) transport of the tracer.

We identify this fiber tract as the central projection of the terminal nerve. The terminal nerve of Protopterus is clearly distinct from the Nervus praeopticus (Pinkus' nerve) which follows an extracerebral course below the telencephalon and enters the brain in the preoptic area. Therefore, Protopterus possesses two distinct rostral cranial nerves: the terminal nerve, associated with the olfactory nerve and tract, as in other vertebrates, and a Nervus praeopticus, which has obviously been misinterpreted as being the terminal nerve of lungfish for almost a century. The Nervus praeopticus has so far only been described in the lungfish (Dipnoi). Hence, Pinkus did not describe what nowadays is considered the terminal nerve, but rather he discovered a structure which has not yet received appropriate scientific interest.

Pinkus, F. (1894) Anat. Anz. 9:562-566; Pinkus, F. (1895) Morph. Arb. 4:275-346; Sewertzoff, A.N. (1902) Anat. Anz. 21:593-608; Locy, W.A. (1905) Anat. Anz. 26:33-63 and 111-123.

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38.14 COMPARATIVE STUDIES OF LHRH AND CRF NEURONAL SYSTEMS IN TWO SPECIES OF TELEOST. T.O. Bruhn, E.L.P. Anthony, and I.M.D. Jackson. Division of Endocrinology, Brown University/Rhode Island Hospital and Department of Biology, Rhode Island College, Providence, RI 02902.

Previously we reported the isolation and characterization of a LHRH-like peptide in codfish (Gadus morhua) brain. Our studies revealed that the peptide was identical to salmon LHRH (sLHRH). We also partially characterized a CRF-like peptide in codfish brain and showed that this material was different from known mammalian CRFs and urotensin I, a CRF-like peptide previously isolated from the urophysis of sucker fish. In the present study we report the immunohistochemical localization of LHRH and CRF in the hypothalamus of two species of teleost, codfish and alewife (Alosa pseudoharengus), respectively. Fish were obtained from the New England Aquarium (Boston, MA); brains and pituitaries were rapidly removed and immersed in either acrolein (5% for 2 hr) or paraformaldehyde/glutaraldehyde (4%/1% for 6-12 hr). Fifty to 100 um sections were cut and processed for immunohistochemistry using standard methods. Antisera #292 (anti sLHRH) and #C-24 (anti-oCRF; gift of Dr. W. Vale) were used at a final dilution of 1:500 in conjunction with the PAP-DAB technique or at 1:2,000 with the ABC-DAB technique. Both antisera were previously characterized by RIA and immunohistochemistry. Specificity was demonstrated by complete absorption of immunostaining by 1 ug peptide/ml undiluted antiserum.

Our studies revealed a striking species variation in the innervation of the pituitary gland. LHRH-immunoreactive (LHRH-i) and CRF-i fibers appear to project directly to the pars distalis of codfish, whereas few immmunoreactive fibers were seen in the neurointermediate lobe. In the alewife, on the other hand, the majority of LHRH-i and CRF-i fibers were found in the neurointermediate lobe; few scattered fibers could be demonstrated in the pars distalis. This different pattern of projection is interesting since perikarya for each peptide were found in similar locations in both fish, the nucleus praeopticus (LHRH) and nucleus lateralis tuberis (CRF), respectively.

Since the hypophysial portal system is absent in most fish, hypophysiotrophic neurons may directly innervate the target organ or reach the pars distalis via the neurointermediate lobe. This anatomical variation suggests a difference between these teleost species in the regulation of pituitary function by LHRH and CRF.

38.15 CENTRAL PROJECTIONS OF THE PRIMARY OCTAVOLATERALIS AFFERENTS IN THE TOADFISH. R. D. Kitch*, S. M. Hightein, and R. Baker (SPON: C. Scudder). Dept. Otolaryngology, Washington Univ., St. Louis, MO. 63110 and Dept. Physiol. and Biophys., New York Univ., N.Y. 10016.

Eighth nerve branches of anesthetized adult toadfish were labeled with HRP and alternate frozen brainstem sections reacted with TMB/DAB. The octavolateralis area was divided into seven nuclei after McCormick (J. Morphol. 171:159 ,'82). The three lateral line nerves terminated in the most dorsally located cell column, in n. medialis and n. caudalis. These nuclei form a long, continuous cell column bounded laterally by the ascending and descending LL tracts. The three LL nerves terminate throughout the length of the entire cell column; the territory of the PLL nerve is predominantly dorsal, that of the fifth nerve division of the ALL nerve is ventral, while that of the seventh nerve division of the ALL nerve occupies an intermediate territory. Each of the LL nerves projects as well to the dorsal region of n. magnocellularis (see below).

Ventral to the LL column, throughout its length, five contiguous nuclei can be distinguished. From rostral to caudal these are the anterior octavus n. (AO), n. magnocellularis (Mg), n. tangentialis (T), descending octavus n. (DO), and posterior octavus n. (PO). The central projections of the three SCCs are overlapping and will be described together. They cover the entire territory of AO, T and PO, and the lateral and ventral aspects of both Mg and DO. The saccular and lagenar projections to the AO and PO overly those of the SCC; however, they do not project to T, and, in contrast to the SCC, project dorsally to DO and Mg. The utricular nerve projections cover the entire territory of AO, T, and PO, go to the medial and ventral regions of Mg, and are seen diffusely throughout DO, overlying both saccular and SCC projections.

All nine of the octavolateralis nerves send primary afferent fibers to the eminentiae granulares (EG). Canal input was substantial and diffuse to both the lateral and medial portions of the rostral EG. LL input was mainly to a more caudal portion of the lateral EG but there was a consistent tract that projected to the rostromedial EG and may represent some overlap of octavus and lateralis innervation. Of the seven nuclei discussed above, only Mg receives dual innervation from lateralis and octavus afferents. (Supported by NIH NINCDS-52946 and NS 21055).

38.16 PROJECTIONS OF THE PINEAL COMPLEX IN THE SILVER LAMPREY. R. L. Puzdrowski and R. Glenn Northcutt. Div. Biol. Sci., Univ. of Michigan, Ann Arbor, MI 48109 and SIO Neurobiol. Unit and Dept. Neurosci.'s, UCSD, La Jolla, CA 92093.

Recently, experimental anatomical techniques have been applied in the investigation of the pineal connections in a variety of vertebrates. As a continuation of these investigations the central connections of the pineal complex of the silver lamprey, Icthyomyzon unicuspis, were determined utilizing HRP techniques.

Both juvenile and adult silver lampreys were used in this study. The central projections of the pineal complex were determined by innoculation of the pineal complex with HRP paste on the tip of a sharpened insect pin. Following survival times of 5-7 days, at 10-18°C, the animals were reanesthetized with MS222 and transcardially perfused with phosphate buffer (pH 7.4) followed by a 2% glutaraldehyde solution. The brains were transversely sectioned at 35µm and alternate sections processed according to a Hanker-Yates ('77) protocol or the Adams ('81) heavy metal intensification protocol.

The pineal tract courses caudally through the tela choroidea along the left side of the habenular commissure. A few fibers penetrate the brain through the caudal most portion of the habenular commissure; however, the majority of fibers continue caudally and enter the brain at the level of the posterior commissure. The pineal tract projects bilaterally to the subcommissural organ, nucleus of the posterior commissure, posterior tubercular nucleus, subhabenular nucleus, dorsal and ventral thalamus and hypothalamus, the optic tectum and the tegmentum. A few fibers were observed decussating in the ventral tegmental commissure. The course of these decussated fibers could not be followed. No retrogradely labeled cells were found in the present study.

With the exception of projections to the optic tectum, the pineal projections of lampreys are similar overall to those reported in teleosts (Ekstrom and van Veen, '83) and amphibians (Eldred et al., '80). It appears that these connections represent the primitive condition for vertebrates and that there has been a reduction in pineofugal connections in the amniotic vertebrates.

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38.17 RETROCEREBRAL PROJECTIONS FROM THE BRAIN AND SUBESOPHAGEAL GANGLION OF THE CRICKET. D. Moore and W. Loher*. Department of Entomological Sciences, University of California, Berkeley, CA 94720

Occupying a critical position in the control of physiological and behavioral events in insects, the retrocerebral complex consists of the paired corpora cardiaca (CC) and corpora allata (CA) as well as nerves connecting these structures to the brain, to each other, and, in some orders, to the subesophageal ganglion (SEG). The CC is a neurohemal organ for the release of neurosecretory material produced by neurons in the brain and also contains intrinsic hormone-producing cells. The CA is an endocrine gland for the production of juvenile hormone, which has significant effects on metamorphosis, egg production, and sexual behavior. Despite the importance of the brain and, possibly, the SEG as regulators of numerous neuroendocrine and endocrine functions, in no insect have the axonal pathways between these ganglia and the retrocerebral complex been completely characterized.

The origins and axonal trajectories of neurons projecting to the retrocerebral complex of the cricket, Teleogryllus commodus, were examined in silver-intensified nickel backfills. Spatially separate groups of somata in the pars intercerebralis (PI) and in the pars lateralis (PL) of the brain, commonly accepted as neurosecretory loci, were found to give rise to axons terminating in the nervus corporis allati 2 (NCA 2), the CA, or the CC. Additional findings demonstrated a distinct group of somata from the PI whose axons run in the esophageal nerve (stomatogastric nervous system), nine somata in the SEG with axons that project through the NCA 2 as far as the CA but do not invade the CC, and also a small cluster of tritocerebral perikarya with axons terminating in the CC. Most significantly, somata residing in the PI and PL were found to be compartmentally organized, based upon the retrocerebral destinations of their axons.

Fibers from the brain neurosecretory centers have apparent terminals in several different retrocerebral locations: the CC, CA, NCA 2 (nerve connecting the CA with the SEG), and the NCA 1 (nerve between the CA and CC). Thus, all of these structures may be considered putative neurohemal release sites. The NCA 2 in crickets has previously been proposed as a neurohemal organ, based on the presence of "synaptoids" as viewed in EM sections of the nerve (Weber, W. & Gaude, H., Z. Zellforsch. 121:561, 1971). We show that these terminals originate from somata in both the PI and PL of the brain. Classically considered as distinct protocerebral loci, the PI and PL in T. commodus exhibit an unexpected proximity; at their closest points, they are separated by no more than 40 micrometers.

Support: NIH postdoc. (DM) and NIH grant HD 03619 (WL).

PEPTIDES: OPIATE RECEPTORS

39.1 **ANTI-MET-ENKEPHALIN ANTI-IDIOTYPIC ANTIBODIES: OPIATE RECEPTOR BINDING STUDIES.** Bingci Liu* and Y.Y. Thomas Su (SPON: L. Denner). Center for Biotechnology, Baylor College of Medicine, 4000 Research Forest Drive, The Woodlands, Texas 77381

Anti-idiotypic (anti-ID) antibodies specific for a variety of hormone andneurotransmitter receptors have been produced following immunization against specific anti-ligand antibodies. These anti-ID antibodies are able to interact with the ligand binding sites on the membrane. In this communication, we report the generation and characterization of anti-Met-enkephalin (anti-ME) anti-ID antibodies.

Partially purified monoclonal anti-ME antibody was used as an immunogen to produce anti-ID antibodies from rabbits. Rabbits were immunized with 0.1 mg of immunogen in Freund's adjuvant biweekly. The first bleeding for testing of antiserum was made one week after the third injection. The effect of antiserum on ^{3}H-ME and anti-ME interaction was employed for testing the production of anti-ID antibodies. In these experiments rabbit anti-ME was used to test against the anti-ID antibodies.

Anti-ID antibodies that inhibited ^{3}H-ME and anti-ME interaction were further testing for their ability in the inhibition of ligand-receptor binding. In these experiments different amounts of anti-ID or preimmune serum (as the control) were incubated with 400 µg of membrane protein overnight 4°C in the presence (nonspecific binding) or absence (total binding) of 250 nM naloxone. Samples without any serum or membrane protein were also prepared. ^{3}H-DADL was then added to the reaction mixtures (final concentration 2 nM in 400 µl of solution) and incubated for one hour at 4°C. ^{3}H-DADL bound to opiate receptors was measured in a scintillation counter. The results from these studies demonstrated that anti-ID serum exhibit a concentration dependent inhibition of ^{3}H-DADL binding to the opiate receptors.

In other experiments the effect of anti-ID antibodies on saturation binding of ^{3}H-DADL to the opiate receptor was also studied. In the presence of anti-ID serum ^{3}H-DADL saturation binding to the opiate receptor was decreased. Scatchard analysis of the bindings yielded a set of parallel straight lines (for the control and the sample) with a K_D of 0.62 and 4.80 nM. It appeared that anti-ID antiserum produced a noncompetitive inhibition of DADL binding. All these studies indicated that anti-ID antibodies specific for the opiate receptor have been produced.

Supported by NIH grant EY03701.

39.2 AUTORADIOGRAPHIC LOCALIZATION OF 3H-alpha-MSH and of 3H-DESACETYL-alpha-MSH BINDING SITES IN RAT BRAIN. W. Lichtensteiger, M. Schlumpf and A. Eberle*. Inst. of Pharmacology, Univ. of Zürich, CH-8006 Zürich, and Zentrum für Lehre u. Forschung, CH-4031 Basel, Switzerland.

Systemic administration of alpha-MSH influences brain function, but it is still uncertain whether these effects reflect actions of the peptide released from pituitary intermediate lobe cells into the circulation, or of peptide released from alpha-MSH containing central nerve terminals. However, manipulation of endogenous alpha-MSH levels by systemic antiserum injection affects the functional state of adult central transmitter systems (Monnet and Lichtensteiger, 1981), while early postnatal antiserum injection causes a permanent alteration of the responsiveness of hypothalamic dopamine neurons to this peptide (Lichtensteiger and Schlumpf, 1986).

In an attempt to further elucidate the interaction of alpha-MSH with the central nervous system in adulthood and ontogeny, we are investigating central binding sites for alpha-MSH. The brain of adult male Long Evans rats was cut on a cryostat and processed for qualitative and quantitative in vitro-receptor autoradiography, using alpha-MSH and desacetyl-alpha-MSH tritiated according to Eberle and Zeller (1985; specific activity 115 Ci/mmol). Specific saturable binding was observed by computer assisted image analysis in several areas of the rat forebrain, i.a., in hippocampus, cortex and mediobasal hypothalamus, and in hindbrain regions. The hippocampal location is interesting in view of earlier EEG data; this area receives a projection of the non-POMC alpha-MSH-like neuron system (Khachaturian, Lewis, Kang Tsou and Watson, 1985). Binding of 3H-desacetyl-alpha-MSH is studied because the desacetylated form of the peptide appears to predominate in brain. Its general distribution resembled that of 3H-alpha-MSH. A detailed characterization of binding sites is under way.

39.3 LOCALIZATION OF MU OPIOID RECEPTORS WITHIN THE A10 REGION OF THE RAT. R.P. Dilts, P.W. Kalivas, Dept. of VCAPP, Washington State Univ., Pullman, WA.

Recent evidence indicates that stimulation of the mu opioid receptor increases output from the A10 dopaminergic cell group, including the ventral tegmental area (VTA) (Latimer et al., J. Pharmacol. Exp. Ther., 1987). To study whether this effect was caused by mu opioid receptors situated upon dopaminergic neurons or whether the enhanced activity was mediated by other neurons present within the A10 region, we utilized quantitative receptor autoradiography. The highly selective mu opioid ligand, ^{125}I-Tyr-D-Ala-Gly-N-MePhe-Gly(ol) (DAGO) was employed to assess the anatomical location of the mu opioid receptors within the mesencephalic tegmentum. Additionally, the neurotoxins 6-hydroxydopamine (6-OHDA) (10 µg free base in 1.5 µl, 0.2% ascorbate) and quinolinic acid (QUIN) (300 nmoles) were utilized to remove, unilaterally, dopaminergic and intrinsic perikarya, respectively.

Quantitative analysis of the autoradiographic pattern of DAGO binding sites within the A10 dopamine cell region showed a moderate density of mu receptors in the nucleus parabrachialis pigmentosa, the nucleus paranigralis and the interfascicular nucleus. Furthermore, particularly intense binding was apparent in the area circumscribing the fasciculus retroflexus.

Unilateral loss of dopaminergic perikarya induced by 6-OHDA treatment failed to significantly alter DAGO binding within the A10 region while invoking a unilateral loss of high affinity neurotensin receptors, assayed with the selective ligand ^{125}I-Tyr^3-Neurotensin (Palacios and Kuhar, Nature 294: 587, 1981). However, unilateral administration of QUIN, directed towards the VTA, produced a maximum 70% loss of DAGO binding on the injected side with a concomitant reduction in NT-3 binding.

These results suggest that the enhanced activity of the A10 dopaminergic cell group produced by intra-VTA administration of mu receptor agonist is mediated, at least in part, by a group of non-dopaminergic neurons intrinsic within the A10 region of the rat.

39.4 MU OPIOID ([^{3}H] DAGO) BINDING IN THICK SLICES OF RAT BRAIN: CHARACTERIZATION AND INHIBITION BY SODIUM IONS, GUANYL-5'-YL-IMIDODIPHOSPHATE, DSP4 AND XYLAMINE. M. Wilkinson, D.A. Wilkinson* and W. Jacobson*. Dept. of Physiology and Biophysics, Dalhousie University Medical School, Halifax, N.S., CANADA B3H 4H7.

We and others have suggested that the examination of receptors in a more physiological environment - such as in ionic buffers and/or in intact cells - may be of some benefit to a more complete understanding of receptor-mediated processes (Life Sci. 39:2037, 1986). For opioid receptors the opportunity to examine agonist binding with some degree of subtype specificity would be valuable.

We have now characterized and quantified the binding of the µ-opioid agonist [^{3}H] DAGO to 300 µm slices of hypothalamus and cerebral cortex. The receptors have many of the opioid characteristics previously demonstrated in homogenate assays. Binding is reversible, saturable, stereospecific and of high affinity (nM). The δ-opioids DTLET and DSLET are 36- and 30-fold respectively, less effective than DAGO in competing for the binding site. Assays can be routinely performed in the presence of physiological concentrations of sodium, though in TRIS buffer the affinity and the number of receptors is increased. Protection of the ligand against proteolytic degradation (bestatin and bacitracin) is unnecessary. Since binding is rapidly reversible and is unaffected by ouabain, cellular uptake of label seems unlikely. Unexpectedly, we observed that GppNHp inhibits [^{3}H] DAGO binding to brain slices. This effect is normally seen only in broken cell preparations. Thus, the drug is either readily able to penetrate cell membranes or it can induce an allosteric modification of the µ-receptor itself.

We have previously demonstrated that the noradrenergic neurotoxins DSP4 and xylamine bind to [^{3}H] naloxone receptors. We now show that the µ-receptors are also blocked by DSP4 and xylamine. This re-emphasizes our contention that care should be exercised in the use of these drugs.

The assay technique is simple, rapid and involves minimal disruption of tissue. It should provide new opportunities for the study of cell surface opioid receptor subtypes in intact tissue.

Supported by the Canadian MRC.

39.5 INTERACTIONS OF DIMERIC DERMORPHIN ANALOGS WITH δ AND µ OPIATE RECEPTORS ON RAT BRAIN SYNAPTOSOMES. L.H. Lazarus, A. Guglietta, W. E. Wilson*, P. Melchiorri*, and R. De Castiglione*. LBNT, NIEHS, Research Triangle Park, NC 27709, Institute of Medical Pharmacology III, University of Rome, 00185 Rome, Italy, and Farmitalia Carlo Erba SpA, 20146 Milan, Italy.

The amphibian skin peptide dermorphin (DM) (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) is the only known opiate peptide with a naturally occuring D-amino acid. Studies in vivo (analgesia, catalepsy) and in vitro (muscle relaxation and receptor binding) indicate that DM acts primarily as a µ-opiate receptor agonist. Recent results on dimeric DM analogs (peptides which contain 2 identical peptide chains coupled through the C-terminal amide nitrogen atoms directly, or with the use of intervening methylene groups) indicated that these compounds retain selectivity for µ receptors in vivo and with isolated tissue preparations; however, several dimeric enkephalin analogs (with a D-amino acid in position 2) selectively binds to δ opiate receptors. In order to delineate opiate receptor selectivities of the dimeric DM analogs, rat brain synaptosomal receptor affinities were determined with the aid of the specific ligands [^{3}H]-DADLE (δ agonist) and [^{3}H]-DAGO (µ agonist). Dimeric-penta-DM_2 (I)(subscript represents the number of CH_2 units bridging the peptides) and dimeric-tetra-DM_2 (II) were slightly more δ receptor specific than dimeric-penta-DM_0(III), [Sar^4]-dimeric-penta-DM_0 (IV) and [D-Arg^2,Sar^4]-dimeric-penta-DM_0(V), which show weak µ-type selectivity.

Opiate Receptor Affinities of Dimeric Dermorphin Analogs

Peptide	Receptor Type δ IC_{50} (pmoles)	Receptor Type µ IC_{50} (pmoles)	Selectivity Ratio δ/µ
DM	2.7	11.1	0.24
I	34.0	18.8	1.81
II	61.0	54.5	1.21
III	5.7	6.8	0.84
IV	1.5	1.8	0.87
V	28.0	32.0	0.88

The affinities of peptide IV for either type of receptor exceeded that of DM, while those of compounds I, II and V were less than DM; analog II exhibited the weakest interaction with the receptors. These results corroborate positively with the consequences of icv administration of the same peptides on inhibition of gastric secretion (Guglietta et al., Neuroscience Abs., 1987): those studies indicated that µ-opiate receptors appear to regulate gastric acid secretion in rats.

39.6 PURIFICATION AND CLONING OF A 23kD MORPHINAN-BINDING PROTEIN FROM RAT BRAIN. E. Hanneman, D. K. Grandy*, J. Salon*, C. Machida*, J. Bunzow*, M. Shih*, J. Bidlack^, E. Herbert* and O. Civelli*. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201 and Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642^.

In an attempt to clone the mu opioid receptor, membrane proteins isolated from rat brain minus cerebellum were stereospecifically eluted from a morphinan (^{14} ß-bromoacetamidomorphine) (ß-AM) affinity column using different agonists. Five major and several minor proteins were isolated as judged by silver-stained SDS polyacrylamide gels. The protein population eluted from the ß-AM affinity column retains stereospecific opiate-binding activity in soluble binding assays. To further purify individual proteins, the ß-AM column eluate was injected onto a C4 reverse-phase HPLC column and eluted in an acetonitrile gradient. The major purified protein (23,000 MW) was digested with trypsin and the sequences of the five largest proteolytic fragments were determined. A search for homology among several protein and nucleic acid data bases suggests that this is a novel protein.

For three of the peptide sequences oligonucleotide probes were synthesized, labeled with ^{32}P, and independantly used to screen a rat brain lambda GT10 cDNA library. Several phage clones which hybridized with all three probes were purified and the insert sizes determined. The longest clone so far characterized is 1.2 kb in length (p23K1). The DNA sequence of the insert confirms the sequences of the peptides obtained by tryptic digestion. The p23K1 insert was nick-translated with ^{32}P-dCTP and used to probe a Northern blot of rat tissue and NG108-15 poly A$^+$ mRNA and a Southern blot of rat genomic DNA. The Northern blot autoradiograph revealed a single mRNA species of 1.2 kb that is found in brain minus cerebellum, cerebellum, liver, and NG108-15 cells. The Southern blot autoradiograph showed several bands resulting from EcoRI, HindIII, and BamHI restriction digestions. These findings suggest that the 23kd protein is not a proteolytic fragment of a larger protein and that it may belong to a multi-gene family.

While the concentration, localization and size of the 23kd protein probably preclude it from being the ligand binding subunit of the mu opioid receptor, it might be involved in modulating the receptor specificity or secondary events following ligand-receptor interaction. To further our understanding of the function of this protein, a polyclonal antibody is being used to analyze its distribution in rat tissues. Supported by NIADDK-NIH DK37231.

39.7 IMINODIPROPIONITRILE CAUSES DOWNREGULATION OF OPIATE MU RECEPTORS IN RAT BRAIN: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. V. Jackson-Lewis, J.L. Cadet, B. Kuyatt, S. Fahn, and E. de Souza. Department of Neurology, Columbia University, New York, NY 10032 and NIDA, Baltimore, MD 21224.

Chronic administration of IDPN leads to the development of a persistent syndrome characterized by random circling and lateral and vertical head shakes which are similar to those induced by some receptor agonists such as the enkephalins. These abnormalities also bear some similarities to human disorders of movement such as spasmodic torticollis or Huntington's chorea. In mice, the IDPN-induced abnormalities can be blocked by the opiate antagonist naltrexone. In vitro homogenate binding studies have revealed a significant downregulation of total and delta opiate receptors in the striata of IDPN-treated animals (Cadet and Rothman, Neurosci. Lett. 72: 84, 1986). In the present study, we have used in vitro autoradiography using ^{3}H-dihydromorphine (DHM) in order to evaluate the effects of IDPN on the opiate mu receptor in some areas of rat brain that might be related to the motor phenomena.

Male rats were treated with IDPN (100mg/kg, ip) or saline until the development of the dyskinetic abnormalites. After an interval of one month, the animals were killed by decapitation and their brains were rapidly removed and frozen at -70°C. In vitro ^{3}H-dihydromorphine (DHM) binding was assessed in 8 um coronal sections. Briefly, slides were preincubated with 50uM GTP and 100mM NaCL in 50mM Tris HCl, pH 7.6, at room temperature. After a 5 minute wash in .17M Tris HCl (pH 7.6), incubation was carried out in the presence of 1.8nM ^{3}H-DHM at room temperature. After two 5 minute washes in buffer at 4°C, the slides were dipped in deionized water and dried. Labelled slides were apposed to LKB Ultrofilm for six weeks. Binding was measured by computer assisted densitometry.

This study revealed that, as previously described, the binding of DHM occurs in patches in the striatum and in laminae 4-6 of the neocortex. Chronic IDPN treatment results in significant decreases in DHM binding in all of these areas. The results of the effects of IDPN on delta and kappa receptors will also be presented.

The results add more evidence for the possible involvement of the opioid systems in the manifestation of the IDPN-induced abnormalities. They also raise the possibility of a role for these systems in human movement disorders.

39.8 EVIDENCE FOR KAPPA OPIOID RECEPTOR SUBTYPES IN GUINEA PIG CORTEX. M.D.Hall*, J.A.M.Smith*, J.C.Hunter*, R.G.Hill and J.Hughes* (SPON:European Neuroscience Association). Parke-Davis Research Unit, Addenbrookes Hospital Site, Cambridge, CB2 2QB, UK.

Kappa opioid agonists produce a characteristic profile of behaviour in animals, mediating analgesia, sedation and diuresis (Martin et al., J.Pharmac.Exp.Ther., 197, 517-532, 1976). In contrast to mu analgesics, kappa ligands have minimal addiction liability and respiratory depressant properties. Kappa receptors have been defined by both in vivo behavioural criteria and in vitro biochemical and pharmacological studies. To date there is limited evidence for the existence of kappa receptor subtypes (Su et al., J.Pharmacol.Exp.Ther., 232, 144-148, 1985). In this study, using a radioligand with high kappa selectivity, [^{3}H]U69593, we provide strong support for the proposal of kappa opioid receptor heterogeneity.

The binding of three radiolabelled probes, [^{3}H]etorphine, [^{3}H]bremazocine and [^{3}H]U69593, was investigated in guinea pig cortical membranes in the presence of unlabelled $_5$ (D-ala^2,N-methyl-phe-gly-ol)enkephalin (DAGO) and (D-ala^2,D-leu^5)enkephalin (DADLE) to saturate mu and delta sites respectively. Binding studies with [^{3}H]etorphine and [^{3}H]bremazocine were performed in the presence of a 160-fold excess of DAGO and DADLE, and parallel studies with [^{3}H]U69593 used a 20-fold excess. Results from saturation analyses are presented below.

	Bmax (fmol/mg prot)	Kd [nM]	n
[^{3}H]Etorphine	127 ± 2	0.69 ± 0.04	4
[^{3}H]Bremazocine	130 ± 2	0.22 ± 0.01	4
[^{3}H]U69593	64 ± 6*	3.9 ± 0.4	4

* $P < 0.01$ (Mann Whitney U-Test)

Scatchard transformation of the specific binding data indicated the presence of a heterogeneous population of kappa sites in the guinea pig cortex. [^{3}H]U69593 recognised only ~50% of kappa sites labelled by both [^{3}H]etorphine and [^{3}H]bremazocine. Similar results have been obtained with our own kappa selective ligand [^{3}H]PD117302 (Hill et al., Brit.J.Pharmacol., 1987, in press). From these results we propose that [^{3}H]U69593 and [^{3}H]PD117302 label kappa-A sites in guinea pig cortex, whereas [^{3}H]etorphine and [^{3}H]bremazocine label, in addition to kappa-A sites, a U69593-insensitive site, kappa-B. These findings were confirmed in competition experiments performed in parallel against [^{3}H]U69593 and [^{3}H]etorphine. Etorphine, naloxone and dynorphin A(1-8) were approximately equipotent at both kappa-A and kappa-B sites, confirming the opioid nature of kappa-B sites. In contrast, U69593 and PD117302 preferentially displaced from kappa-A sites, with only weak activity at kappa-B sites.

39.9 EVIDENCE FOR MULTIPLE KAPPA OPIATE BINDING SITES IN RAT MIDBRAIN. T. Devlin* and W.J. Shoemaker. Neuroscience Program, Univ. of Connecticut Health Center, Farmington, Ct. 06032.

In the course of studies to determine possible alterations in opiate receptors following prenatal ethanol exposure, it became evident that the determination of kappa-type opiate receptors using ethylketocyclazocine (EKC) was inadequate (SN Abst. 12:613,1986). The heterogeneity of kappa-type opiate binding sites in rat midbrain was, therefore, investigated. Displacement binding assays were carried out using the opiate agonists ^{3}H-EKC and ^{3}H-bremazocine in the presence of various blocking ligands known to block mu and delta receptors. 24 point Scatchard plots were generated from displacement data, and kinetic analysis was carried out using the LIGAND computer program. ^{3}H-bremazocine binding, in the absence of any blocking agents and the presence of .4M NaCl, best fit an apparent single site model with a K_d of .44 nM and a B_{max} of 132 fmoles/mg protein. Incubation with 1 uM morphiceptin, a mu-type receptor ligand, blocked 28% of total ^{3}H-bremazocine binding, while incubation with .1 uM DSLET, a moderately selective delta ligand, blocked 36% of total ^{3}H-bremazocine binding. The K_d values calculated under these two blocking conditions were .12 nM and .15 nM respectively. Incubation with morphiceptin and DSLET together blocked 45% of total ^{3}H-bremazocine binding, with a single site model best fitting the data. Residual ^{3}H-bremazocine binding in the presence of morphiceptin and DSLET (55% of total ^{3}H-bremazocine binding), defined as "kappa" binding, was further investigated. It was found that kappa binding sites resolved into both a DADLE-sensitive and a DADLE-insensitive population. When 5 uM DADLE was used as a blocking ligand in assays, an additional 19% of the total ^{3}H-bremazocine binding was blocked, above and beyond the 45% blocked in the presence of morphiceptin and DSLET. Similar results were obtained using ^{3}H-EKC where, under mu and delta receptor blocked conditions, residual ^{3}H-EKC binding resolved into both a DADLE sensitive and a DADLE insensitive site. The effect of Na^+ on binding of ^{3}H-bremazocine binding in the presence and absence of mu and delta blocking ligands was also investigated. When ^{3}H-bremazocine binding was carried out in the absence of NaCl, the capacity of binding decreased by an average of 50% and the change in the K_d indicated a shift to a slightly lower affinity state. This effect confirms earlier reports of enhanced agonist binding to kappa sites in the presence of Na^+, and will be useful in formulating new protocols to resolve opiate receptor subpopulations. In summary, mu and delta sites comprise 45% of the total binding sites in rat midbrain, and, kappa sites can be further divided into at least two distinct populations. (Supported by NIAAA# 06927)

39.10 COMPARATIVE DISTRIBUTION OF KAPPA OPIOID RECEPTOR IN HUMAN FOREBRAIN USING [^{3}H]U69,593 AND [^{3}H]BREMAZOCINE. C. Pilapil, J. Magnan* and R. Quirion. Douglas Hospital Res. Centre and Dept. of Psychiatry, Faculty of Medicine, McGill Univ., 6875 LaSalle Blvd., Verdun, Québec, Canada H4H 1R3 and Departement de Pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada.

While various highly selective mu and delta radiolabelled probes are available for autoradiographic studies, a selective kappa radioligand was not available. Previous studies had to be performed using non-selective probes such as [^{3}H]ethylketocyclazocine or [^{3}H]bremazocine in presence or saturating concentrations of mu and delta opiates to block possible binding, of these ligands to mu and delta opioid receptor sites. Recently, the characterization of a new possibly highly selective kappa ligand, [^{3}H]U69,593 has been reported. In the present study, we describe the autoradiographic distribution of putative kappa opioid receptor binding sites in human brain using the highly selective kappa agonist [^{3}H]U69,593 and the previously used radioligand [^{3}H]bremazocine. Control human brains (n=4, 52-71 years old) were obtained at autopsy (post mortem delay = 12.4 hours). For in vitro receptor autoradiography, forebrain sections were incubated for 60 min at 25°C in 50 mM Tris.HCl buffer, pH 7.4 containing either 1.0 nM [^{3}H]U69,593 (42 Ci/mmol; New England Nuclear, Boston, MA) or 1.0 nM [^{3}H]bremazocine (27.8 Ci/mmol, NEN). 100 nM DAGO (Peninsula Labs, Belmont, CA) and 100 nM DSLET (peninsula Labs) were added to [^{3}H]bremazocine incubation buffer to prevent binding of this ligand to putative mu and delta sites. Sections were then rinsed for 12 min in cold incubation buffer, dipped in cold distilled water to remove ions, dried and juxtaposed against tritium-sensitive film. Specific binding was determined as the difference in radioactivity bound in the presence and absence of 5.0 µM (-) cyclazocine. Our results show that the autoradiographic distributions of [^{3}H]U69,593 and [^{3}H]bremazocine (plus blockers) binding sites are identical strongly suggesting that the two radioligands bind to the same population of sites in human brain. In the forebrain, high densities of sites are present in the deep layers of the cortex, claustrum, nucleus basalis of Meynert and amygdaloid body. Much lower densities are observed in the caudate, putamen, globus pallidus and nucleus accumbens. Interestingly, the distribution of these sites is apparently heterogeneous in the caudate. Mu and delta opioid binding sites are differentially distributed in human brain (Pilapil et al, in press). Thus, [^{3}H]U69,593 appears to be a highly specific ligand for the characterization of kappa opioid receptors.

40.1 INTERACTION OF EFFERENT ACTIVITY AND HUMORAL FACTORS IN CONTROLLING THE STRUCTURE OF THE RETINA OF *LIMULUS POLYPHEMUS*.

B.G. Calman*, S.C. Chamberlain, H.K. Lehman, and R.B. Barlow Jr.. Institute for Sensory Research, Syracuse University, Syracuse, N.Y. 13244-5290.

The structure of the retina of the lateral eye of *Limulus polyphemus* is controlled by the diurnal light cycle and by a circadian rhythm of efferent impulses from the brain. The rhabdom and aperture are long and narrow in the daytime light-adapted state, and short and wider in the nighttime dark-adapted state. When an eye is removed from a light-adapted animal in mid-afternoon and placed in darkness in organ culture at 15°C, the rhabdom and aperture progressively shorten and after approx. 20 hrs. approach the nighttime state. An acetone extract of Limulus blood added to the organ culture medium stops the drift in structure toward the nighttime state. The aperture length is particularly sensitive to shortening in organ culture and to the effect of the blood extract. The aperture length in organ culture after 24 hrs. darkness is 5-10 *u*m., near the normal value for dark-adapted animals at midnight. With the addition of the blood extract, the aperture length is maintained at 40-45 *u*m., in the range of daytime light-adapted values.

The addition of the acetone blood extract to organ culture also enhances *in vitro* membrane shedding from the rhabdom of the lateral eye retina at light onset. If lateral eye slices are maintained in organ culture in darkness for 24 hrs., and then exposed to light for 15 min. before fixation, little membrane shedding is observed. However, if blood extract is added to the incubation medium, the number and size of membrane whorls formed at light onset is greatly increased. Work is in progress to fractionate the crude acetone extract and identify an active factor.

These results suggest that the structure of the retina is controlled by two systems with opposite effects; efferent activity, which occurs only at night, moving the retina to the nighttime high sensitivity state, and humoral factors moving the retina to the lower sensitivity daytime state in the absence of efferent input.

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40.2 THE TWO-LIGHT EXPERIMENT IN *DAPHNIA MAGNA*. T.R. Consi, B. Passani* and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

The single compound eye of the crustacean *Daphnia magna* performs a variety of oculomotor behaviors, including fast rotatory movements (eye "flick") in response to light flashes, and fixation and tracking when stimuli of longer duration are presented (T.R. Consi and E.R. Macagno, *Neurosci. Abstr.* 12:461, 1986). As previously observed, a spatially restricted flash of light elicits eye rotations of different size or different direction, depending upon what particular region of the eye is stimulated and the intensity of the stimulus. In the experiments reported here, a narrow stimulus (10° in the dorso-ventral direction and 30° to each side of the midplane of the eye) of 66 msec duration was used. With this stimulus, only the ommatidia located adjacent to the midplane are stimulated (D, A, B and C ommatidia in a dorsal to ventral direction). A stimulus located a few degrees dorsal to the D ommatidium causes no response, independent of stimulus intensity; when the stimulus is placed further dorsally, a dorsal rotation is elicited. In contrast, when the A or B ommatidia are stimulated, a ventral eye movement is produced. For both dorsal and ventral rotations, the size of the response increases with intensity up to a maximum.

To assess how information from different spatial locations is integrated by the oculomotor system, we have examined responses to pairs of stimuli delivered simultaneously to two different regions of the visual field. The two experimental conditions used were: (1) stimulation of the A and B ommatidia, which individually elicit a ventral eye flick when illuminated; (2) stimulation of either the A or the B ommatidium and stimulation of the D ommatidium in the region that elicits a dorsal eye flick. The paired light flashes were delivered at increasing intensities from threshold to saturation of the response. We found, for the first experimental paradigm, that the magnitude of the eye movement produced by two stimuli which individually elicit a ventral rotation is not a linear summation of the two single-stimulus responses. Instead, the magnitude is the same as that of the response to the single stimulus that produces the larger ventral rotation. This occurred at light intensities at which either stimulus alone induces a rotation well below the maximum eye movement possible. When two stimuli that produce rotations in opposite directions were flashed simultaneously, however, either very little or no eye rotation was observed, even at light intensities for which the individual dorsal and ventral rotations were of different magnitudes.

These initial observations indicate that the integration of information from different spatial locations by the *Daphnia* oculomotor system, as measured by eye movement responses, is complex and non-linear. Further experiments, utilizing pairs of stimuli of different intensities and at more positions around the eye, are currently underway in order to elucidate the neural mechanisms that control eye movements in *Daphnia*.

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40.3 LATERAL INHIBITION IN THE COMPOUND EYE: NON-LINEAR INTERACTION BETWEEN RECEPTOR CELL TERMINALS STUDIED WITH WHITE NOISE ANALYSIS. M. Järvilehto*, M. Weckström*, E. Kouvalainen*, and K. Djupsund* (SPON: S. M. Martin). Department of Zoology and Department of Physiology, University of Oulu, 90220 Oulu, Finland.

Spatial information is known to be processed already in the first synaptic ganglion in the insect peripheral retina (Zettler F., Järvilehto M., Z. vergl. Physiol., 76:233, 1972). Lateral interaction has previously been studied with respect to the maximum amplitude of the response, and the mechanisms responsible are under debate. The influence between the functional elements in the ganglion has been suggested to act through gap junctions between the photoreceptor terminals (Ribi, W., Cell Tissue Res., 195:299, 1978) or via extracellular field potentials channeled by proposed high resistance barriers (Shaw, S. R., Nature, 255:480, 1975). The mechanism of lateral interaction is also undoubtely a function of frequency. We therefore studied lateral interaction in the frequency domain in order to reveal the responsible mechanism by investigating the differences in the linear transfer functions. Blowfly photoreceptors were stimulated with pseudorandomly (white noise) modulated light from a green (555 nm) LED. Transfer functions as well as coherence functions were calculated by FFT and compared at the center of the receptive field (on-axis) and at 2°/4° off-axis stimulation. The mean amplitude and the peak-to-peak level of the intracellularly recorded responses at off-axis stimulation were adjusted by the mean and the modulation degree of the stimulation intensity to match the on-axis responses, thus providing the same operating point on the intensity-response function. The transfer function obtained on-axis is similar to a system of five cascaded low-pass filters (corner frequency at 63 ±12 Hz) with at least one second-order term and a pure delay element. Surprisingly, the corner frequency of the 4° off-axis stimulation is decreased about 30 Hz, indicating inhibition at higher frequencies. The phase part of the function shows an increasing time lag in off-axis responses. The coherence function (a measure of linearity) begins to decrease at about the same frequency as the gain part of the function, pointing out an additional non-coherent input which is different from the initial membrane noise found in on-axis stimulation. The findings were verified by comparing the recorded impulse responses of equal amplitudes and also those obtained by inverse FFT with each other.

The non-coherent input inhibiting the responses at higher frequencies, a finding that has not been previously documented, is characteristic of capacitive coupling between photoreceptors. This is caused by high resistance barriers between receptor cell terminals in different neuro-ommatidial cartridges surrounded by glial cells. Our findings also explain the lateral inhibition reported in the lamina monopolar cells.

40.4 SEX-SPECIFIC NEURONS IN THE VISUAL SYSTEM OF BLOWFLIES (Calliphora erythrocephala) REPRESENT CONCENTRICALLY ORGANIZED RETINOTOPIC DOMAINS AND ARE SEGREGATED OUT TO END AT SPECIFIC REGIONS OF PREMOTOR DESCENDING NEURONS. N. J. Strausfeld. Arizona Research Lab. Div. Neurobiol. Univ. Arizona, Tucson, AZ 85721.

Certain species of Diptera exhibit visually induced behavior during which the male, but not the female, computes interception trajectories during high-speed pursuit of a small contrasty object such as another fly (Land, M.F., Collett, T.S. J. Comp. Physiol. A., 89: 331, 1974). Land and Eckert, H (J. Comp. Physiol. A., 156: 525, 1985) have demonstrated that male flies, such as C. erythrocephala have a specialized dorso-frontal area of retina where the divergence angle between neighboring photoreceptor axes is smaller than elsewhere, thus comprising a region of high acuity. Since each photoreceptor has a precise structural relationship with a set of columnar interneurons that extends through successive layers of synaptic neuropil retinotopic organization in the neuropil can be accurately extrapolated out into visual space by virtue of the mapped optical alignments of receptors. Iontophoresis of cobalt ions or Lucifer Yellow dye into the lateral deutocerebrum has revealed 13 morphologically distinct types of neurons that originate exclusively from the male optic lobes, in addition to those known to be cobalt-coupled to descending neurons (DNs: Strausfeld, N.J. Nature: 283, 381, 1980). Each species of male-specific neuron comprises a population of between 1 (unique) and 30 nerve cells, the latter having a columnar organization and small overlapping dendritic domains. The populations, resolved by cobalt uptake and silver intensification, can be stained in conjunction with the retinotopic inputs from the retina. Hence, each male-specific cell type can be related to the photoreceptors and their optical alignments into visual space. Using Land and Eckert's eye maps, each assembly of male-specific neurons has been projected out into the visual hemisphere to identify idealized receptive fields - assuming that these coincide with the dendritic spread of the neurons. These maps demonstrate that the assemblies of male-specific neurons subtend concentric fields, the innermost belonging to columnar neurons, the outermost and largest belonging to male lobula unique neurons. These neuronal types are further distinguished by their projections centrally onto premotor DNs. Small columnar neurons terminate at multibranched dendritic arbors, in parallel with other columnar neurons common to both sexes and representing the entire monocular visual field. In contrast, unique neurons or small populations of male-specific tangential cells characteristically end at the main trunks of descending neurons or even more proximally at their outgoing axons from the brain. This arrangement suggests that large-field sex-specific neurons are arranged to gate the activity of DNs, whereas small field endings interact peripherally with a variety of other parallel visual inputs.

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40.5 EVOLUTION OF RHABDOMERE ASYMMETRY AND PHOTORECEPTOR AXON PROJECTIONS IN THE EYES OF PRIMITIVE DIPTERA. S.R. Shaw and I.A. Meinertzhagen, Dept. of Psychology, Dalhousie Univ., Halifax, N.S., Canada B3H 4J1.

Under the lens in a compound eye of an advanced fly, each photoreceptor has a separate light-absorbing rhabdomere, one of seven arranged in a characteristic asymmetric trapezoidal pattern, such that each rhabdomere images a slightly different point in space. Conversely, certain rhabdomeres in adjacent ommatidia point in the same direction, and it is the axons of such groups that selectively associate synaptically in the cartridges of the lamina. The advanced Muscomorpha evolved from ancestors of the more primitive subdivision, Nematocera, most of which have symmetrical rhabdomere patterns. Extending our interest in the cellular evolution of this system (Shaw, S.R. and Meinertzhagen, I.A., PNAS 83: 7961, 1986), we have investigated the point at which the asymmetric pattern arose, using in vivo optical inspection or microscopy. In the nematoceran family closest to the common ancestor (Anisopodidae), the pattern is horseshoe-shaped but not trapezoidal, as in parts of the eye in the related Bibionidae (Zeil, J., J. comp. Physiol. 150: 379, 1983). In the advanced muscomorphan series, the families that diverged earliest (Stratiomyidae, Xylophagidae, Rhagionidae, Tabanidae) already show the advanced pattern, as do all later groups, making it taxonomically diagnostic for Muscomorpha.

The asymmetric pattern carries maximum optical advantage only if the axons converging upon one cartridge have a matching asymmetric distribution, rotated 180° (Kirschfeld, K., Exp. Brain Res. 3: 248, 1967), while the prototype (bibionid) projection is reportedly less well optimized and more diffusely connected (Zeil, 1983). We therefore investigated whether the "modern" projection developed simultaneously with the asymmetric rhabdomere configuration, or whether it evolved later. Light-facilitated dye uptake (Wilcox, M. and Franceschini, N., Science 225: 851, 1984) can be adapted to chart the axonal projection, and shows that a member of one of the oldest muscomorph families (Stratiomyidae) already possesses a fully developed modern projection.

A possible anomaly remains that in Bibionidae, regrouped photoreceptor axon bundles first appear to associate with one cartridge before diverging again as in higher flies, to synapse in others (Zeil 1983). Having connections in two cartridges would degrade visual resolution. Our EM observations on Bibio confirm Zeil's interpretation of Golgi preparations, that the first of these associations is non-synaptic, comprising small bundles of photoreceptor axons lying in the spaces between cartridges. An evolutionary transition to the advanced form of the photoreceptor projection thus becomes simpler to imagine, starting from the non-divergent projection of dipteran ancestors.

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40.6 CORRELATIONS BETWEEN STRUCTURE, TOPOGRAPHIC ORGANIZATION, AND SPECTRAL SENSITIVITY OF PROTHORACIC, INTERGANGLIONIC INTERNEURONS OF THE CRICKET. G. Atkins* and G.S. Pollack. Department of Biology, McGill University, Montreal, Canada H3A 1B1.

Recent studies have identified nine sound-sensitive, interganglionic interneurons in the cricket Teleogryllus oceanicus (Atkins, G. and G.S. Pollack, Soc. Neurosci. Abst. 12:858). Each neuron receives input in the prothoracic ganglion, but only two are potentially directly post-synaptic to auditory receptor cells. Five of the neurons are most sensitive to high frequencies (> 12 kHz) whereas four respond best to low frequencies (3-10 kHz). The morphology of the individual neurons was studied by filling them with Lucifer Yellow and examining their processes in wholemount and in sectioned preparations. Comparison of the structural characteristics of these neurons to their spectral-sensitivity reveals several correlations. (1) The processes of high frequency neurons are primarily located in the dorsal portion of the prothoracic ganglion whereas low frequency neurons are positioned ventrally. This arrangement indicates that sound stimuli of low and high carrier frequencies are processed in different regions of the cricket's nervous system, and is the first example of topographic arrangement of higher order, sound-sensitive interneurons in insects. (2) The descending axons of the neurons are also spatially separated; axons of high frequency neurons are positioned laterally in the pro-mesothoracic connective whereas those of low frequency neurons are positioned medially. (3) The overall structure of the cells is also correlated to their spectral-sensitivity. High frequency neurons share structural features (soma position, neurite trajectory, axon position) which, in the locust (another orthopteran insect) are indicative of inhibitory neurons (Pearson, K. and R.M. Robertson, Cell Tissue Res., in press). This raises the possibility that the high frequency cells might have inhibitory effects. None of the low frequency neurons have inhibitory-type morphology.

40.7 NEURAL CIRCUITS OF THE BOLLWORM MOTH ACOUSTIC SYSTEM. Herndon R. Agee. Insect Attractants Behavior, and Basic Biology Research Laboratory, USDA, ARS, P.O. Box 14565, Gainesville, FL. 32604.

The axonal terminations of the paired acoustic receptors (A1 and A2) and the B cell from the tympanic ear of the bollworm moth, Heliothis zea were studied after infiltration with cobalt chloride to determine their dendritic patterns in the central nervous system.
The most sensitive acoustic receptor (A1) produced a monosynaptic axon that had branches in the meso-metathoracic ganglia and was tracked to the anterior junction of the subesophageal-protoracic ganglia connective.
Neurophysiological data (Agee, H. R., J. Agric. Entomol. 2:277-285, 1985) indicated that the A1 axon was present in the dorsal midline of the brain. However, I have not been able to get the cobalt chloride to infiltrate the A1 axon beyond the subesophageal ganglion, probably due to injury to the connection.

The A2 axon (20 decibel less sensitive acoustic receptor, Agee, H. R., J. Econ. Entomol. 60:366-369, 1967) after entering metathoracic ganglion branches posteriorly and anteriorly. The anterior branches terminate in the same region of the mesothoracic ganglions as the dendrites of the motor neurons that drive the flight muscles of the metathoracic ganglion.

The non-acoustic B cell axon leaves the tympanic ear and follows a path parallel to the A1 axon through the CNS to the anterior limits of the subesophageal - prothoracic connective. There are some short dendritic branches of the B axon in the mesothoracic and prothoracic ganglia.
Neurophysiological data indicate that the B axon also enters the brain as does the A1 axon.

Behavioral data (Agee, H. R., J. Agric. Entomol. 2:345-350) and these histological and neurophysiological data indicate that the directed evasive maneuvers made by the bollworm moth when only the A1 receptors detect ultrasound signals are controlled by neural connections in the brain of the moth. The A2 cell axon does not extend beyond the mesothoracic ganglia. The A2 receptor is an important part of the sensorimotor circuit that generates the undirected evasive reactions that moths display when the receptor detects intense bursts of ultrasound (60-80+ dB) (Agee, H. R., 1969, Ann. Entomological Soc. Amer. 62:801-807).

40.8 MOLECULAR STUDIES OF INSECT PHEROMONE RECEPTION: VARIATION AND ONTOGENY OF SENSORY HAIR PROTEINS. R.G. Vogt*, A.C. Koehne* and G.D. Prestwich* (SPON: A. Carlson). Dept. of Chemistry, SUNY, Stony Brook, NY 11794.

The extracellular space of the pheromone sensitive sensory hairs of male gypsy moths, Lymantria dispar, contain high concentrations of two small soluble proteins (both 15K daltons). N-terminal sequence analysis shows these proteins are aprox. 50% homologous with each other, and about 60% homologous (in combination) with the pheromone binding protein (PBP) of the silk moth Antheraea polyphemus. (Vogt,R.C. et al., Proc. Natl. Acad. Sci USA 82:8827, 1985). Antisera prepared against the A. polyphemus PBPs cross reacts specifically with the gypsy moth proteins, as well as with specific antennal proteins of other moths, but not with antennal proteins of non-lepidopteran insects. Antisera prepared against the respective gypsy moth PBPs show similar specific cross reactivity patterns (Western blot analysis). Presumably due to differences in the primary antigen structures, these three antisera each reveal different proteins while maintaining specificity to antennal tissue. These studies are documenting a class of proteins, PBPs, which function in lepidopteran sensory hairs to effect pheromonal signal processing at the molecular level.

Gypsy moth adult development requires about 9 days (27°C). We have established criteria for selecting animals at specific, visually recognizable stages during the last half of adult development. Electrophoretic studies reveal that the gypsy moth PBPs are first synthesized 3 days prior to adult emergence (E-3), synthesis continuing at a high rate for at least several days into the brief adult life. However, the PBP concentrations in the antenna (aprox. 10 mM) plateau by E-1, remaining at that level for the duration, indicating an extremely high rate of turnover for these proteins. This turnover suggests that a metabolically active cell membrane at the base of each hair (Steinbrecht, R.A., Naturwissenschaften 73:275, 1986) functions as a high capacity pump for the delivery and removal of PBPs from the hair lumen. Considering the PBPs' role as a pheromone carrier, this also suggests an additional cleanup mechanism for ridding the hair lumen of residual pheromone molecules, by pumping them out with the PBPs, thus maintaining a low noise level within the hair and a high sensitivity to incoming signal.

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40.9 FREQUENCY-DEPENDENT RESPONSES TO PULSED SEX PHEROMONE STIMULATION IN CENTRAL OLFACTORY NEURONS OF *MANDUCA SEXTA*. T.A. Christensen and J.G. Hildebrand. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Female moths, among other insects, release sex pheromones that attract males over long distances. Several recent lines of evidence indicate that the pheromone plume is highly variable and furthermore that males orient to an intermittent stimulus more accurately than to a uniform concentration. We are interested in how the male moth extracts from this odor trail the information necessary to locate a "calling" female. Using intracellular methods, we recorded the responses of olfactory neurons in the CNS of male *Manduca sexta* to pulsed stimulation with sex pheromones. All recordings are from a subset of projection neurons (PNs) which represent a major pathway of pheromonal information flow between the first-order olfactory centers (the antennal lobes) in the deutocerebrum and higher-order centers in the protocerebrum. Electrodes were directed toward the area of the macroglomerular complex, the sexually-dimorphic area in the male antennal lobe neuropil to which the pheromone-responsive afferent axons project. Each cell was tentatively identified as a PN by its response to electrical stimulation of the antennal nerve. PNs are characteristically inhibited (indirectly) by electrical stimulation of sensory afferents and the IPSP is followed by brief excitation, and sometimes by later inhibition (Christensen & Hildebrand, *J. Comp. Physiol.*,in press).

PNs followed 1:1 with repeated pheromone stimuli as short as 15 msec and up to frequencies around 10 Hz. The cells responded to each stimulus pulse with a burst of spikes preceded by a transient IPSP. The excitatory burst elicited by the last stimulus pulse was often followed by a period of inhibition before the cell returned to its background rate of spiking. As the inter-stimulus interval was decreased, the inhibitory period between pulses was reduced and each excitatory burst began to merge with the one preceding it. At frequencies well above a given cell's cut-off range for repeated bursting, responses consisted of a single phasic burst of spikes followed by a prolonged inhibition.

In the presence of bicuculline, the IPSPs are blocked and the burst structure is altered. This indicates that GABA-mediated inhibition, as previously described (Waldrop *et al.*, *J. Comp. Physiol.*, in press), helps maintain the separation between bursts, thereby reflecting the changing temporal characteristics of the pheromone stimulus. The inhibitory neurons that help structure the bursts are, in all probability, the GABAergic local interneurons in the antennal lobe (Hoskins *et al.*, *Cell Tissue Res.*, 244, 243-252 (1986).

40.10 NEURONAL PROCESSING BY CENTRAL OLFACTORY NEURONS RELATED TO THE INITIATION OF MATING BEHAVIOR IN THE MALE SILKWORM MOTH. +R. Kanzaki* and T. Shibuya*. (SPON:H.Itagaki) Biol. Sci., Univ. of Tsukuba, Ibaraki 305 Japan
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Olfactory stimuli play a major role in initiating insect behaviors such as mating, feeding, oviposition and so on. Recently, there has been considerable progress in unraveling central olfactory mechanisms underlying mating behavior which occurs in response to sexual pheromones.

Male silkworm moths (Bombyx mori) show a remarkable mating behavior consisting of wing vibration, walking and occasional abdomen curvature in their so-called "mating dance" when the receptor cells of their antennae are stimulated with sexual pheromone (bombykol and bombykal) released by females. The mating dance can also be initiated by synthetic bombykol.

To elucidate central mechanisms by which mating behavior of male moths is initiated, we have examined the activity of brain neurons related to stimulation of the antennae with pheromone. We have recorded from more than 600 neurons located in the deutocerebrum (1st order olfactory center) and the protocerebrum (higher olfactory center) and have characterized the morphology of 101 of these by intracellular staining with Lucifer yellow.

Two types of deutocerebral interneurons (Ia and Ib) were identified that projected from the deutocerebrum to the protocerebrum. Both types have arborizations in the macroglomerular complex, a male-specific neuropile region, and respond to pheromone (bombykol) stimuli with accelerated firing followed by inhibition. The excitatory part of the response includes an initial phase of higher frequency firing followed by a prolonged phase of firing at a lower frequency (PTI type). Local interneurons restricted to deutocerebral glomeruli were also identified.

Neurons were identified whose cell bodies and processes were restricted to the protocerebrum. These responded to antennal stimulation by bombykol with tonic increases in firing. Other protocerebral neurons were identified that have axons descending in the ventral nerve cord. These also responded with accelerated firing that often outlasted the bombykol stimulus. Dose-response curves generated with bombykol stimulation show that accelerated firing of descending protocerebral neurons parallels increased wing vibration, one of the components of mating behavior in male moths, that is observed using similar stimuli. We think that these neurons may be important for initiating and maintaining wing vibration during the mating dance.

40.11 TEMPORAL ANALYSES OF THE ACTIONS OF PROPANOL ON FLY TASTE RECEPTOR CELL RESPONSES TO SODIUM CHLORIDE. D.M. Bourassa* and L.M. Kennedy. Dept. Biology, Clark Univ., Worcester MA 01610.

Normal alcohols (n-alcohols) suppress fly behavioral and taste receptor cell action potential responses to sucrose(1,2,3). The receptor cell effects are biphasic over time-- firing is first suppressed and then increased and irregular, as are effects of the selective, sweetness-suppressing taste modifiers, gymnemic acids, ziziphins and hodulcin(3,4). For the initial suppression produced by n-alcohols, the duration and magnitude increases as alcohol chain length or concentration increases(3). Using the same paradigm as (3), we studied the effects of propanol, on Phormia regina taste receptor cell action potential responses to NaCl in order to test differential actions of the alcohol.

Single taste sensilla in isolated proboscises were tested in two-trial sequences. In the first trial, sensilla were treated with Tris (15mM,pH 7) for 2 min and then beginning at 1 min posttreatment, responses to NaCl 500mM were recorded continuously for 10 min. In the second trial, sensilla were treated with propanol 1.2 or 9M (in Tris), or with Tris, for 2 min and then responses to NaCl recorded as before. For each fly, the numbers of spikes in 0.1 or 1.0 sec excerpts, taken at 15 or 30 sec intervals from second trial responses, were expressed as ratios to the numbers of spikes in excerpts taken at the same times from first trial responses. Ratios were averaged over 1 min intervals.

For responses to sucrose after propanol 1.2M, the duration of the inital suppression is 1-2 min posttreatment(3). For responses to NaCl after propanol 1.2M, we found no suppression during the 10 min posttreatment. Yet when n-alcohols are mixed with the NaCl stimulus, responses are suppressed immediately after application of the mixture(2). For responses to sucrose after propanol 9M, there is a recovery from the initial suppression at 10 min posttreatment(3). We found that responses to NaCl after propanol 9M also are suppressed initially and then recover at 5.5 min posttreatment. The initial suppression produced by n-alcohols is similar for sucrose and NaCl in the effects of chain length(2). After propanol, the initial suppression is similar in the biphasic form and in the effects of alcohol concentration. Yet the time course of suppression differs for responses to NaCl or to sucrose. These data have important implications for studies of the selective actions of taste modifiers.

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41.1 COOLING SENSITIVE MECHANORECEPTORS AND TEMPERATURE COMPENSATION IN THE GRASSHOPPER, SCHISTOCERCA AMERICANA. C.I. Miles, Dept of Zoology, University of Washington, Seattle, Wa. 98195.

Mechanosensory hair neurons of grasshoppers are sensitive to temperature, responding to hair deflections with higher firing frequencies if temperature increases. Stronger hair deflections will also produce higher firing frequencies. A central nervous system (CNS) neuron which receives inputs from such neurons might thus be incapable of distinguishing changes in their firing frequencies due to different stimuli from those due to temperature changes. A population of mechanosensory hairs located on the dorsal head surface, the wind sensitive head hairs, have especially high temperature sensitivities. For a CNS neuron receiving their inputs, the situation is further complicated by the temperature variability of the head. In freely moving animals, the temperature of the wind hairs can be up to 5°C different from the brain, and wind hairs are exposed to temperature fluctuations not experienced by the brain.

The Tritocerebral Commissure Giant (TCG) is a brain interneuron which receives wind hair input (Bacon & Tyrer, J. comp. Physiol., 126:317-325, 1978). If temperature of the wind hairs is changed while that of the TCG is not, the interneuron's spike output shows very little sensitivity to the temperature-induced changes in the levels of its sensory inputs. This study describes a mechanism by which this compensation could be produced.

A subgroup of wind hair neurons were found which exhibit responses to temperature change alone, without deflections of their associated hairs. These neurons display a cooling response, or increase in firing frequency with decreasing temperature. They also decrease their firing rate, or may cease firing entirely if temperature increases. When their hairs are deflected, these neurons can be as temperature sensitive as other wind hairs, but only if the deflections are brief. During tonic deflections, they are nearly insensitive to temperature change.

Information about head surface temperature provided by cooling sensitive wind hairs could enable the TCG to compensate for temperature's effects on the mechanosensory responses of its other wind hair inputs, as long as at least some of the cooling sensitive neurons are not stimulated with brief hair deflections. A natural wind stimulus would likely deflect individual hairs differently, due to the varied orientations of hairs on the head surface and the contours of the head. Fluctuations in head surface temperature should thus not affect the function of the wind hair-TCG system or the behaviors it mediates.

41.2 MECHANICAL AND PHYSIOLOGICAL PROPERTIES OF SLOWLY AND RAPIDLY ADAPTING SENSORY NEURONS OF THE DIPTERAN WING BLADE. M. H. Dickinson* (SPON: R. Pinter). Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

Mechanosensory fields of most animals are rarely homogeneous assemblages of neurons with identical physiological properties. Rather, individual cells within a single modality often differ with respect to absolute threshold, directional sensitivity, and dynamic response. This herteogeneity may result from differences in either extrinsic physical properties of the accessory structures utilized in mechanical coupling, or cell intrinsic membrane properties involved in the processes of transduction and encoding.

The wing blade of many dipteran species contain eight identifiable campaniform sensilla, mechanosensory structures that encode deformation of the cuticle. Four of the neurons adapt rapidly to step deformations and can follow sinusoidal stimulation at high frequencies. The other four cells are slowly adapting, respond maximally to lower frequencies, and show a lower threshold sensitivity. A preparation has been developed for examining the role of campaniform dome compliance setting the dynamic response properties within these two distinct groups of neurons. The campaniform dome is indented directly with sinusoid or noise stimuli using a microprobe that allows measurement of the force of indentation while simultaneously recording the impulse frequency of the neuron (see Chapman and Duckrow 1975, J. Comp. Physiol. 100:251-268). Both the compliance of the dome and the firing rate of the sensory cell may then be examined over a wide frequency range.

Preliminary evidence suggests that the rate sensitivity of these campainiform neurons is not primarily a function of the dynamic characteristics of the dome compliance, an observation that is supported by direct electrical stimulation. Thus the differences between rapidly and slowly adapting behaviors must be assigned to membrane intrinsic electrical properties. However, dome compliance may be an important parameter in setting the treshold sensitivites between the two classes of neurons.

Future studies will examine the roles that these two classes of neurons might play in the flight behavior of flies.

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41.3 EXTRACTION OF SENSORY INFORMATION FROM A COMPUTATIONAL MAP BY INTERNEURONS IN THE CRICKET CNS. J.P. Miller and G.A. Jacobs. Zoology Dept., UC Berkeley, Berkeley, Ca. 94720

The cercal sensory system of crickets is a model for the study of computational maps of sensory space, and for the mechanisms by which single neurons extract and encode information from such maps. This system mediates the detection of the direction, velocity and acceleration of wind currents directed at the animal. Previous studies have shown that the excitatory receptive fields of several primary sensory interneurons (INs) are determined by the location of their dendrites within the afferent map, inhibition from other INs plays a significant role in shaping the directional sensitivity of some cells,and the threshold and response characteristics of several primary INs vary over a broad range. Shimozawa & Kanou (1984) have classified cells as either velocity or acceleration detectors, and demonstrated the basis for this variation in threshold and response characteristics. For that work, sinusoidally oscillating air currents were used as stimuli. We have tested and extended this earlier work using constant velocity wind stimuli. Intracellular recordings were obtained from nine different types of identified INs, including several from which no data was previously available. 1.) Most cells showed marked directional selectivity, but several showed very little. 2.) In all cells with marked directional selectivity, inhibition was observed to play an important role in shaping the response characteristics. The amplitude of inhibition was demonstrated to be dependent upon wind direction and velocity. 3.) With few exceptions, the predictions of Shimozawa & Kanou were observed to be correct: ie. a.) INs 10-2 and 10-3 (innervated by long hairs) had very low thresholds, had opposite directional selectivities, and generated responses proportional to wind velocity. b.) LGI and MGI (innervated by short hairs) had high thresholds, and generated responses proportional to wind acceleration. 4.) Other cells were found which had intermediate and complementary properties. For example, cell 11-1 had relatively high threshold, showed very little directional sensitivity, and generated "on-off" responses proportional to wind acceleration. 5.) Several aspects of neuronal response properties were different than predicted by Shimozawa & Kanou. a.) All cells showed substantial adaptation to maintained wind current, ie: all were "phasic". b.) 9-3 was the "least phasic"; 10-2 and 10-3 were intermediate; LGI, MGI and 11-1 were among the most phasic. The different cell types have complementary response characteristics which allow the system as a whole to sense and encode a wide range of direction, velocity and acceleration parameters of wind stimuli. Based upon this data, a preliminary assessment of feature extraction and information coding by this system will be presented. (Shimozawa & Kanou, J. Comp. Physiol. A 155:485-505.)

41.4 **INTEGRATION OF DIRECTIONAL WIND FIELD INFORMATION IN THORACIC INTERNEURONS OF THE COCKROACH** **R.E. Ritzmann, A.J. Pollack* and J. Westin. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.**

We have identified several thoracic interneurons in the cockroach that respond directly to ventral giant interneuron (vGI) activity. At least one population of these interneurons, members of the dorsal posterior group (DPG), also excites the leg motor neurons that are activated during the GI mediated escape response. Both the wind receptive fields and the synaptic responses to individual GI's of several of these interneurons have been examined. These data support the contention that the DPG interneurons serve to integrate the information on wind direction which is encoded in the vGIs.

In an attempt to understand the details of this integration process, we have studied the potential connectivity of each GI with the various DPG interneurons. For two of the thoracic interneurons in question, this study is now complete. Both Lambda and the Reverse-J cells receive excitatory inputs from each of the 8 vGIs but fail to receive inputs from any dGI interneurons. In studies where two vGIs were impaled in conjunction with DPG interneurons, evidence was found for summation of inputs both from vGIs in the same connective and from pairs including one vGI in each connective.

These experiments also permit us to compare the relative strength of synaptic excitation between specific pairs of vGIs. Two questions were addressed for this aspect of the study: (1) What are the relative amplitudes of EPSPs generated in thoracic interneurons by stimulating each of the two impaled GIs intracellularly? In order to eliminate spatial considerations, especially in cases where GIs are tested in both connectives, all DPG recordings were taken on the midline of the thoracic ganglion. (2) What are the relative contributions to generation of action potentials in the thoracic interneuron for inputs from each GI? Since, in most cases, inputs from individual GIs do not depolarize the thoracic interneurons to threshold, they were depolarized by current injection to a level that allowed at least one of the two GI inputs to evoke action potentials. In this way we hope to establish a ranking of synaptic efficacy for each of the vGIs. This, along with the wind fields that have been established for each vGI, should begin to explain the origin of wind fields which we have observed for the thoracic interneurons.

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41.5 DUM INTERNEURONS ARE EXCITED BY VENTRAL GIANT INTERNEURONS OF THE COCKROACH A.J. Pollack* and R.E. Ritzmann (SPON: J. Westin). Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

A considerable amount of data has been brought forward implicating the giant interneurons (GIs) of the cockroach P. americana in the initial movements of the wind-evoked escape response. In an attempt to identify the neurons that integrate the information encoded in GIs, we have recorded from a variety of thoracic interneurons while stimulating GIs intracellularly. Among those we have tested are a variety of Dorsal Unpaired Median (DUM) cells. Several of these cells have been shown to release octopamine which, at least in the periphery, can augment excitatory synaptic responses from other neurons.

The results of our tests indicate that none of the DUM cells that have large somata, including those with peripheral axons, receive inputs from either ventral or dorsal GIs. These cells are only weakly wind sensitive, and intracellular stimulation of GIs consistently fails to generate PSPs. Nevertheless, we have recorded from 4 different DUM cells which do respond to ventral GI stimulation with large short latency EPSPs. Each of these cells has a small soma and no peripheral axons. Three of the cells are local intraganglionic interneurons in the metathoracic ganglion. One of them has processes in both T2-T3 thoracic connectives.

Evidence exists indicating that in locust all of the DUM cells synthesize the neuromodulatory transmitter octopamine (Goodman et.al., 1979, Science 204:1219). If the DUM interneurons that receive inputs from GIs also release octopamine, it would be tempting to hypothesize that they may serve to modulate synaptic activity in the CNS in the same way that the larger peripheral DUM cells modulate synaptic activity in the periphery.

(Supported by NIH grant NS-17411 to R.E.R.)

41.6 DESCENDING PATHWAYS CARRYING WIND AND TACTILE SENSORY INFORMATION IN THE NERVE CORD OF THE COCKROACH Periplaneta americana. J.A. Burdohan* and C.M. Comer. Dept. Biological Sciences, Univ. of Illinois at Chicago, Chicago, IL 60680.

Much of the work on the wind-evoked escape behavior of the cockroach has focused on its control by a set of giant interneurons (GIs) which ascend the ventral nerve cord (VNC) carrying cercal-derived wind sensory information. However, recent behavioral studies in our lab (Comer et al., Neurosci. Abstr. 12:203, 1986) have demonstrated that after complete elimination of the ascending GI system by surgical section of the abdominal portion of the VNC, animals can still turn and run in response to wind puffs. Such "non-GI" wind-evoked responses are similar in form to normal escape, but are somewhat longer in latency. They were nearly eliminated by subsequent removal of the antennae, although evasive turning and running in response to tactile stimulation of other rostral structures persisted. We have begun characterizing descending wind and tactile sensory pathways of the sort implied by our behavioral results, and provide here an initial electrophysiological description of their properties.

Recordings (extracellular and/or intracellular) were made simultaneously from the neck connectives and from the connectives just rostral to the metathoracic ganglion. Robust unit responses were recorded at these sites in response to wind puffs similar to those used in our behavioral experiments, and in response to tactile stimulation of the antennae, the head, and the pronotum. These responses persisted following complete section of the nerve cord between the abdomen and the thorax. At the level of the metathoracic ganglion, descending wind-evoked activity arrives approximately 50 msec later than ascending GI activity. This accords reasonably well with the shift in latency of wind-evoked escape responses seen following elimination of the GI pathway.

The source of input to these wind sensory units was ascertained by experiments involving removal or covering of rostral sensory structures. A major source of input is the antennae, as their removal leads to a dramatic decline in descending wind-evoked responses. Additional wind-evoked activity is mediated by sensory hairs on the head and pronotum. These descending units appear to have a slightly higher threshold for wind than the GIs. We are now beginning to examine single descending units in relation to leg motor neurons and the GIs.

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41.7 ANATOMICAL AND ELECTROPHYSIOLOGICAL CORRELATES OF A SOMATOTOPIC AFFERENT-TO-MOTORNEURON MAP IN LARVAL MANDUCA SEXTA. G.A. Jacobs, B.A. Peterson and J.C. Weeks. Dept. of Zoology, Dept. of Physiology-Anatomy, and Dept. of Entomological Sciences, Univ. of California, Berkeley, Berkeley, CA 94720.

Each abdominal segment of the tobacco hornworm caterpillar, Manduca sexta, bears hundreds of singly innervated sensory hairs that relay mechanosensory information. The only larval abdominal appendages are the paired sets of prolegs, which are used for locomotion and other behaviors. The highest density of hairs on the abdomen of 5th instar larvae occurs on the distal tip of each proleg, the planta, with each planta bearing ca. 40-50 long, stout hairs in a compact array. Tactile stimulation of the planta hairs elicits a proleg retraction reflex that is mediated by monosynaptic excitatory connections between the planta hair afferents and proleg retractor motorneurons (J. Comp. Physiol. 1987, 160:315-330). Anatomical contact between the afferents and motorneurons occurs in ventral (sensory) neuropil, to which the motorneurons send a small dendritic projection. Intracellular motorneuron recordings indicate that the size of the afferent-evoked EPSP varies consistently as a function of the location of the hair within the planta array. To investigate the possible anatomical basis of this pattern, we determined the central projections of individual afferents by cobalt filling via the cut hair shaft. The long axis of the planta hair array is in the anterior-posterior direction and the dorsal-ventral axis is narrow. The terminal arbors of the afferents formed a well defined somatotopic map in sensory neuropil, with the anterior-posterior axis of the hair array mapped along the anterior-posterior axis of the ganglion. Afferent position was also reflected in the extent of contralateral branching within the ganglion, and in differences in interganglionic axonal projections. The map orientation was the same as that reported by Levine et al (J. Comp. Physiol. 1985, 157:1-13) for non-proleg abdominal hairs, but planta afferents had larger arbors than did non-proleg afferents, and the planta hair map occupied a disproportionately large portion of neuropil given the small size of the planta hair array on the body surface. Thus, as might be predicted on behavioral grounds, the proleg region of the body is relatively overrepresented in sensory neuropil. The finding of strong somatotopy in the planta hair afferent projections is consistent with an anatomical contribution to the observed pattern of EPSP sizes in proleg motorneurons, a hypothesis that can be further tested experimentally.

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41.8 INTERSEGMENTAL PROCESSING OF MECHANOSENSORY INFORMATION BETWEEN LOCAL CENTRES CONTROLLING THE LEGS OF THE LOCUST. G. J. Laurent* (SPON: M. Burrows) Dept. Zool., Univ. Cambridge, CB2 3EJ, England.

The local interneurones involved in the control of the movements and position of one leg must be informed of the state of the adjacent legs so that coordinated movements can occur. A population of more than 30 paired intersegmental interneurones that could subserve that role between the meso- and meta-thoracic segments is being studied in the locust. The range of their inputs from various extero- and proprio-ceptors on a middle leg has been defined and the way the sensory signals are integrated by them, studied in detail.

Each interneurone in this population can be identified by its morphological features elaborated from a common ground plan (Laurent, 1987, J. Comp. Neurol., 256, 412-429) and by its mechanosensory receptive field on one middle leg (Laurent, 1987, J. Neurosci., in press). In this way, a given interneurone is, for example, excited by extension, and inhibited by flexion of the tibia; another interneurone is excited by touching a patch of hairs on the dorsal femoro-tibial joint, and inhibited by pressure applied on the ventral surface of the tarsus.

The excitatory regions in the receptive fields of these interneurones are due to direct excitation from proprioceptive or exteroreceptive afferents. The spikes of the afferents evoke excitatory post-synaptic potentials in the interneurones with a consistent central latency of about 1msec. The projections of the interneurones and the corresponding afferents overlap in the neuropile.

By contrast, the inhibitory regions in their receptive fields are due to disynaptic inhibition mediated by a layer of spiking local interneurones. A spike in a local interneurone, evoked directly by a set of afferents causes an inhibitory post-synaptic potential in a given intersegmental interneurone with a latency of less than one msec. The inhibition can result from converging inputs from several local interneurones.

These intersegmental interneurones send an axon to and arborize in the ipsilateral metathoracic neuropile. Immunocytochemical labelling combined with unitary intracellular staining has allowed GABA to be identified as one of the putative transmitters, suggesting inhibitory effects.

This work was supported by an SERC (UK) grant to M Burrows.

41.9 PROPRIOCEPTIVE AND TACTILE INTEGRATION BY IDENTIFIED INTERNEURONS IN THE SAND CRAB EMERITA. D.H. Paul (SPON: N.M. Sherwood). Biology Department, University of Victoria, B.C., Canada V8W 2Y2.

The tailfan (telson flanked by the uropods) in crustaceans such as the crayfish is a major source of sensory information which is used in balancing, steering and initiating locomotory behaviors. In crayfish, who swim by tailflipping, ~55% of the 50 paired neurons with somata in the terminal ganglion (G6) and axons projecting anteriorly through the connective are individually identified mechanosensory interneurons, MSI (Sigvardt et al., 1982. J comp Physiol 148: 143). G6 of crayfish is probably representative of the ancestral neuronal organization of this ganglion with respect to sensory integration. In sand crabs, the uropods are reoriented to flank the abdomen, not the telson, and Emerita swims by beating the uropods (Paul, 1981. J exp Biol 94: 159). Because the arrangement of sensory fields on the tailfan is phylogenetically stable, homologous nerves of G6 in different taxa are recognizable (Paul et al., 1985. Biol Bull 168: 106). In Emerita uropod stretch receptors, absent from crayfish, provide reafference from uropod movements through one of these nerves (Paul, 1976. J exp Biol 65: 243). The questions raised are: Does uropod stretch receptor input impinge on homologs of any of crayfish's identified MSIs? And, if so, are these the neurons that in crayfish handle proprioceptive information from other sources?

Cobalt backfills of one hemiconnective into G6 of Emerita reveal a population of ~ 25 projection neurons with somata clustered into 3 contralateral groups similar in position to the caudal, medial, and rostral clusters of neurons projecting anteriorly from G6 in crayfish (Sigvardt et al.). My data on structures of individual MSIs, from connective backfills and dye injections into single cells, suggest that most members of the rostral cluster are retained in Emerita and that the reduction in numbers has been among interneurons with caudal and medial somata. Preliminary physiological data, from repeated intracellular recordings of exteroceptive and proprioceptive input to 11 different MSIs, suggest that stretch receptor afference impinges on homologs of certain cells that in crayfish respond only to exteroceptive input. I am currently persuing this investigation in order to discover how signals from the phylogenetically new stretch receptor are integrated with other afference from the tailfan.

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41.10 DIRECTIONAL CODING BY CRAYFISH MECHANOSENSORY INTERNEURONS. Lon A. Wilkens. Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121.

The sensitivity of crayfish mechanosensory interneurons to directional stimuli, as observed initially in the caudal photoreceptor (CPR) (Wilkens and Larimer, 1972), is useful in characterizing morphologically identified cells (Sigvardt, Hagiwara and Wine, 1982; Reichert, Plummer and Wine, 1983; Wine, 1984). Until now, descriptions of directionality have been limited to the rostral/caudal axis, due in part to the difficulty of sequentially generating quantifiable stimulus currents from various directions. For aquatic animals relatively finite recording chambers must be employed which have inherent resonant frequencies and produce interference in the form of reflected traveling waves. In the present experiments, these difficulties have been overcome by the use of stimulus currents in the vertical axis generated by modified sinusoidal movements of the chamber floor. With this arrangement there are no traveling waves and water displacements are synchronous and uniform throughout the chamber. The tailfan, along with the 6th abdominal ganglion, is mounted vertically on the end of a horizontal supporting rod. Rotation of the support rod (up to ±90°), as water moves up and down in the plane of the tailfan, effectively simulates currents from any direction desired. Interneuronal responses are recorded en passant by electrodes attached to the support rod.

The CPR has been reported variously as being headward and/or bidirectionally sensitive. In the present experiments the CPR is consistently most responsive to transverse currents directed toward the tailfan appendages ipsilateral to the cell axon and dendrites. Headward and tailward responses are greatly attenuated and the opposite is direction without effect. The contribution of bilateral excitatory and inhibitory input from receptive fields on the telson and uropods has been assessed by unilateral section of the ganglionic roots while recording simultaneously from both CPRs. Ipsilateral input underlies the CPR response in the "preferred" direction, but responses based solely on excitatory input are less narrowly tuned. Contralateral input alone depresses the endogenous activity of the CPR for stimuli in the opposite direction. In simultaneous recordings from up to 6 sensory interneurons, the direction of peak sensitivity is seen to vary from one cell to the next. These results demonstrate in part that directional specificity is constant along with cell morphology and the somatotopic axon position in crayfish interneurons. Supported by grants from the Whitehall Foundation and Weldon Springs Fund.

41.11 FUNCTIONAL MORPHOLOGY OF NONSPIKING UROPOD STRETCH RECEPTORS IN THE SAND CRAB (EMERITA ANALOGA). L.J. Wilson* and D.H. Paul. Dept. of Biology, University of Victoria, Victoria, B.C. Canada. V8W 2Y2.

The uropod stretch receptor (USR) in Emerita is an elastic strand, arising from the dorsal surface of the telson and inserting on the uropod, which is innervated by 4 giant mechanosensory cells (SRs) with somata in the terminal abdominal ganglion (Paul,1972. Science 176:680). Reafference from USRs is required for the power stroke of the uropod in the 'treading water' swimming pattern (Paul, 1976.J Exp Biol 65:243). We are using light and electron microscopy (EM) in conjunction with cobalt and horseradish peroxidase peripheral filling techniques to examine the structure of the USR, looking for characteristics of nonspiking cells and morphological corellates of stretch.

Each SR has 3 morphologically distinct zones within the strand. 1)Zone of dendrite entry(ZDE):large (»10μm) branches resemble axons of motoneurons except that the dendrite membrane is thickened in all zones. Each has abundant neurofilaments and neurotubules, peripheral mitochondria, glial ensheathment and sparse extracellular matrix (ECM). SRs may have evolved from motoneurons. 2)Transitional zone(TZ):medium sized (1-10μm) branches are surrounded by connective tissue containing fewer glial cells and more ECM. ECM fibres in the TZ and ZDE exhibit the 64nm banding pattern of collagen. 3)Zone of dendrite termination(ZDT):dendritic endings are embedded without glial wrapping in dense ECM oriented parallel to the long axis of the strand. The ECM is similar to structures called 'vacuolated strings' containing 0.1μm SR endings of thoracico-coxal receptors (TCRs) of other decapods (Whitear,1965.Phil Trans Roy Soc B 248: 437). The vacuolated strings in Emerita contain small (0.25-0.5 m) dendritic processes and 0.1μm holes which may also be neuronal. Strand stretching causes cross-sectional profiles of the vacuolated strings to become longer and thinner. This may deform the dendrites either by compression by ECM or by stretching if the endings are attached to the ECM (Krauhs & Mirolli,1975.J Neurocytol 4:231). ECM fibres of the ZDT, including those in the vacuolated strings, belong to the special category of invertebrate elastic fibres that: 1)have a distinctive EM appearance, 2)are physically elastic and 3)stain with potassium permanganate/spirit blue (Elder & Owen,1967. J Zool Lond 152:1;Elder,1973.Biol Bull 144:43).

Thus, large branches of the SRs are anchored by collagen as they enter the strand while their termini are embedded in an elastic matrix. We continue to study how the site of receptor potential generation changes with stretch. The USR is less specialized than its putative homolog the TCR and appears to have the minimum elements essential for mechanosensory transduction.

Supported by a grant from NSERC to D.H. Paul.

41.12 MECHANOSENSITIVE SENSILLA INNERVATING THE SWIMMERETS OF THE LOBSTER. K.A. Killian and C.H. Page. Rutgers University, Bureau of Biological Research, Piscataway, NJ 08854

Mechanical stimulation of the swimmerets modulates postural activity produced within the abdominal nerve cord (Kotak, V.C. and Page, C.H., J. Comp. Physiol. A. 158:225-233, 1986). To better understand this response, the properties of several tactile sensitive receptors located on these appendages were investigated. Swimmerets consist of two flat leaf-like rami, the exopodite and endopodite, attached to a stalk-like coxa. We have examined the three most characteristic types of receptors responding to tactile stimulation: cuticular receptors underlying the rami surfaces and large and small feathered hairs which fringe the outer edges of each ramus.

An isolated third swimmeret with attached ventral nerve cord was prepared. Responses of receptors to mechanical stimulation were recorded with a suction electrode en passant from the first root. The mechanical stimulator consisted of a small (2 mm) diameter wooden probe glued to the center of a miniature loudspeaker. The blunt probe was used to stimulate cuticular receptors directly while hairs were deflected by slipping a small loop attached to the distal tip of the probe over individual hairs. Preliminary experiments indicate that both hair types also respond to near field pressure waves.

Large feathered hairs responded phasically by discharging a single action potential in response to mechanical stimulation. Small feathered hairs and cuticular receptors exhibited a more phasic-tonic response dependent on the strength of the applied stimulus. Receptive fields of individual cuticular receptors varied in size from large fields that include almost an entire surface of a ramus, to small restricted fields of a few millimeters diameter. Those receptors with larger fields innervate the more pliant cuticle within the center of the ramus while those with smaller fields are located under hard cuticular bands along the outer edges of the ramus. The sensory neurons that innervate the large feathered hairs responded to all directions of deflection of the hair. Their responses to sinusoidal movements were maximal between the frequencies of 100 and 200 Hz. The threshold for discharge within this range of frequencies was approximately 2-10 degrees which corresponds to 5-25 microns of linear movement measured at the midpoint of the hair shaft. The latency for recording spikes in the afferent axons was long and variable (15-30 msec) suggesting spike conduction is very slow in these fibers. This slow conduction velocity agrees with the unusually long latencies Kotak and Page (1986) observed for initiation of abdominal flexion inhibition responses produced by deflection of the feathered hairs. (Supported by NIH grant NS 19983-04 to CHP).

41.13 MODULATION OF THE RECEPTOR POTENTIAL IN CRUSTACEAN MECHANO-SENSITIVE AFFERENT NEURONS BY PROCTOLIN, SEROTONIN AND OCTOPAMINE. V.M. Pasztor and B.M.H. Bush* Biology Department, McGill University, Montreal, Canada, and Physiology Department, Bristol University, England

We have shown recently that the sensory response of mechano-sensitive afferents can be modulated by proctolin, serotonin and octopamine. (Nature, 326:793 - 795, 1987). Using intracellular recordings from the three primary afferents of a simple lobster stretch receptor, lacking centrifugal control, we observed that the two neuroamines depressed stretch-evoked impulse discharge, whereas the pentapeptide proctolin enhanced it. The drugs, which are known to be present in Lobster, were bath applied at concentrations of 10-6M. This mechano-receptor, the oval organ, is a favorable system for studying peripheral events because, not only are the afferents large enough to permit the introduction of microelectrodes near the transducer site, but their unusually large space constants (>10 mm) result in the receptor potentials being conducted along the afferents with very little decrement. Thus both receptor potentials and impulses are accessible for experimentation. The present study was undertaken to determine the target of neuromodulation: transducer membranes, adaptation systems or spike initiation zones.

Responses to trapezoidal stretches recorded in the presence of 10-6M TTX to block spiking, showed that the amplitude, but not the shape of the receptor potential was increased or decreased by 20 - 30% by physiological concentrations of the neuroactive substances. The rate and extent of both short and long-term adaptation was unchanged.

Spiking patterns, induced by standard depolarizing currents injected into the afferents near the spike initiation zone, showed no modulation by any of the three substances.

The lack of modulation of either adaptation or spiking threshold implies that the increased sensory responsiveness induced by proctolin, and the decrease seen with octopamine and serotonin may be explained by modulation of channel populations primarily linked with mechano-transduction.

41.14 ANATOMICAL AND PHYSIOLOGICAL EVIDENCE FOR A NOVEL INHIBITORY CIRCUIT IN THE CRAYFISH LATERAL GIANT ESCAPE RELFEX. E.T. Vu, S.C. Lee, and P.B. Krasne. Neuroscience Program, Dept. of Psychology, and Brain Research Institute, University of California, Los Angeles, CA 90024.

The key role of the pair of lateral giant (LG) command neurons in the neural network underlying the LG-mediated tailflip escape reflex of the crayfish has prompted us to undertake a detailed anatomical study of the LG dendrites and their synaptic inputs. By physiological criteria, the sensory inputs received and integrated by the LG dendrites in each abdominal ganglion have long been thought to be mediated purely be excitatory, electrical synapses. In contrast, our electron microscopy (EM) studies have revealed a complex mixture of synapse types onto the LG's. Of particular interest, some EM profiles believed to be excitatory afferents to the LG's have been seen to make synapses upon presumed GABAergic neurons which in turn form nearby synapses on the LG's. Since GABA is known to produce inhibition in LG cells, these observations suggest the possible existence of a "feed-forward" inhibitory circuit onto the LG's which parallels, and is driven by, the physiologically demonstrated excitatory one. The expected consequence of such a circuit would be the occurrence of inhibition of the LG's shortly after activation of their excitatory inputs, thus possibly helping to temporally tune the LG command cells to respond best to sharply arising stimuli. Physiological evidence for this inhibition, which we call "post excitatory inhibition", has been obtained. Supported by USPHS grant NS08108 (FK) and a Predoctoral NSF Fellowship (EV).

DRUG EFFECTS ON RECEPTORS I

42.1 PHENCYCLIDINE ACTIONS ON MODULATION OF THE NMDA-ACTIVATED CATIONIC CHANNEL. M. Bertolino, S. Vicini and E. Costa. FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Medical School, Washington, D.C. 20007.

Phencyclidine (PCP) reduces $^{45}Ca^{2+}$ ionic fluxes induced by the glutamate receptor agonist N-methyl-D-aspartic acid (NMDA) in cultured granule cells from post-natal rat cerebellum (1). The action of PCP on NMDA-activated cationic channel was investigated with the single-channel patch-clamp recording technique on outside-out patches excised from post-natal rat cerebellar neurons and neonatal rat cortical neurons in primary culture. The recording solution contained in mM NaCl 145, $CaCl_2$ 2, KCl 5, Glucose 5, HEPES-NaOH 5 at pH 7.4. The pipette solution contained $CsSO_4$ 145, $CaCl_2$ 1 mM HEPES-NaOH at pH 7.2 to avoid potassium and chloride fluxes. The NMDA-activated channel opened with a mean frequency of 0.5 sec^{-1} in cerebellar neurons, 1.03 sec^{-1} in cortical neurons in presence of NMDA 2 uM. At -50 mV holding potential and room temperature with NMDA 2 uM, the main conductance state of the channel was 50 pS in both models and the mean open time was 3.8 ± 1.1 msec (mean ± SD, n=10) and 6.4 ± 2.6 (mean ± SD, n=40) in cerebellum and cortex, respectively. In cortical cell, low PCP concentrations (2 uM) reduced the channel opening frequency by 60% (n=5) while high PCP concentration (20 uM) reduced the channel open time (n=8) BY 50%. Both effects were observed only when the channel was conducting in the inward direction (negative membrane potential), meaning that the action of PCP is voltage dependent. It is possible that the reduction of open time could be due to an open channel block mechanism with a relatively slow off-rate while the more specific action on opening frequency could be due to the allosteric modulation of the channel-receptor complex. Since the report that NMDA channel opening could be modulated by glycine presumably acting as an allosteric endogenous modulator (2), we investigated the combined action with PCP of this amino acid. At negative holding potential glycine (1 uM) was not able to completely counteract the action of phencyclidine while at positive membrane potential PCP failed to decrease channel opening frequency while glycine was still increasing it. This suggests that the two compounds are acting at different recognition sites located presumably in the NMDA receptor.

1) Wroblewski, J.T. et al. (1986), Clin. Neuropharmac. 9: Supp. 4, 494-496.
2) Johnson, J.W. and Ascher, P. (1987), Nature 325: 529-531.

42.2 EVIDENCE FROM 2-DG AUTORADIOGRAPHY THAT PHENCYLIDINE'S FUNCTIONAL EFFECTS ARE MEDIATED BY SPECIFIC PCP RATHER THAN SIGMA RECEPTORS. C.Ray*, G.D.Vogelsang and M.F.Piercey, (Spon. P.Broderick), CNS Research, The Upjohn Company, Kalamazoo, MI. 49001.

Sokoloff's 2-deoxyglucose (2-DG) autoradiographic technique (J. Neurochem. 28:897, 1977) was used to identify neural structures underlying the behavioral effects of the psychedelic drug phencyclidine (PCP, "angel dust") and to compare the distribution of PCP's functional effects to the distribution of specific 'PCP' and sigma receptors, both of which bind PCP (Vignon et al. Brain Res. 378:133, 1986). Phencyclidine, 5 mg/kg, was administered i.p. to male Sprague-Dawley rats 15 min prior to 25 uCi i.v. (^{14}C)-2-DG. Animals were sacrificed 45 min later. Autoradiograms of coronal sections were prepared from the prefrontal pole to the cervical cord. Computerized image analysis yielded quantitative measurements of regional energy metabolism. PCP dramatically increased metabolism in discrete brain regions, nearly all of which were located in diencephalic and telencephalic structures rich in PCP receptors (Largent et al., JPET 238:739, 1986). These effects were greatest in the limbic circuit described in 1937 by the neurologist James Papez (mammillary bodies, anterior thalamus, cingulate gyrus, entorhinal cortex, hippocampus, fornix), which was dramatically excited throughout, and the terminal zones of dopaminergic projections (caudate, n. accumbens, olfactory tub., prefrontal cortex). In general, anterior cortical regions, (especially sensory-motor cortex), were only weakly stimulated or even depressed, compared to more caudal cortical zones, (particularly striate 18), giving rise to an anteroposterior gradient similar to that reported in schizophrenia. Brainstem areas rich in sigma receptors (Largent et al., ibid) were generally unaffected. The inferior colliculus and the lateral habenula were inhibited. Chi-square analysis revealed a strong positive correlation for the areas stimulated with the presence of PCP receptors and a negative correlation with the presence of sigma receptors. Haloperidol (HAL), which binds to sigma but not PCP receptors, antagonized PCP's stimulant effects in most dopaminergic areas, but not in Papez' circuit. Even HAL's effects were negatively correlated with the presence of sigma receptors. HAL tended to depress PCP's cortical stimulation throughout without altering the anteroposterior gradient; indeed, some anterior cortical regions were severely depressed below controls. HAL did not significantly affect PCP's intense cingulate stimulation. Although HAL stimulated the lateral habenula, it only partially reversed the depression evoked by PCP. It is concluded that PCP elicits its extreme psychotropic effects by intense stimulation of Papez' limbic circuit and dopamine release, all of which are probably mediated either directly or indirectly through the PCP receptor.

42.3 **BLOCKADE OF CALCIUM CURRENT BY COCAINE IN CULTURED SENSORY GANGLION NEURONS OF THE MOUSE.** **R.Y.K. Pun. Dept. of Physiology and Biophysics, Univ. of Cincinnati, Cincinnati, Ohio, 45267-0576.**

The use of cocaine in the United States has reached an epidemic level. The number of people hospitalized, seeking help, and the number of deaths related to drug overdose has increased dramatically. In humans, inhalation of cocaine produces a bout of euphoria followed by irritability and dysphoria. In some cases, use of the drug also may produce psychotic symptoms such as acute anxiety, paranoia, or hallucinations. The cellular mechanism underlying the actions of cocaine is not fully understood, but it is thought to be mediated by enhancement of the responses to monoamines since cocaine is a potent blocker of re-uptake in synaptosomal preparations.

Another possibility to explain the enhanced synaptic response is that cocaine increases transmitter release by altering Ca^{++} influx at the presynaptic terminals. To test this hypothesis, I have studied the actions of cocaine on the transmembrane Ca^{++} current in primary cultures of mouse dorsal root ganglion (DRG) neurons.

Spinal cords with the sensory ganglia attached were isolated from 12-14 days old fetal mice. The cords were then enzymatically treated, dissociated and maintained in culture. Experiments were performed on cells plated for 3-8 weeks. Only DRG cells were studied because they are known to have larger Ca^{++} currents. Whole-cell recording was done with patch pipettes filled with a HEPES-buffered EGTA solution containing cesium (110mM) and TEA (20 mM). Voltage-clamp (VC) experiments were performed under the discontinuous single electrode VC mode with the Axoclamp amplifier. All experiments were done in the presence of TTX (2μM) at 22°C. To facilitate recording, the growth medium was changed to a HEPES-buffered medium containing TEA (50 or 100 mM), 4-AP (1mM), and $CaCl_2$ (3 or 5mM).

In DRG neurons during current-clamp recording, long duration action potentials (APs > 100msec) could be evoked by short depolarizing pulses at low stimulation frequencies (0.1 to 0.2Hz). Cocaine at 100 and 500μM was found to reduce the duration of the AP by about 65 % and 80 %, respectively. Neurons less than 30μm in diameter were used for VC studies since larger cells displayed axonal Ca^{++} spike which was not adequately clamped. In the majority of the cells examined, at holding potentials of either -40 or -90mV, a slowly inactivating inward current was recorded when depolarizing steps to -30 or -20mV were made. Peak current was obtained by depolarizing to 0mV (see Jia and Nelson, J. Neurophysiol., 56, 1257, 1986). On several occasions, a current of larger amplitude with a more rapidly inactivating time course could be evoked. The amplitude of both the slowly and rapidly inactivating currents were attenuated by cocaine (100 and 500μM). The antagonistic action of cocaine on presynaptic Ca^{++} currents cannot account for the potentiating effects of cocaine on monoamine responses, and may well reflect its local anesthetic action. Experiments are being conducted currently to examine the effects of lower concentrations of cocaine on the Ca^{++} current and on the process of synaptic transmission.

42.4 THE COCAINE BINDING SITE ASSOCIATED WITH DOPAMINE UPTAKE INHIBITION AS LABELED BY ^{3}H-GBR 12935. J. Sharkey* and M.J. Kuhar. Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224

Recent studies have indicated that the reinforcing properties of cocaine and related compounds are mediated by dopamine uptake inhibition, as defined by ^{3}H-mazindol binding in the rat striatum. (Ritz MC and Kuhar MJ, 1987: Fed. Proc 46(3): 508). However, in addition to labeling dopamine uptake sites, ^{3}H-mazindol also labels norepinephrine and serotonin uptake sites. To further define the mechanisms underlying behavioral reinforcement, we have examined the effects of cocaine and related compounds at the dopamine uptake site using the new and putatively more selective ligand ^{3}H-GBR 12935.

In rat striatal membranes, ^{3}H-GBR 12935 exhibited saturable, high affinity binding (K_D= 0.5 nM; B_{max}=400 fmol/mg tissue). Comparable binding characteristics were also observed within the nucleus accumbens, an area thought to subserve cocaine's reinforcement behaviors in the rat. In these brain areas, ^{3}H-GBR 12935 binding was competitively displaced by mazindol, cocaine and its analogues. Competition studies indicate that the affinities of cocaine, its stereoisomers and 7 structurally-related analogues at the ^{3}H-GBR 12935 binding site significantly correlate with their potencies at the ^{3}H-mazindol binding site and in cocaine reinforced behaviors ($r=0.84$, $p< 0.01$; $r= 0.92$, $p< 0.001$ respectively). This is consistent with the correlation, previously reported, between the relative potencies of these compounds in producing behavioral reinforcement and in displacing ^{3}H-mazindol from the dopamine uptake site.

In summary, 3H-GBR 12935, a more potent and selective dopamine uptake inhibitor than mazindol is similar to mazindol in that it presumably labels the site mediating the reinforcing behaviors of cocaine. Moreover, these data further support the hypothesis that inhibition of dopamine uptake systems underlies the reinforcing properties of cocaine.

42.5 COCAINE INHIBITS QNB BINDING TO MUSCARINIC RECEPTORS IN RAT BRAIN AND HEART. M.C. Ritz*, J. Sharkey* and M.J. Kuhar (SPON: Stephanie Bird). Molecular Pharmacology Laboratory, Neuroscience Branch, NIDA Addiction Research Center, Baltimore, Maryland, 21224

The action of cocaine at monoamine uptake sites has been well established in both CNS and peripheral tissues. However, the effects of cocaine at pre- or postsynaptic cholinergic binding sites have not been well described. The results of current experiments from our laboratory indicate that cocaine binds to muscarinic receptors in rat brain and heart tissues.

^{3}H QNB was used to label muscarinic M_2 in rat heart and brainstem. Tissue homogenates were incubated with 0.1 nM ligand at 37 degrees C. Nonspecific binding was defined by the addition of 5 uM atropine. Drug affinities at these binding sites were determined by analysis of competition curves. (+)- and (-)-cocaine had K_i values of 0.4 uM and 4.6 uM, respectively, in the brainstem. Similar K_i values of 0.57 uM and 3.5 uM were observed for these stereoisomers in the heart. In comparison with its binding characteristis at monoamine uptake sites, cocaine exhibits a reverse stereospecificity at muscarinic receptor sites. This is similar to our previous observation of reverse stereospecificity at ^{3}H hemicholinium labeled choline uptake sites in the brainstem. The affinity of cocaine at choline uptake sites, however, is relatively low and suggests that there is little cocaine binding at these sites at pharmacologically relevant blood concentrations. Cocaine competition at ^{3}H QNB binding sites was not modulated in the presence of 10 uM Gpp(NH)p, indicating that cocaine does not act as an agonist at these sites.

These data represent the first demonstration of cocaine binding to muscarinic receptor sites in cardiac and cerebral tissues. The affinity exhibited by cocaine at these muscarinic sites is similar to that reported for its actions at the adrenergic, serotonin and dopamine uptake sites. Micromolar concentrations have been shown to be pharmacologically relevant and are associated with the psychostimulant effects of cocaine in humans. It is possible that inhibition of muscarinic binding in the heart may be related to the cardiotoxic effects of cocaine.

42.6 COCAINE SUPPRESSES THE ACTIVITY OF NORADRENERGIC AND SEROTONERGIC NEURONS RECORDED FROM MOUSE BRAIN SLICES IN VITRO. K. Knudsen* and M.E. Trulson (SPON: L.L. Keeley). Dept. Anat., Coll. Med., Texas A&M Univ., College Station, TX 77843.

The psychotropic and behavioral effects of cocaine are thought to be mediated by its actions on central monoaminergic neurons, in particular, norepinephrine (NE)-containing neurons in the locus coeruleus (LC) and serotonin (5HT)-containing neurons in the dorsal raphe nucleus (RD). Recent studies have reported that cocaine suppresses the activity of NE- and 5HT-containing neurons in intact, anesthetized rats. However, it is not possible to determine from these studies whether the action of cocaine on the monoamine neurons is direct or indirect. We examined the effects of cocaine on 5HT- and NE-containing neurons recorded from mouse brain slices in vitro. Mice were decapitated and the brains were removed and cut into 400 μm coronal sections. Extracellular unit activity was recorded as previously described (Eur. J. Pharmacol. 124, 1986, 189; Brain Res. Bull., 18, 1987, 179). After obtaining a stable baseline of unit activity cocaine was added to the incubation medium by a nonpulsating exchange pump. Cocaine produced a dose-dependent decrease in the activity of LC cells, with an ED_{50} value of 6.7 μM. The suppression of LC unit activity was reversed by addition of the α_2 antagonist, piperoxane (5 μM) in all cells tested. Cocaine also produced a dose-dependent decrease in the activity of RD neurons, with an ED_{50} value of 4.3 μM. The suppressant effect of cocaine on RD neurons was also reversed by piperoxane in approximately 60% of the cells tested. The suppressant effect of cocaine on both 5HT- and NE-containing neurons persisted in an altered incubation medium containing low calcium (0.5 mM) and high magnesium (10 mM). The suppressant effect of cocaine on 5HT- and LC-containing neurons was rapidly reversible by drug washout. Administration of procaine, at a dose of 10 μM, produced no significant change in the discharge rate of 5HT- nor NE-containing neurons. These data demonstrate that cocaine suppresses the activity of both 5HT-containing RD neurons and NE-containing LC neurons by a direct action on the cells. The effect is not mediated by afferent input to these neurons since most afferents would be severed in the brain slice preparation. Any afferent inputs that may remain intact in the tissue slice preparation are rendered inactive in the low calcium/high magnesium medium. Furthermore, the in vitro slice preparation demonstrates that the changes in the activity of the monoamine neurons is not attributable to altered blood pressure or other physiological variables. The fact that procaine, a structurally related local anesthetic, produced no significant effect on the monoamine neurons indicates that the suppression of unit activity by cocaine is not due to the local anesthetic properties of the drug.

42.7 BENZODIAZEPINE RECEPTOR SUBTYPES AND MODULATION OF ELECTRICAL ACTIVITY IN CORTEX, HIPPOCAMPUS AND OLIVO-RUBRO-CEREBELLAR LOOP OF RABBITS. D.DeMedici* and M. Massotti (SPON:R.del Carmine). Lab. di Farmacologia, Istituto Superiore di Sanita', 00161 - Roma, Italy.

After administration of diazepam (0.1-10 mg/kg iv) and flunitrazepam (0.03-3.0 mg/kg iv), which bind at central BDZ_1 and BDZ_2 and peripheral BDZ receptor subtypes, increase of the amplitude (from 50-100 to 150-200 µV) and decrease of the frequency (from 40-50 to 20-30 Hz) of the electrical activity can be recorded from red nucleus and nucleus interpositus. Clonazepam (0.02-5.0 mg/kg iv), which binds at central BDZ_1 and BDZ_2 receptor subtypes, and the triazolopyridazine derivative CL 218,872 (0.2-10 mg/kg iv), which selectively binds at central BDZ_1 subtypes, both elicit a slight increase of the amplitude (to 80-120 µV) without significantly affecting the frequency in both areas.

At the level of the associative cortex, diazepam, flunitrazepam and clonazepam induce changes of electrical activity consisting of a lengthening (from 0.5-1.0 to 2-4 sec) of the spindle bursts (7-12 Hz; more than 300 µV) and the appearance of 25-30 Hz activity. On the contrary, selective activation of the BDZ_1 receptor subtype by Cl 218,872 induces the above reported modifications of the spindle bursts only.

All drugs also elicit disruption of the hippocampal theta waves. A slowing of the electrical activity emerges with the presence of sharp waves.

Present data would suggest that activation of BDZ_1 receptors modulates the appearance of the spindle bursts in the associative cortex with a limited effect on the activity of the olivo-rubro-cerebellar loop.

The BDZ antagonist Ro 15-1788 counteracts the EEG modifications induced by the highest doses of the various agonists in all brain areas with different potency. Doses of 0.1 and 0.2-0.4 mg/kg iv antagonize the effects of diazepam and CL 218,872, respectively. On the contrary, higher doses (0.4-1.0 mg/kg iv) are required to inhibit the effects of both flunitrazepam and CL 218,872 in all leads.

This work has been supported in part by the CNR contract n. 86.02039.56.

42.8 A NOVEL GABA ANTAGONIST: 1,7-DIMETHYL-6-IMINO-2-THIOXOPURINE. G.J. Patel*, P.C. Harrison*, K.G. Grozinger* and V.B. Ciofalo. Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877.

Two compounds, 1,7-dimethyl-6-imino-2-thioxopurine (compound A) and 1-methyl-6-imino-2-thioxopurine (compound B) were found to have significant affinity for GABA receptors (IC_{50}'s for displacement of [3H] muscimol = 640 and 220 nM, respectively) in an in vitro rat brain receptor binding preparation. Since a compound interacting at the level of GABA receptors in the brain could be a GABA agonist or antagonist, we selected compound A as representative of the two compounds and conducted the following in vitro and in vivo studies.

Compound A failed to display significant affinity for benzodiazepine, opiate, noradrenaline, dopamine or serotonin receptors in rat brain in vitro receptor binding assays. In in vivo studies, intracerebroventricular (i.c.v., third ventricle) administration of compound A resulted in convulsions in rats ($ED_{50} \leq 2$ µg). Bicuculline, a known GABA receptor antagonist, also produced convulsions in rats when it was administered i.c.v. at similar dose levels ($ED_{50} \leq 2$ µg). Compound A (2 µg, i.c.v.) induced convulsions were completely antagonized by a known GABA-receptor agonist, muscimol (400 ng, i.c.v.) in a preliminary study.

Compound A was also tested for its ability to selectively block muscimol induced contralateral turning in rats after unilateral administration of muscimol into the substantia nigra, pars reticulata (SNR). The contralateral turning is believed to be the result of unilateral stimulation of GABA receptors within the SNR by muscimol. Bicuculline, administered unilaterally into the SNR, has been reported to selectively block this muscimol-induced turning (Arnt and Scheel-Kruger, Psychopharmacol. 62: 267-277, 1979), suggesting that this animal model can be used to test novel GABA antagonists. Compound A (0.5 µg/0.5 µl) administered unilaterally into the SNR 30 minutes after muscimol (25 ng/0.5 µl), completely blocked muscimol induced contralateral turning in rats. Saline (0.5 µl) did not antagonize muscimol-induced turning in rats which lasted for more than 60 minutes. Muscimol treated rats displayed a frequency of 9-16 turns/minute at the time of compound A or saline administration. Collectively, these data suggest that compound A is a novel GABA-receptor antagonist.

42.9 COMPARISON OF TRIOXABICYCLOOCTANE BINDING TO THE PUTATIVE CHLORIDE CHANNEL IN TORPEDO ELECTRIC ORGAN AND RAT BRAIN. R.G. Thompson, D.E. Menking* and J.J. Valdes. Dept. of Environ. Health Sciences, The Johns Hopkins University and Biotech. Div., Chem. Res. Devel. & Engr. Ctr., Aberdeen Proving Ground, MD 21010-5423.

Trioxabicyclooctanes of the structure $X\text{-}(OCH_2)_3\text{-}Y$ represent a class of potent convulsants that are believed to antagonize GABA neurotransmission by blocking the conductance of anions through the Cl^- channel. The most potent of these compounds occur when a phosphorous substituent (P, P=O, P=S) in the X position is coupled with a branched alkyl group ($t\text{-}C_4H_9$, $i\text{-}C_3H_7$) in the Y position. In view of observations that ^{35}S-t-butylbicyclophosphorothionate (^{35}S-TBPS) appears to bind at two different sites in rat brain, we sought to determine and compare the relative potency and selectivity of different bicyclophosphate congeners for displacing ^{35}S-TBPS from both the GABA-independent voltage sensitive Cl^- channels found in Torpedo electric organ and the GABA-A type receptor-gated channels found in rat brain.

^{35}S-TBPS binding in electric organ gave estimates of 2.4 uM and 35.4 pmol/mg protein for the ligand's binding constant and maximal number of binding sites, respectively. The most potent of the bicyclophosphates in displacing ^{35}S-TBPS from electric organ membranes were unlabelled TBPS, with an IC_{50} of 3 uM, and the t-butylphospho-oxide derivative with an IC_{50} of 84 uM. All of the bicyclophosphates proved at least two orders of magnitude less potent than the hexachlorocyclohexane insecticide, lindane, which showed an IC_{50} of 45 nM. In addition, none of the bicyclophosphates were found to affect binding of ^{3}H-PCP to the Na^+ channel in electric organ membrane preparations.

Binding of ^{35}S-TBPS to rat brain membranes yielded an apparent dissociation constant of 150 nM and a maximum of 18.5 pmol bound per gram of original tissue. In contrast to the results obtained in electric organ, the t-butylphosphorothionate and the t-butyl and isopropyl- phosphooxide derivatives appeared equipotent with lindane in displacing ^{35}S-TBPS with IC_{50}s between 366 and 457 nM. GABA was also observed to inhibit ^{35}S-TBPS binding with an IC_{50} of 30 uM and this effect was reversed in a dose-dependent manner by the GABA antagonist bicuculline, which also significantly increased binding when added to the incubation alone. In the reverse situation where the ability of bicyclophosphates to affect GABA receptor binding was assessed, only TBPS in high concentrations (IC_{50}~100 uM) was found to inhibit the binding of ^{3}H-muscimol to rat brain membranes. We conclude that the pharmacological binding profile of voltage-gated channels in Torpedo electric organ is significantly different from the GABA receptor-gated channels in rat brain with the isopropylbicyclophosphooxide providing the best discrimination between them (selectivity index >2000). Further studies are underway in efforts to discriminate between receptor and voltage-gated sites in brain.

42.10 INVOLVEMENT OF GABAERGIC TRANSMISSION IN THE ELECTROPHYSIOLOGICAL EFFECTS OF NICOTINE. R.K. Freund, D. A. Jungschaffer*, and J. M. Wehner. Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

Bath-application of nicotine to submerged hippocampal slices from mice produces an increase in the amplitude of the evoked population spike (PSA) and the appearance of multiple spikes measured in the CA1 pyramidal cell layer (Freund and Wehner, J. Neurogenet., in press). A number of GABA-related agents were used to investigate the possibility that nicotine may interfere with GABAergic transmission to produce these effects in DBA/2Ibg mice. Bath-application of GABA (400 uM) reversed the increase in the initial PSA and abolished the secondary spiking induced by nicotine (800 uM). Similarly, the GABA uptake inhibitor nipecotic acid (1-5 mM) reversed these effects of nicotine. L-C-Allylglycine (4 mM), an inhibitor of GABA synthesis, and bicuculline methiodide (1-8 uM), a GABA receptor antagonist, both produce electrophysiological effects similar to those produced by nicotine. These results suggest that the excitatory effects of nicotine on CA1 pyramidal cells may be mediated at least in part by disrupting inhibitory GABAergic transmission, since agents known to interfere with GABA transmission mimic the effects of nicotine. Furthermore, these effects of nicotine appear to involve a reduction in local GABA levels and not to be direct effects at the level of the GABA receptor, since pharmacological manipulations which should increase synaptic GABA levels resulted in effective inhibition in the presence of nicotine.

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42.11 ANTAGONISM BY Ro 15-4513 OF ETHANOL EFFECTS ON GABA-EVOKED RESPONSES IN FROG SPINAL CORD. R. Afar*, A. Sevigny*, P. Polc* & A.L. Padjen. Department of Pharmacology & Therapeutics, McGill University, Montreal, Quebec.

Recent reports demonstrated that Ro 15-4513, a "partial inverse agonist" at GABA-benzodiazepine receptor, antagonizes some effects of ethanol on the central nervous system (Polc, 1985; Suzdak et al., 1986). In the present work, we have examined the interaction of Ro 15-4513 with ethanol effects on GABA-evoked responses on dorsal root ganglia, and on GABAergic and taurinergic synaptic transmission in frog spinal cord. The experiments were done on isolated dorsal root ganglia and hemisected spinal cord of frogs; electrical signals were recorded by sucrose gap technique.

Ethanol (50 - 100 mM) caused a concentration dependent increase of dorsal root ganglion responses to GABA (10 - 100 μM), with a leftward shift of concentration - response curve; at 20 μM of GABA, the increase was 63 ± 15 %, SE, n=8, $p<<0.01$; responses to 1 mM of GABA and taurine on dorsal root terminals were only occasionally increased by ethanol (cf. Padjen et al. 1987). Addition of Ro 15-4513 (100 nM) significantly and reversibly suppressed this increase; e.g. at 20 μM GABA, ethanol increase was attenuated by 22 ± 8 %, SE, n=8, $p<0.05$. Depolarizing effect of ethanol on dorsal root ganglia (up to 1.5 mV) was insensitive to bicuculline and was not affected by Ro 15-4513.

Selective effects of ethanol (100 mM) on synaptic potentials (increase in amplitude and decrease in duration of GABA and taurine mediated dorsal root potentials and suppression of spontaneous activity, cf. Padjen et al. 1987), were inconsistently reversed by Ro 15-4513 (up to 2 μM).

In conclusion, our results demonstrate:

1. Partial reversal of ethanol effects on GABA-evoked responses by Ro 15-4513, in agreement with the hypothesis that GABA-benzodiazepine receptor is a site of ethanol action in the nervous system. 2. Direct depolarizing effect of ethanol on primary afferents appears to be mediated by another site.

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42.12 Ro5-4684 AND MIDAZOLAM RELAX AIRWAY SMOOTH MUSCLE IN VITRO: A ROLE FOR A BENZODIAZEPINE PERIPHERAL RECEPTOR IN BRONCHODILATION? D. Raeburn* and L.G. Miller (SPON: D. Kline). Depts. of Pharmacology and Medicine, LSUMC, New Orleans, LA 70112.

A peripheral, non-neuronal benzodiazepine (BZD) binding site or receptor has been described in cardiac muscle and vascular smooth muscle. In these tissues BZDs have Ca^{2+} antagonist-like properties especially at higher (>1 μM) concentrations and it is postulated that a μM affinity BZD receptor may be associated with Ca^{2+} channels in peripheral organs (Holck, M. and Osterrieder, W., Eur. J. Pharmac. 118: 293, 1985). Ro5-4684 is a selective agonist at the peripheral receptor where its actions are blocked by the selective antagonist PK11195 (Le Fur, G. et al. Life Sci. 32: 1849, 1983). We decided therefore to examine the actions Ro5-4684 and midazolam in guinea-pig tracheal smooth muscle to assess their potential as Ca^{2+} antagonists in the airways. Preliminary binding studies using [^{3}H]-Ro5-4684 indicate the presence of a peripheral receptor with a K_D of approximately 4 nM and B_{max} of 6 pmol/mg protein. In tension studies however, no activity was seen for Ro5-4684 at concentrations below 1 μM. Higher concentrations relaxed the airway smooth muscle under basal tone, the effect was augmented significantly ($p < 0.05$) by epithelium removal (pD_2 +EPI, 4.19 ± 0.14; -EPI, 4.42 ± 0.12). Similar results were obtained in tissues precontracted with methacholine. The relaxant effects of Ro5-4684 were antagonised by PK11195 (10 nM - 1 μM) but were unaffected by the central BZD receptor antagonist (Ro151788, 0.1 μM). Midazolam relaxed airway smooth muscle in the same concentration range as Ro5-4684 (pD_2 +EPI, 4.18 ± 0.03; -EPI, 4.38 ± 0.02). PK11195 (0.1 μM) reduced the maximum response to midazolam in -EPI tissues but had no effect in intact tissues. Ro151788 (0.1 μM) was without effect in the presence or absence of the epithelium. In K^+-depolarized tracheal strips bathed in a zero Ca^{2+} solution, the re-addition of Ca^{2+} (0.1 - 2.5 mM) produced a concentration related contractile response. Midazolam (100 μM) significantly ($p < 0.05$) attenuated the response to Ca^{2+} in the K^+-depolarized tracheal strips, the effect was greater at low Ca^{2+} concentrations. The rate of Ca^{2+} entry was reduced by midazolam, the time to half maximum response increasing by 60 to 250% over control. These results indicate a similar profile of activity for BZDs in airway and vascular smooth muscle. The compounds appear to be Ca^{2+} antagonists in airway smooth muscle. However, Ro5-4684 and midazolam may be acting via different mechanisms. If BZDs are Ca^{2+} antagonists then they are not typical as shown by their ability to reduce basal tone in airway smooth muscle, a property not found in the "classical" Ca^{2+} antagonists.

DRUG EFFECTS ON RECEPTORS II

43.1 ION CHANNEL KINETICS FOR TWO POTENT, SEMIRIGID NICOTINIC AGONISTS. C.E. Spivak and J.A. Waters*. Neuropharm. Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224, Dept. Pharmacol. & Exp. Ther., Univ. of MD Sch. Med., Baltimore, MD 21201; NIADDK, Bethesda, MD 20205.

The mechanism for recognition of an agonist and the consequent opening of the ion channel by the nicotinic receptor is obscure at the molecular level. The kinetics of both steps depend upon the agonist (e.g. Colquhoun, D. and Sakmann, B. J. Physiol. 369:501 1985). Thus, a single transduction process is seen to be composed of four or more kinetic constants, each of which may have independent structure-activity relationships. An understanding of each of these relationships can yield insight into the mechanism of each step.

Semirigid agonists are most useful in probing the mechanism for molecular recognition. Of the semirigid nicotinic agonists, one of the most potent is isoarecolone methiodide (ISO) (Spivak, C.E. et al., Eur. J. Pharmacol., 120:127 1986), which is 50-times more potent than carbamylcholine at the frog neuromuscular junction. In this abstract, we compare this agonist to the analogue, dihydroisoarecolone methiodide (H_2-ISO), which is 9.1-times more potent than carbamylcholine.

Patch clamp records were obtained at junctional regions (only) of dissociated interosseal muscle fibers (Allen, C.N. et al., J. Physiol. Paris, 79:338, 1984) from toes of the frog Rana pipiens. The agonist concentration was 100 nM, and the temperature was 10°C. The effective bandwidth was 7.5 kHz, so gap durations as brief as 40 microseconds were recorded reliably. With both agonists, closed time histograms showed three (major) components, given below as time constants and relative areas (in parentheses):

	ISO	H_2ISO
tau (fast)	0.036 ms (0.51)	0.055 ms (0.43)
tau (med.)	0.78 ms (0.30)	0.78 ms (0.35)
tau (slow)	163. ms (0.19)	510. ms (0.22)

Bursts were defined as open events separated by 3 ms or less. Two components were resolved:

	ISO	H_2ISO
tau (fast)	0.46 ms (0.12)	0.62 ms (0.28)
tau (slow)	7.6 ms (0.88)	10.6 ms (0.72)

These two structurally similar agonists clearly produced similar, though distinctly different effects on gating kinetics.

Events per burst histograms showed two clear geometric distributions, indicating two open states of the ion channel. A five-state cyclic model, therefore, was adopted, and the rate constants were estimated.

43.2 COMPARATIVE EFFECTS OF OXYBUTYNIN (DITROPAN®) AND ITS ENANTIOMERS IN THE GUINEA PIG BLADDER. J.S. Peterson*, D.E. Wilkins*, V.C. Lowe*, J.F. Kachur*, J.P. Carter*, W.J. Rzeszotarski*, L. Noronha-Blob* and R.C. Hanson*. (Spon: W.J. Kinnier). Nova Pharmaceutical Corp., 6200 Freeport Centre, Baltimore, Maryland 21224-2788.

Oxybutynin HCl (OXY), in its racemic mixture form, has been used for many years in the treatment of symptoms related to neurogenic bladder and stress or urge incontinence. Its therapeutic effects have been ascribed to antimuscarinic, antispasmodic and local anesthetic actions. In order to judge the effect of stereochemistry on the biological activity of OXY, it was necessary to resolve the enantiomers. The (+) and (-) optical isomers of OXY were prepared and evaluated in several in vitro assays and in the in vivo cystometrogram (CMG) using the anesthetized guinea pig.

Isomers of OXY were prepared as HCl salts by first resolving cyclohexylmandelic acid and then coupling it to 4-diethylamino-1-hydroxy-2-butyne using Mitsunobo conditions (triphenylphosphine/diethylazodicarboxylate) producing the desired esters in high yield.

Guinea pig bladder detrusor strips were prepared to examine antimuscarinic and antispasmodic activities in vitro against carbachol induced and potassium evoked contractions, respectively. Inhibition of phosphoinositide (PI) breakdown was measured in minced bladder tissue using acetylcholine (100 μM) to stimulate PI turnover. Cystometrograms were performed on urethane anesthetized guinea pigs in vivo to determine peak intravesicular pressure when contracting in response to a slow infusion of saline.

Test ($n \geq 3$; $\bar{x}$ ± S.E.M.)	(+/-)OXY	(+)OXY	(-)OXY
Antimuscarinic; K_b (μM)	.042 ± .01	.557 ± .255	.021 ± .002
Antispasmodic; IC_{50} (μM)	11 ± 2	14 ± 2	12 ± 4
PI breakdown; K_i (μM)	.017 ± 0.8	.654 ± .044	.004 ± .0002
CMG; ID_{50} (mg/kg, i.v.)	0.14 ± 0.03	2.13 ± 0.69	0.10 ± 0.03

Stereoselectivity is evident, (-)OXY > (±)OXY > (+)OXY, for muscarinic receptors in guinea pig bladder while no selectivity was observed for antispasmodic actions. The similar rank order of potency of these compounds in the in vivo assay suggests that muscarinic receptors play an important role in mediating bladder contractions.

43.3 EFFECT OF MONOAMINE UPTAKE INHIBITORS ON NOREPINEPHRINE STIMULATED PHOSPHATIDYLINOSITOL HYDROLYSIS IN RAT CEREBRAL CORTICAL SLICES. M.Mosaddeghi* and R.A.Gonzales, Dept. of Pharmacology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

It has recently been discovered that phosphoinositides are a storage form of molecules that carry signals across the membrane as second massengers in response to extracellular agonists. In brain, neurotransmitter activated phosphatidylinositol (PI) hydrolysis probably plays a role in the regulation of neuronal function. The effect of NE uptake inhibitors cocaine (COC), desipramine (DMI), and nisoxetine on norepinephrine (NE) stimulated PI hydrolysis was investigated. Rat cortical tissue slices were labelled with [^{3}H]inositol and incubated in the presence of 8 mM LiCl and various concentrations of NE and the monoamine uptake inhibitor as described by Gonzales and Crews (J. Neurochem. 45:1076, 1985). All tissue slices were treated with pargyline for 30 minutes during labelling. Labelled tissue slices were incubated with the uptake inhibitor for 10 minutes before stimulation with NE. Norepinephrine stimulated PI hydrolysis was stopped by adding 1.0 ml chloroform-methanol (1:2) solution. [^{3}H]Inositol phosphates were eluted from Dowex 1-X8 with 6 ml of 1.0 M ammonium formate/0.1 M formic acid and radioactivity was determined. Cortical tissue slices incubated with 1 uM and 10 uM COC in the presence of NE caused an increase in the maximum PI hydrolysis by 15 and 30 percent respectively, in addition to a shift to the left of NE dose response curve. The EC50 for NE stimulated PI hydrolysis shifted from 3.82 uM for control to 2.63 and 2.07 uM in the presence of 1 and 10 uM COC, respectively. The potentiating effect of COC was dependent upon the concentration of COC and the level of stimulation by NE with a maximum effect at 100 uM COC in the presence of 0.3 uM NE. The norepinephrine concentration-effect curve was shifted to the right 100-fold in the presence of 0.1 uM prazosin. The potentiating effect of 10 uM COC on NE-stimulated PI hydrolysis was not seen in the presence of 0.1 uM prazosin. Nisoxetine (0.1 uM) caused a significant shift to the left of the concentration effect curve for NE-stimulated PI hydrolysis with a decrease in the EC50 from 2.54 uM for the control value to 1.04 uM with presence of nisoxetine and a 12 percent increase in the maximum response. DMI at concentrations of 0.1 and 1.0 uM did not significantly affect NE-stimulated PI hydrolysis. The negative effect of DMI may be due to its alpha adrenergic blocking properties. These data suggest that some of the central effects of the COC may be mediated through alpha-1 adrenergic receptors. However, the possible interaction between COC and prazosin at pre- or postsynaptic sites is unknown. (Supported by LSUMC BRSG and NIAAA grant AA07223)

43.4 INHIBITION OF BINDING OF [^{3}H] BTX-B TO VOLTAGE-SENSITIVE SODIUM CHANNELS BY CONFORMATIONALLY RIGID 2,4-OXAZOLIDINEDIONES. T. M. DeLorey, G. B. Brown, and W. J. Brouillette, Department of Chemistry and The Neurosciences Program, University of Alabama at Birmingham, Birmingham, AL 35294.

A number of clinically effective anticonvulsants, as well as other classes of pharmacological agents, have been investigated as competitive binders of the voltage-sensitive sodium channel. Especially significant are the findings that the commonly used anticonvulsants diphenylhydantoin (IC_{50}=40 μM) inhibit the binding of [^{3}H] batrachatoxin A 20-α-benzoate ([^{3}H] BTX-B) in rat brain synaptosomes at concentrations consistent with mean therapeutic brain levels. However, the anticonvulsants sodium valproate, ethosuximide, phenobarbital, and trimethadione (3,5,5-trimethyl-2,4-oxazolidinedione) do not alter [^{3}H] BTX-B binding at concentrations up to 1 mM. We examined this effect for a series of synthetic 2,4-oxazolidinediones. We found that 5-ethyl-5-phenyl-2, 4-oxazolidinedione and 5-ethyl-3-methyl-5-phenyl-2,4-oxazolidinedione, like trimethadione, exhibited only slight effects on [^{3}H] BTX-B binding. However, two analogs with conformationally restricted 3- and 5-alkyl substituents, namely 1-aza-8,9-dioxo-7-oxa-6-phenyl-bicyclo [4.2.1] nonane and 1-aza-9,10-dioxo-8-oxa-7-phenylbicyclo [5.2.1] decane, exhibited significant inhibition of [^{3}H] BTX-B binding (approximate IC_{50}=300 and 150 μM, respectively). These results suggest that the correct conformation of side-chain alkyl substituents in these systems may be essential for efficient binding to the voltage-sensitive sodium channel.

In addition, these oxazolidinediones and their analogs were evaluated on their ability to effect GABAergic binding.

Currently, NIH is evaluating these compounds on animal seizure models. With this information it should be possible to establish correlation between compound structure, site of action and efficacy as anticonvulsants.

43.5 **A RAPID SCREENING PROCEDURE FOR THE DETECTION OF COMPOUNDS ACTIVE AT THE VOLTAGE-SENSITIVE SODIUM CHANNEL.** G. B. Brown and J.E. Gaupp*, The Neuropsychiatry Research Program, University of Alabama at Birmingham, Birmingham, AL 35294.

The objective of these studies has been to develop a rapid, reliable and simple screening assay of broad scope and sensitivity for the detection of compounds acting at the voltage-sensitive sodium channel that can accomodate the screening of large numbers of samples. These requirements have been met in the form of an *in vitro* radioligand binding assay in which the binding of [^{3}H]batrachotoxinin-A benzoate (BTX-B) serves as a sensitive indicator of test compound interactions at any of at least five different binding domains on the sodium channel. Previous work from this laboratory and others has shown that binding of [^{3}H]BTX-B is modulated by several other classes of sodium channel toxins and ligands, including α-scorpion toxins, pyrethroid insecticides, local anesthetics and certain anticonvulsants, and the heterocyclic guanidinium channel blockers tetrodotoxin and saxitoxin. Each of these is known to bind to a different site on the sodium channel and, in doing so, to either increase (α-scorpion toxin, pyrethroids) or decrease the affinity for the binding of [^{3}H]BTX-B. Using a radioligand binding paradigm, a tracer amount of [^{3}H]BTX-B is equilibrated with the vesicular synaptoneurosome preparation from rat brain cerebral cortex in the presence of TTX, deltamethrin, and α-scorpion toxin from *Leiurus quinquestriatus*, each at a concentration approximately at the mid-point of the respective dose-response curve for enhancement or inhibition of BTX-B binding. By selecting these particular concentrations, the level of BTX-B binding in control assays is in a delicate balance and highly sensitive to the presence of agents added in test assays which might act at any of these sites. Assay tubes are incubated at room temperature for 1 hr and the samples collected by automatic filtration on a 30-place manifold prior to scintillation counting. To further simplify the practical aspects of the assay, conditions are described by which the synaptoneurosomes may be prepared in bulk and successfully stored in a frozen state at -70°C for up to 4 months. The time required from set-up of the assay to initiation of sample counting is less than two hours with the use of pre-prepared synaptoneurosomes, thus large numbers of samples may be processed easily. This assay should prove to be very useful for programs which require preliminary screening of multiple samples such as might arise in testing of chromatographic fractions from the purification of sodium channel-active agents, screening of panels of antibodies directed against the sodium channel or its ligands, or in the identification of new potentially therapeutic compounds arising from a broad-based synthetic program.

43.6 THE EFFECTS OF BETA-ADRENERGIC ANTAGONISTS ON NEUROMUSCULAR TRANSMISSION IN RAT SKELETAL MUSCLE. JF Howard, Jr., BR Johnson and SR Quint[1]. Dept. of Neurology, Neurobiology Curriculum and [1] Division of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27514.

Beta-adrenergic blocking drugs are used to treat a variety of medical disorders including hypertension, migraine, glaucoma, hyperthyroidism and angina. Patients with one of the above medical disorders and in addition a neuromuscular disorder have reported symptomatic worsening of strength when treated with beta-blocking drugs. Although much is known about beta-adrenergic blocker action in the central and sympathetic nervous systems, little is known about the effects of these drugs on neuromuscular transmission in skeletal muscle. If beta-blockers reduce the safety factor for neuromuscular transmission then these drugs should be used with caution in those patients whose transmission is already compromised by a disease state. In this study we examined the effects of the beta-blocking drugs atenolol, labetolol, metaprolol, nadolol, propranolol and timolol on neuromuscular transmission in rat skeletal muscle.

Conventional intracellular recording techniques were used to measure end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) in forearm flexor digitorum longus muscle from adjult rats (T-24-27 deg.C); EPP and MEPP waveform properties and quantal content were analyzed with an on-line computer. At test concentrations from 1-100uM, all drugs produced a dose dependent reduction in the amplitude of both MEPPs and EPPs. Resting potentials of motor end-plate regions were not affected by any drug treatments. The drugs most effective in reducing MEPP amplitude were, in descending order of potency, propranolol, metaprolol, nadolol, timolol, labetolol and atenolol. At the highest test concentration timolol, nadolol and propranolol decreased MEPP rise time while all drugs but atenolol decreased MEPP duration. Atenolol was the only drug to increase MEPP duration. Labetolol produced a large increase in MEPP frequency, propranolol a smaller increase, while atenolol and metaprolol reduced MEPP frequency. The most effective blockers for EPP amplitude were propranolol, timolol, metaprololol, labetolol, nadalol and atenolol, in order of descending potency. Again, at 100 uM all drugs but atenolol decreased EPP rise time and duration. Atenolol had no effect on EPP rise time but greatly increased EPP duration. Propranolol, labetolol and metaprolol decreased quantal content.

Our results demonstrate that different beta-blocking drugs may have reproducibly different pre- and post-synaptic effects on neuromuscular transmission in rat skeletal muscle. These differential effects may be related to the differences these drugs display in their beta-receptor selectivity, agonist activity and/or local anesthetic activity. Although the concentrations we found to affect neuromuscular transmission are higher than those used to treat medical disorders, the effects noted here may be important for patients with diseases such as myasthenia gravis or myasthenic syndrome where neuromuscular transmission is already impaired. Supported in part by NS00941.

43.7 IRREVERSIBLE AGONIST BINDING TO THE ß-ADRENORECEPTOR: EVIDENCE FOR SUSTAINED ACTIVATION. K.M. Standifer, Y. Chida*, J. Pitha*, and S.P. Baker. Dept. of Pharmacology, University of Florida College of Medicine, Gainesville, FL 32610, and GRC-National Institute on Aging, Baltimore MD 21224.

Irreversible antagonists have been shown to be useful probes for the ß-adrenoreceptor. However, it is well known that agonist interactions with the receptor are modulated by a variety of factors, whereas antagonist interactions are not. Therefore, it would be of interest to investigate the effects of an agonist that binds irreversibly to the ß-adrenoreceptor. In the present study, the effects of 2 carbostyril congeners, Carbo-Amine (C-Am) and Carbo-Bromoacetyl (C-Br) were investigated using the rat reticulocyte ß-adrenoreceptor system.

Competitive binding studies using [^{125}I]iodocyanopindolol (ICYP) in the presence of 100 µM guanyl-5'-yl imidodiphosphate gave IC_{50} values of 7.6 ± 0.3, 21 ± 0.6, and 813 ± 66 nM for C-Br, C-Am, and (-)isoproterenol (iso), respectively. The EC_{50} values for adenylate cyclase activation by these drugs was C-Br, 8.1 ± 2.1 nM; C-Am, 17.8 ± 3.1 nM; and iso, 241 ±17 nM. Furthermore, both compounds exhibited the same intrinsic activity as iso. The initial rate of adenylate cyclase activation by 1 µM of C-Br and C-Am and 10 µM iso was blocked with concurrent addition of 20 µM propranolol. If, however, propranolol was added 7 min into the assay, cAMP production by C-Br and C-Am remained linear throughout the 14 min time course, while further cAMP production by iso was immediately blocked.

Pretreatment of membranes with 1 µM of either C-Br or C-Am for 30 min followed by washes showed a 70-92% loss of receptor sites, with no change in the K_D for ICYP binding to receptors remaining. In both cases, receptor loss was prevented by simultaneous addition of 10 µM nadolol with drug, and could not be reversed by the addition of nadolol subsequent to the 30 min incubation. However unlike C-Br treated membranes, C-Am binding was reversed by incubation at 45°C in the presence of nadolol.

This data indicates that the 2 carbostyril congeners are potent full agonists at the ß-adrenoreceptor, and that C-Br appears to be an irreversible agonist.

43.8 BHT-920 IS A FULL AGONIST AT THE D-2 DOPAMINE RECEPTOR IN RAT INTERMEDIATE LOBE. L.D. Artman*, M.S. Ackerman*, R.G. MacKenzie and J.W. Kebabian. Abbott Laboratories, Abbott Park, IL 60064.

The azepine derivative, BHT-920, is an agonist at the alpha-2 adrenoceptor and is reported to act as a dopamine D-2 agonist selective for presynaptic autoreceptors (Anden, N.-E. et al.. Naunyn-Schmiedeberg's Arch. Pharmacol. 321:100, 1982). Recently, BHT-920 was also shown to inhibit acetylcholine release from striatal slices which is indicative of a post-synaptic action at the D-2 receptor (Schmidt, C.J. et al. Naunyn-Schmiedeberg's Arch. Pharmacol. 334:377, 1986).

The present study tested BHT-920 in biochemical models for activation of the two dopamine receptor subtypes, D-1 and D-2 (Kebabian, J.W. and Calne, D.B. Nature, 277:93, 1979). D-1 activation was measured as stimulation of adenylate cyclase in homogenates of fish retina whereas D-2 activation was measured as inhibition of cholera-toxin stimulated adenylate cyclase activity in homogenates of rat intermediate lobe. These dopamine receptors in these tissues are postsynaptic to the dopamine-containing terminals. In addition, binding to the D-2 receptor in membranes from rat neurointermediate lobe (NIL) was studied by competitive binding assays using [125I]-N-(p-aminophenethyl)spiperone to label the D-2 receptors.

BHT-920 was found to be inactive at the D-1 receptor at concentrations up to 100uM. At the D-2 receptor, BHT-920 inhibited cyclase activity to the same extent as the full D-2 agonist (-)-apomorphine (APO) and was only slightly less potent (ED_{50}s:BHT-920 = 235 nm; apomorphine = 158 nM). The BHT-920 effect was not mediated via alpha-2 adrenoceptors because (1) it was reversed by the dopaminergic antagonist fluphenazine but not by the alpha antagonist phentolamine and (2) alpha-2 adrenergic agonists such as clonidine and BHT-933 had no effect on cyclase activity at concentrations up to 100uM. The potency hierarchy established in the cyclase assay was maintained in the binding assay in which APO>BHT-920>>BHT-933. We conclude that BHT-920 is a dopaminergic agonist with high selectivity for the D-2 receptor and question its description as a selective autoreceptor agonist.

43.9 REVERSAL OF ANTICHOLINESTERASE-INDUCED CONTRACTIONS IN ISOLATED TRACHEALIS. S.A. Reutter*+, M.G. Filbert*, D.H. Moore*, and M. Adler. Neurotoxicology Branch, Pathophysiology Division, USAMRICD, APG, MD, 21010-5425

Inhibitors of cholinesterase (ChE) produce profound, sustained contractions of isolated airway smooth muscle, in the absence of any external stimulus. The response is blocked by muscarinic antagonists and is thought to be mediated by accumulation of basally released acetylcholine (ACh) following blockade of ChE (Adler et al., in: Cellular and Molecular Basis of Cholinergic Function, 1987). The present study was designed to investigate the mechanism(s) by which antiChE-induced tracheal contractures can be prevented or alleviated and thus to identify compounds useful for prophylaxis and treatment of tracheobronchial constriction following ChE inhibition. Isometric contractions were recorded from isolated bovine trachealis (mucosa removed) in standard tissue chambers at 37° C. The efficacies of several types of drugs that have been used for clinical or experimental treatment of bronchoconstriction were tested on tissue strips contracted with the organophosphorous compound soman (100 nM) or the carbamate physostigmine (3 uM). Maximal concentrations of the beta-adrenergic agonist isoproterenol (Iso) completely relaxed soman-treated tissues in less than 10 min; the EC_{50} was 2 uM. Theophylline (Theo) was also effective in alleviating soman-induced contractions, but it was considerably less potent, with an EC_{50} of 1 mM. Combined treatment with Theo and Iso was additive. Dibutyryl cyclic-AMP (dBcAMP) (up to 1 mM for 4 h) produced no significant decrement in soman-induced tension. The Ca^{2+} blockers verapamil, nifedipine and diltiazem (1 to 50 uM for up to 1 h) did not produce significant or sustained relaxation in tissues exposed to either physostigmine or soman, but Ca^{2+} blockers did antagonize contractions induced by sub-maximal concentrations of acetylcholine (≤100 nM). Prostaglandin E_2 (10 uM), which has been reported to produce transient, partial relaxation of soman-induced contractions in canine tissue (Moore, D.H. and Hawkins, T., Proc. I.U.P.S., 16:213.24, 1986), had no effect on similarly contracted bovine tissue. Of the compounds tested only Iso and Theo were effective in alleviating contractions induced by ChE inhibitors. The action of Iso is mediated by activation of beta adrenergic receptors. Theo may potentiate such beta responses via inhibition of phosphodiesterase. However, the absence of similar relaxation by dBcAMP suggests that Theo may instead be acting on adenosine receptors. Contrary to their reported efficacy in asthma, Ca^{2+} blockers do not appear to be effective against antiChe-induced contractions.

+NRC Associate USAMRICD

43.10 Characterization and Localization of [^{3}H]-Clozapine Binding Sites in Rat Brain. E.J. McConnell*, F.M. Filloux*, T.M. Dawson, and J.K. Wamsley. [SPON: B.I. Grosser] Dept. of Psychiatry, Univ. of Utah School of Medicine, Salt Lake City, UT 84132.

Clozapine has been shown to be an effective antipsychotic in European clinical studies. Its exact mechanism of action is currently unknown, but a portion of [^{3}H]-clozapine binding has been shown to be displaceable by the muscarinic agent, atropine, and to a lesser extent by [+]-butaclamol, a dopaminergic antagonist. The authors have used the technique of in vitro receptor autoradiography and membrane homogenate studies to localize and further characterize [^{3}H]-clozapine binding sites in rat brain.

Tissue sections of forebrain corresponding to the striatum, for initial biochemical studies, and from various areas of forebrain for anatomical distribution studies. For routine autoradiographic studies the slide mounted tissue sections were incubated in a 50mM Tris-HCl buffer [pH 7.4] containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$ and 1.0nM [^{3}H]-clozapine. Specific binding was assessed by simultaneously incubating adjacent tissue sections in the same solution with the additional presence of 10^{-6}M atropine or 10^{-6}M [+]-butaclamol. Binding displaceable by atropine accounted for 39% of the total binding and that displaceable by butaclamol accounted for 13% of the total. In order to further characterize the nature of [^{3}H]-clozapine binding sites, displacement studies using muscarinic, dopaminergic, serotonergic, benzodiazapine and sigma opiate agents were conducted on whole brain homogenates. Synaptosomal membranes were obtained by centrifugation of homogenized forebrain at 40,000xg. The resulting pellet was resuspended in Tris-HCl buffer and .250ml aliquots of the membrane preparation were incubated in the presence of 1nM [^{3}H]-clozapine. Various displacers at 10^{-6}M concentrations were added followed by rapid filtration and rinsing with fresh buffer. [^{3}H]-clozapine was displaced only by atropine [39%], SCH23390 - D-1 antagonist - [14%], and MJ 14802-1 - a selective sigma opiate agent - [15%][$p<0.01$, $p<0.01$, $p<0.05$ respectively]. The other compounds, including sulpiride [D-2 antagonist], did not significantly displace [^{3}H]-clozapine binding under these conditions. Examination of the autoradiograms revealed the presence of heterogeneously distributed [^{3}H]-clozapine binding sites in several areas of the brain. [^{3}H]-clozapine binding sites were concentrated in basal ganglia structures (i.e. caudate putamen, nucleus accumbens, and olfactory tubercle), hippocampus and cortex.

These results suggest that[^{3}H]-clozapine binds to muscarinic receptors, D-1 receptors and sigma opiate receptors; but not to D-2 receptors in specific brain regions. It is possible to speculate that the antipsychotic action of clozapine is based primarily on its capacity to block sigma opiate receptors rather than D-2 receptors.

43.11 Δ^9-TETRAHYDROCANNABINOL INCREASES ARACHIDONIC ACID LEVELS IN GUINEA PIG CEREBRAL CORTEX SLICES. M. Reichman*, W. Nen*, and L.E. Hokin* (SPON: A.W. Clark) Dept. of Pharmacology, Univ. of Wisconsin Med. School, Madison, WI 53706

Although there is much information on the behavioral and cellular effects of the major active ingredient in marihuana, Δ^9-tetrahydrocannabinol (THC), its mechanism of action remains elusive. There is considerable evidence that THC increases the mobilization of arachidonic acid (AA) in non-neural cell cultures; however, the effects of THC on AA mobilization in CNS preparations with intact synapses have not been examined. To study the effect of THC on lipid metabolism in the mammalian brain, we prelabeled guinea pig cortex slices with [^{14}C]AA and measured changes in the disposition of radioactivity in membrane lipids upon incubation with the psychoactive (-) as well as the non-psychoactive (+) stereoisomers of THC.

Cerebral cortex slices (350 x 350 µm) were incubated with 1 µCi/ml of [^{14}C]AA for 1 hr at 37°C in Krebs-Henseleit bicarbonate saline (KHBS). The labeled slices were washed twice by incubation in 1% BSA in KHBS for 10 min to remove excess label. Prelabeled slices were incubated at 37°C in KHBS in the presence of vehicle or increasing concentrations of THC for 30-120 min. At the end of the incubation, the lipids in the tissue were extracted and the DPM values in the individual phospholipids and neutral lipids were measured following their separation by thin-layer chromatography.

The psychoactive stereoisomer of THC significantly increased [^{14}C]AA levels compared with the vehicle (0.25% dimethylsulfoxide) controls. At 30 min, 8.0 µM (-)THC significantly increased unesterified [^{14}C]AA levels by 42% ± 5%. The level of radioactivity in monoacylglycerol (MG) was elevated 25% ± 5% while the radioactivity in diacylglycerol (DG) and triacylglycerol (TG) decreased by 25% ± 5% and 18% ± 5%, respectively. There were no significant changes in the phospholipids at 30 min. At 1 hr, free [^{14}C]AA levels were elevated 2-fold. The levels of MG increased by 80% ± 5%, and DG and TG levels both decreased by 33% ± 4%. At 1 hr, we observed a loss of label from phosphatidylinositol and phosphatidylethanolamine as well. To determine if the THC-induced stimulation in unesterified [^{14}C]AA was stereospecific, slices were incubated with (+)THC or (-)THC for 1 hr. The effect of (-)THC was dose-dependent and saturable over a range of concentrations between 2 µM and 32 µM. The EC_{50} and maximal response were 4 µM and 12 µM, respectively. Twelve µM (-)THC produced a stimulation of 125% ± 19%. On the other hand, (+)THC was significantly less potent: 8.0 µM (+)THC increased free AA levels by 29% ± 6%. In marked contrast to the saturable effect of the active stereoisomer, the (+)THC-induced increases in free [^{14}C]AA were linear up to 160 µM, which was the highest concencentration tested. At this concentration, free AA levels were increased 3.2-fold.

The stereospecificity of THC in stimulating free AA levels in brain cortex slices and the finding that the effective concentrations were within the range found in plasma at the time of intoxication suggest that the mechanisms underlying cannabinoid action may involve the mobilization of AA from membrane lipid stores.

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43.12 EFFECT OF ACETYLCHOLINE ON Ca $^{2+}$- ACTIVATED K^+CHANNELS IN BOVINE CHROMAFFIN CELLS. M. I. Glavinovic, Dept. Anaesthesia Research and Physiology and Biomedical Engineering Unit, McGill University, Montreal, Quebec, Canada.

There is no doubt that synaptic vesicles prepared from cholenergic nerve terminals contain acetylcholine (ACh). Acetylcholine appears also to be stored free in the nerve terminal cytoplasm. During intense electrical nerve stimulation, the cytoplasmic ACh concentration can change substantially with a complex time course (Dunant et. al, Brain Res. 125, 123-140, 1977; Dunant et al. J. Physiol. 325, 441-460, 1982). The cytoplasmic ACh concentration is not known with precision but may be high (Katz & Miledi; Proc. Roy. Soc. Lond., 196, 59-72, 1977). Large conductance Ca^{2+} activated K^+ channels are now known to exist in a variety of membranes including those from secretory cells (chromaffin cells, Marty, Nature, 291, 497-500, 1981; motor nerve terminals, Mallart, J. Physiol., 368, 577-591, 1985). Blockade or activation of such channels by ACh may alter the resting membrane potential and the amplitude as well as the time course of the action potential and hence also the amount of transmitter released by nerve stimulation. This is an attempt to determine whether such an action of ACh on Ca^{2+} activated K^+ channels is present.

Experiments were done on excised inside-out patches from bovine chromaffin cell membranes. The microelectrodes were filled with Locke solution which consisted of (mM): 154, NaCl; 2.2, $CaCl_2$; 1.2, $MgCl_2$; 2.5, KCl; 2.15, K_2HPO4; 0.85, KH_2PO_4; and 10, dextrose. The internal solution contained (mM): 160 KCl/KOH; 10, Hepes; 1.0, $MgCl_2$; 5 EGTA; and 0.382 $CaCl_2$ at pH 72, and nominally had 0.3 µM free Ca^{2+}. When ACh was used in the internal solution, a sufficient amount of 1-M stock solution was added to the standard internal solution, to make the desired concentration. Solutions were changed at the intracellular membrane surface of the patch by placing the recording pipette and attached membrane in a microchamber. Experiments were performed at room temperature (20-23°C).

ACh concentrations ranging from 20 to 200 µM did not alter the probability of the open state (P) of these channels. Higher concetrations (2mM) led to a small (10-15%) decrease of P which was reversed with return to control solution. The amplitude of the single channel currents did not change at any ACh concentration used. There was no evidence of a flickery block either. It is concluded that even large changes in cytoplasmic ACh, that may occur with stimulation and ACh release do not change the current passing through large conductance Ca^{2+} - activated K^+ channels.

Supported by MRC and MDA (Canada).

43.13 UTILIZATION OF MUSCLE MEMBRANE INPUT RESISTANCE IN THE STRETCHER MUSCLE OF THE SHORE CRAB (PACHYGRAPSUS CRASSIPES) TO CHARACTERIZE IVERMECTIN AND RELATED COMPOUNDS. J.W. Bowman* and D.P. Thompson. The Upjohn Company, Kalamazoo, MI 49001.

The avermectins are a group of naturally occurring macrocyclic lactones, produced by Streptomyces avermitilis, that exhibit potent nematocidal activity. Ivermectin (IVM), 22,23-dihydroavermectin B_1, is the derived commercial product. Preliminary studies indicate IVM's mode of action as potentiating the release and binding of the inhibitory neurotransmitter GABA (gamma-aminobutyric acid) and the subsequent opening of GABA mediated Cl^- channels, leading to a reduction of muscle membrane resistance (MMR) (Wang, C.C. & Pong, S.S., Proq. Clin. & Biol. Res., 97, 373-395, 1982). More recent work indicates that IVM may have a broader mode of action, opening non-GABA mediated Cl^- channels as well (Scott, R.H. & Duce, I.R. Pest. Sci., 16, 599- 604, 1985).

Our lab has developed an efficient assay to monitor MMR in GABA innervated muscle fibers of the shore crab, Pachygrapsus crassipes. We have used this model to "fingerprint" IVM and related compound effects on MMR and specific ionic conductances. The experimental set up utilizes the exposed stretcher muscle from an isolated crab walking leg. Two microelectrodes placed 50µ apart are inserted into an individual muscle fiber. A 500 msec, 100 nA hyperpolarizing current pulse is applied through one electrode and MMR is monitored via the second electrode.

Results show that IVM reduces MMR in a dose dependent manner. At 1 µM, IVM induces a 70% drop in MMR from baseline levels within one min and an 85% reduction by 15 min. Replacement of IVM with Cl^- channel blockers (picrotoxinin or lindane) results in partial recovery of the MMR to 40% of baseline. Other specific channel blockers including tetraethylammonium, 4-aminopyridine, tetrodotoxin, etc. and various ion substituted ringer solutions have been utilized to determine the ionic dependence of IVM action on shore crab muscle. Results of these studies point to the central nature of the Cl^- channel in the action of IVM on this preparation. Additionally, utilizing GABA to desensitize the GABA receptor, our results suggest that IVM may influence non-GABA mediated Cl^- channels as well as those associated with GABA.

Data generated testing IVM analogs reveal that small structural changes of the molecule result in significant loss of biological activity. The nature of these quantitative structure- activity relationships will be discussed.

44.1 DEGRADATION OF "NEW" RECEPTORS INSERTED INTO DENERVATED VERTEBRATE NEUROMUSCULAR JUNCTIONS AND THEIR RESPONSE TO REINNERVATION. S.-L. Shyng* and M.M. Salpeter. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

A progressive change in degradation rate of acetylcholine receptors at vertebrate endplates seen after denervation could reflect the combined rates of "original" junctional receptors present at denervation, and "new" receptors inserted into the denervated junction (Levitt & Salpeter, Nature 291:239, 1981). Studies on degradation of original receptors using a gamma-counting technique have shown that for about 10 days after denervation the original receptors retain an 8-day degradation half-life (t1/2) equivalent to that of innervated junctional receptors. Thereafter degradation accelerates to a t1/2 of 2.5 days. Reinnervation reverses the t1/2 of these prelabeled receptors back to 8 days (Salpeter et al., J. Cell Biol. 103:1399, 1986).

Here we determined the degradation rate of new receptors. The gamma counting technique, which assumes uniform distribution of extrajunctional label, could not be used since postdenervation extrajunctional receptors develop preferentially near the endplate. EM autoradiography was used to study only the receptors localized on the postjunctional folds. First we inactivated all original receptors in the sternomastoid muscle of anaesthetized mice by topical application of non-radioactive α-bungarotoxin (BGT). The nerve was cut and ligated to prevent reinnervation. New receptors were similarly labeled with I-125-BGT 6 and 14 days later, and their degradation half-life determined by EM autoradiography. At both times after denervation, these new receptors had a t1/2 of 1 day, when the "original" receptors were degrading with a t1/2 of 8 and 2.5 days respectively. Thus "new" receptors inserted into denervated junctions do indeed degrade as do embryonic or postdenervation extrajunctional receptors. EM autoradiography also shows that new and original receptors are interspersed on the postjunctional membrane.

To determine the effect of reinnervation, we again inactivated all original receptors with cold toxin, and denervated the muscle by nerve crush. To prevent reinnervation until a sufficiently large number of "new" receptors was accumulated, the nerve was re-crushed every second day for 10 days. Two days later, the new receptors were labeled, and their degradation followed by gamma counting. (Gamma counting used here could cause an underestimate of the t1/2 but could not cause degradation to slow down). The new receptors initially degraded with a t1/2 of 1 day (as seen above) and by 6 days after last crush, the t1/2 slowed to 5 days. Fine structure showed reinnervation occurred between 3 and 6 days after last crush. We conclude that, as with original receptors, reinnervation can slow the degradation of new receptors already inserted into the postjunctional membrane. (NIH grant NS 09315)

44.2 IN VITRO BLOCKADE OF NEUROMUSCULAR TRANSMISSION BY MONOCLONAL ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES. R. Maselli,* B. Jow,* D. P. Richman,* D. J. Nelson.* (SPON: B.G.W. Arnason), Dept. of Neurology, The Univ. of Chicago, Chicago, IL 60637.

We studied the miniature endplate potentials (MEPPs) of the chicken posterior latissimus dorsi (PLD) muscle exposed in vitro to either anti-AChR mAb 370A, which blocks the binding of alpha bungarotoxin (BT) and induces hyperacute experimental allergic myasthenia gravis (EAMG) in chickens or anti-AChR mAb 132A, which has no effect on the binding of BT and does not induce the hyperacute disease. Only PLD muscles exposed to mAb 370A (n=6) showed a decrease in MEPP amplitudes: before mAb exposure, 0.729± 0.02 mV (mean ± SEM of 57 endplates); after exposure, 0.475 ± 0.02 mV (52 endplates) ($p < 0.005$). No reduction of MEPP amplitude was found after exposure of the preparations to mAb 132A (n=4). In addition, we examined the effects of both mAbs on the amplitude and decay phase of endplate currents (EPCs) in fibers which had been previously treated with 2M formamide to block excitation contraction coupling. Results of studies using mAb 370 (n=4) showed a decrease in current amplitude with no change in either reversal potential or the rate of EPC decay. No significant change in current amplitude or rate of decay was observed in the presence of the mAb 132A (n=3). Using a patch pipette internal perfusion technique, we were able to observe channel activation in single cell-attached rat myotube patches in the presence and absence of mAb. Results with mAb 421H, which induces EAMG in chickens and rats, showed a partial block of channel activation and a decrease in mean channel open time when the membrane patch was exposed to mAb (0.05 mg/ml) simultaneously with the cholinergic agonist suberyldicholine (400 nM). Thus, anti-AChR mAbs can produce in vitro reduction of MEPP and EPC amplitudes as well as single channel activity which appears to depend on pharmacologic blockade of the cholinergic binding site. Antibodies of this type may play a role in the disordered neuromuscular transmission in myasthenia gravis.

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44.3 LIPID SOLUBILITY AND NOT STEREOSELECTIVITY APPEARS TO DETERMINE BLOCKING KINETICS OF SINGLE ACETYLCHOLINE RECEPTOR CHANNEL CURRENTS. L. A. Merriam and J. E. Fiekers. Dept. Anatomy and Neurobiology, UVM College of Medicine, Burlington, VT. 05405.

Lincosamide antibiotics have been shown to have a direct action on the postsynaptic acetylcholine (ACh) receptor-channel complex during neuromuscular transmission, resulting in voltage- and concentration-dependent alterations of miniature end-plate current (mEPC) and end-plate current (EPC) decay. The two clinically used lincosamides, lincomycin and clindamycin exhibit marked differences in end-plate current decay characteristics: lincomycin produces a current decay which is described by two exponential components, while that for clindamycin can be described by a single exponential. Lincomycin and clindamycin differ in chemical structure at the C-7 position. Lincomycin has an R-hydroxy group at the 7 position, while clindamycin has an S-chloride group which imparts a greater lipid solubility. Voltage-clamp studies of mEPC and EPC decays show that while there was little difference between the channel blocking rates of the lincomycins and clindamycins, the unblocking rates for clindamycin and epiclindamycin were slower than the less lipid soluble lincomycin antibiotics.

In order to examine directly the influence of lipid solubility and stereoselectivity on blocking kinetics of the ACh receptor-channel complex, we have measured ACh-induced single channel currents in the presence of lincomycin and clindamycin and their stereoisomers epilincomyin and epiclindamycin in rat myotubes. Bursts of ACh-induced currents recorded in the presence of lincomycin were interrupted by brief closed gaps due to blocking of the open channel by lincomycin. In contrast, the single channel currents recorded in the presence of clindamycin were shorter in duration than the control currents. This is consistent with the slower unblocking rate for clindamycin seen in end-plate current decay studies. These effects of the lincosamide antibiotics were both concentration and voltage-dependent. High concentrations of clindamycin reversed the voltage-dependence of the mean open time such that at hyperpolarized membrane potentials the mean duration of open channels was less than at depolarized potentials. These data obtained from single channel analysis confirm the results seen in voltage clamped end-plates. The compounds with comparable octanol/H2O ratios exhibited greater similarity in blocking and unblocking characteristics than did the compounds with similar stereospecificity, suggesting that lipid solubility has a greater role than stereoselectivity in determining the kinetics of single channel blockade.

44.4 THE CHANNEL CURRENTS ACTIVATED BY ADENOSINE 5'-TRIPHOSPHATE IN CULTURED XENOPUS SKELETAL MUSCLE CELLS. Yukio Igusa.* (SPON:M.Kimura) Dept. of Physiol., Jichi Med. Sch. Tochigi-pref., Japan,329-04.

At the muscarinic neuromusclar junction, high amounts of adenosine 5'-triphosphate (ATP) are contained in cholinergic synaptic vesicles (Whittaker et.al.,1972;Dowdall et.al.,1974) and ATP and ADP are caused extracellulary by repetitive stimulation of the phrenic nerve of rat(Silinsky,1975),suggesting that ATP with ACh are released from motor nerve ending. Recently it is found that there are cation channels activated by ATP in cultured chicken skeletal muscle cells (Kolb & Wakelam,1983). The purpose of the present study is to investigate whether ATP act to the ACh recepter channels in the nicotinic neuromusclar junction. Single channel currents activated by ATP (10^{-7}-10^{-3} M) and by ACh (0.2μM, 10^{-10} M) were recorded in cultured muscle cells using the cell-attached patch clamp technique at room temperature. Dissociated myotomal muscle cultures were prepared from 19-22 stage embryos of Xenopus laevis. Two types of channel currents activated by 10μM ATP, high conductance (High-γ; 60pS) and low conductance (Low-γ; 42pS), were observed during 1-6 days cultures after dissection. Reversal potentials corrected by -85mV of the resting potential were around -10mV in both currents. Both types of ATP-activated currents were reduced to appear by treatment of 20-50μM d-tubocurarine (Curare). Since these characteristics of ATP-currents were essentialy the same as those of the ACh receptor channel currents in cultured Xenopus muscle cells, it is strongly suggested that ATP activate the nicotinic ACh receptor channel. At 10^{-7}-10^{-5} M ATP, occurrence frequency of both currents increased linearly proportional to the logarithm of the concentration of ATP. Mean open times of both currents were 0.93±0.29ms and 0.86±0.34ms in High-γ and Low-γ currents, respectively. Both values,especially of Low-γ currents, were significantly shorter than those in ACh receptor channels. When the patch was exposed in 10^{-7}-10^{-5}M ATP combined with 10^{-10} M ACh, occurrence frequencies of both currents were higher than those at ATP alone, indicated that ATP and ACh mutually facilitate the opening of the ACh receptor channel. At 10^{-5}M ATP with 10^{-10}M ACh,the patches showed distinct two classses of event occurrence which the frequencies were high (about 23 events/sec) or low (about 1.5 events/sec). Frequencies of channel currents decreased to 1-1.5 events/sec at 10^{-5}-10^{-3} M ATP with or without 10^{-10}M ACh. Two types of channel currents having the same conductances as the ACh recepter channels were also recorded by 0.5mM AMP-PNP. Neither AMP(1-100μM) nor ADP(1-100μM) were effective on the examined muscle cells.

44.5 INTERACTION OF GABA AND GLUTAMATE IN THE GATING OF CHLORIDE CONDUCTANCE IN THE ROSTRAL WHITE CELLS OF APLYSIA. W.M. King and D.O. Carpenter. Wadsworth Laboratories, NYS Department of Health and School of Public Health, University at Albany, Albany, NY 12201.

Rapid microperfusion and voltage-clamp techniques have been used to investigate the effects of gamma-aminobutyric acid (GABA) and l-glutamate (GLU) on the neurosecretory rostral white cells (RWCs) of the parieto-visceral ganglion of Aplysia californica. Ganglia were desheathed surgically after incubation for 30 min in artificial sea water to which sucrose had been added to yield a hypertonic solution with a total osmolarity of approximately 1.7. The neuronal shrinkage so induced greatly reduced damage to the RWCs during desheathing.

In previous investigations we have shown that Cl^- conductances elicited by GABA and GLU in the medial pleural neurons of Aplysia differ in dose-response relations, kinetics of activation and desensitization, and voltage-dependence. Despite these differences, however, prior exposure to GABA could completely block the response to GLU while previous exposure to GLU reduced the response to GABA. In the RWCs we have found the 2 mM GLU elicits a response similar to that in the medial pleural cells, i.e., an initial rapidly activating and decaying inward current that is sensitive to $[Na^+]_o$ followed by a more slowly activating and decaying current that varies with $[Cl^-]_o$ and intracellular Cl^- injection. In both groups of cells the initial inward current varied greatly in amplitude relative to the slower Cl^- current. In contrast, GABA, which elicits Cl^- current in the medial pleural cells with a threshold concentration of 1-3 micromolar, was without effect at a 100 micromolar concentration in the RWCs. At this concentration GABA could completely block the Cl^- current response to GLU in the medial pleural cells but had no effect on the response to GLU in the RWCs. Dose-response experiments, however, revealed that higher concentrations of GABA elicited large desensitizing Cl^- conductance in the RWCs with a threshold concentration of 1-3 mM. In addition, exposure to 10 mM GABA completely blocked the response of the RWCs to 2-10 mM GLU, while exposure to 10 mM GLU reduced their response to GABA to approx. 50% of control. Thus, the responses of the RWCs to GABA and GLU as well as their interactions were similar to those in the medial pleural cells except that the dose-response curve for GABA was shifted to the right. For each group of neurons both activation and cross-inhibition occurred within the same dose range while that dose range differed between the groups for GABA but not GLU.

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44.6 PROPERTIES OF $GABA_A$-ACTIVATED CONDUCTANCE IN RAT LOCUS COERULEUS NEURONS IN VITRO. S. S. Osmanovic and S. A. Shefner. Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

Effects of gamma-aminobutyric acid (GABA) on rat locus coeruleus (LC) neurons were studied in a totally submerged brain slice preparation under current- and voltage-clamp conditions. GABA was applied in known concentrations (10 μM-2 mM) in the bath or by pressure ejection from a micropipette located near the recording site. GABA increased conductance, inhibited spontaneous firing and hyperpolarized or depolarized the membrane depending on the internal Cl^- concentration. The mean reversal potential for the GABA-induced changes in membrane potential (E_{GABA}) was -72 mV in cells impaled with K-acetate microelectrodes (n=20) and -48 mV in cells impaled with KCl electrodes (n=15). E_{GABA} was shifted in a depolarizing direction when the external Cl^- concentration was reduced as predicted by the Nernst equation (about 55 mV per ten-fold change in external Cl^-). The GABA concentration-conductance curve was sigmoidal with an apparent EC_{50} of 330 μM (n=25 cells). Bicuculline (10-40 μM) and bicuculline methiodide (40-100 μM) antagonized GABA-induced effects and shifted concentration-conductance curves to the right (n=9).

GABA currents elicited by short (5-100 ms) pressure pulses from pipettes containing GABA (1-10 mM) decayed with an exponential time course which could be fitted by a single exponential in most LC neurons. GABA-induced currents were voltage-dependent and showed outward rectification as indicated by a decrease in the slope of the I_{GABA}/V relation with membrane hyperpolarization. The magnitude of the GABA-induced conductance change was decreased by membrane hyperpolarization and increased by depolarization. The conductance change measured at the peak of the GABA-induced current was linearly related to the membrane potential, decreasing two-fold per 40 mV hyperpolarization. Furthermore, the time constant of decay of the GABA-induced current was increased by depolarization and decreased by hyperpolarization. The voltage dependency of GABA-induced currents was observed both in cells impaled with KCl and K-acetate microelectrodes, which indicates that this phenomenon can not be due to changes in intracellular Cl^- concentration due to leakage from the recording electrode.

We conclude that GABA increases Cl^- conductance in LC neurons through activation of bicuculline-sensitive ($GABA_A$) receptors. The voltage dependency of this GABA-activated Cl^- conductance suggests that changes in membrane potential can significantly alter the extent of GABA-mediated inhibition of LC neurons.

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44.7 SPATIAL DISTRIBUTION OF GABA RECEPTORS AT THE TIME OF THEIR APPEARANCE ON SPINAL NEURONS DIFFERENTIATING IN CULTURE. Greg L. Harris and Nicholas C. Spitzer. Department of Biology, University of California, San Diego, La Jolla, CA 92093.

Spinal neurons of *Xenopus* embryos differentiate in dissociated cell culture and are useful for studies of the development of neurotransmitter sensitivity. Cultures contain sensory, motor and interneurons (Spitzer & Lamborghini, 1976; Bixby & Spitzer, 1984; Lamborghini & Iles, 1985) and cells are initially insensitive to γ-aminobutyric acid (GABA), glutamate (Glu) and glycine. During the first day in culture they become sensitive to one or more of these neurotransmitters (Bixby & Spitzer, 1984). The present study examines the spatial localization of the initial expression of GABA sensitivity, with respect to the cell soma, outgrowing neurites and growth cones.

Dissociated cell cultures were prepared from the neural plates of embryos at stage 15. Neurons begin to extend processes after 6 hrs (Spitzer & Lamborghini, 1976), corresponding to the time of initial neurite extension *in vivo* and were studied at times ranging from 6 to 32 hrs after plating. An intracellular electrode in the soma was used to record changes in membrane potential and input resistance following the application of GABA or Glu (50-100 μM) to the soma, neurites or growth cones. Focal application was achieved by pressure ejection of neurotransmitter from fine tipped micropipets (<1 μm tip diameter). The lengths of neurites examined ranged from 15-185 μm. Application of KCl to neurites revealed that depolarizations could spread passively and were easily detectable at sites 185 μm from the soma.

At early stages of development, only 25% of neurons responded to GABA (6-9 hrs; n=12); these cells were sensitive on their cell bodies but not along their neurites or at their growth cones. Between 9 and 12 hrs, 60% of neurons responded to GABA (n=27); 40% of these were sensitive only on the soma, and 60% were sensitive both on the soma and along their processes. Sensitivity of the growth cone was always accompanied by sensitivity of the full extent of the neurite, at this and subsequent stages of development. By one day in culture, 80% of neurons responded to GABA (20-26 hrs; n=10) and 90% of these were sensitive over their entire surface. At later times, all neurons responded to GABA (26-32 hrs; n=13) and 90% of these were sensitive over their entire surface.

These cultures contain neurons that respond to GABA with an exotic cation-selective depolarization (putative sensory neurons), as well as those which respond to Glu and exhibit the more common chloride-selective hyperpolarizing response (probable motor neurons; Bixby & Spitzer, 1984). Responses obtained from different points along a neurite were the same, with respect to the neurotransmitter to which the membrane was sensitive and the polarity of the response to GABA. Furthermore, both neurites of bipolar cells exhibited the same sensitivities.

These results are consistent with a temporal and spatial gradient for the acquisition of GABA sensitivity, which begins in the cell soma and spreads rapidly along the neurites and to the growth cones. We are presently characterizing more fully the acquisition of GABA sensitivity at the level of single receptor channels.

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44.8 NERVE GROWTH FACTOR POTENTIATES BRADYKININ INDUCED RELEASE OF CALCIUM FROM INTRACELLULAR STORES IN PC12 CELLS. A. Bush, L. Borden*, A. Rukenstein*, L.A. Greene, F.R. Maxfield*. Dept. of Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

Although calcium is known to be a critical determinant of neuronal function, little is known about the release of Ca^{2+} from intracellular stores in neurons (For review see McBurney, R. Neering, I. TINS, 1987, 10, 164). We have been studying this process in the PC12 neuronal cell line. When PC12 cells are exposed to bradykinin (bk), a nine residue neuropeptide, phospholipid turnover is activated. Inositol trisphosphate (IP_3) is released into the cytosol where it subsequently stimulates Ca^{2+} release.

Untreated PC12 cells were plated on polylysine-collagen coated glass coverslips attached to 35mm culture dishes and loaded with Fura-2AM. All Ca^{2+} measurements were conducted at room temperature (23-27°) since we have found that the cells remove the dye rapidly at higher temperatures. Groups of 10-20 PC12 cells were examined using a Leitz Diavert microscope spectrofluorometry system. Bath application of bk produces a dose dependent increase in cytosolic $[Ca]_i$; the EC-50 is approximately 50 nM. The average peak increase in Ca^{2+} (peak minus resting) is approximately 400 nM. Resting $[Ca]_i$ is 37 nM ± 2 nM; n=75 (mean ± S.E.M.). The peak response is generally attained within the first 20 seconds after bk addition followed by a steady return towards baseline. Two minutes after application of 10 nM bk, most cells have reestablished a $[Ca]_i$ level within 20% of prestimulation baseline. The release of Ca^{2+} does not require extracellular Ca^{2+}.

NGF treatment enhances the potency of bk. A large enhancement was seen when cells were cultured in the presence of NGF for 1-3 weeks. The EC-50 for bk was reduced to 5nM while the maximal increase in Ca^{2+} elicited by a saturating dose was unchanged. In addition, one hour preincubation with NGF augmented the response. After one hour preincubation with NGF at 37°, the average maximal increase in response to 10nM bk was 250nM±25; control (bk alone, no NGF) was 107nM±25. After one hour preincubation at 24°, the increase in response to 1nM bk was 163nM±31; control was 44nM±15. Under our conditions, NGF by itself did not affect $[Ca]_i$.

Preliminary experiments suggest that NGF sensitizes Ca^{2+} release neither by up-regulating bk receptors nor by generating greater amounts of IP_3. Preincubation of cells with NGF for one hour at 37° does not significantly change [^{3}H]bk binding (1 and 10 nM). Although NGF alone appears to slightly stimulate phospholipid turnover, there is no potentiation of the IP_3 generated by bk.

44.9 SENSITIVITY OF CULTURED SPINAL NEURONS TO PRIMARY SENSORY TRANSMITTERS, MONITORED BY INTRACELLULAR CALCIUM. M.D. Womack*[1], A.B. MacDermott, and T.M. Jessell (SPON: A. Calof). Center for Neurobiology and Behavior and Howard Hughes Medical Institute, Columbia University, New York, NY 10032; [1]Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Dorsal root ganglion (DRG) neurons that terminate in the dorsal horn release transmitters that elicit fast and slow epsp's in spinal cord neurons. The transmitter mediating fast epsp's is probably l-glutamate while several neuropeptides may mediate slow epsp's. Transmitters co-released from sensory neurons may act on the same subset of post-synaptic spinal cord neurons. However the consequences of activation of excitatory amino acid and peptide receptors on neurons in the spinal cord have not been well defined.

Peptide agonists have been shown to cause release of calcium from intracellular stores in many non-neuronal cell types. We have, therefore, studied the effect of sensory neuropeptides on intracellular calcium concentration, $[Ca^{2+}]_i$, in spinal cord neurons using the fluorescent indicator Fura-2 and have compared the changes in $[Ca^{2+}]_i$ in response to peptides and to l-glutamate. Neurons were isolated from either the whole spinal cord or the dorsal horn of embryonic or newborn rats by enzymatic dissociation and plated onto a monolayer of cortical astrocytes in supplemented F12 medium. After 3-14 days in vitro, neurons were incubated with Fura-2 AM (2 μM) for 10 min. at 37°C. The fluorescence of single neuronal somata was measured on an inverted microscope fitted with a photomultiplier. The excitation wavelength was alternated between 350 and 380 nm by means of a motorized filter changer operating at 0.5 Hz and the 350/380 ratio was used to determine $[Ca^{2+}]_i$.

In individual cultures, between 10 and 70% of Fura-2-loaded spinal cord neurons exhibited spontaneous oscillations of $[Ca^{2+}]_i$ which were inhibited by 1 μM TTX. Application of l-glutamate (50-100 μM) by pressure ejection (0.5-1.5 sec., 1.5 psi) resulted in a rapid increase of $[Ca^{2+}]_i$ in about 80% of neurons tested. The $[Ca^{2+}]_i$ rose to a concentration of about 1 μM then recovered to resting levels in 10-15 seconds. A lower proportion (15-20%) of spinal cord neurons responded to substance P (SP) (100 nM) (3-5 sec, 1.5 psi). Responses to SP varied with increases in $[Ca2+]_i$ ranging from less than 500 nM to greater than 1 μM with the duration of enhanced $[Ca^{2+}]_i$ ranging from 15 to 40 seconds. Other peptides released from DRG neurons also enhanced $[Ca^{2+}]_i$ in spinal neurons. Neurokinin A (100 nM) evoked increases in $[Ca^{2+}]_i$ in about 20% of neurons examined; over 80% of these neurons also responded to SP. GRP and CCK (100 nM) and enhanced $[Ca^{2+}]_i$ in a smaller percentage of spinal neurons.

These observations indicate that i) spontaneously released transmitters and exogenous application of l-glutamate and peptides increase $[Ca^{2+}]_i$ in cultured spinal neurons, ii) a higher percentage of neurons respond to l-glutamate than to peptides, and iii) the duration and magnitude of changes in $[Ca^{2+}]_i$ elicited by l-glutamate and peptides vary considerably.

44.10 A ROLE FOR PHOSPHATIDYLINOSITOL (PI) TURNOVER IN THE MUSCARINIC BLOCKADE OF THE M-CURRENT IN HIPPOCAMPAL PYRAMIDAL CELLS. P. Dutar and R.A. Nicoll, Depts. of Pharmacol. and Physiol., Univ. of Calif., San Francisco, CA 94143.

In hippocampal pyramidal cells, the cholinergic agonist carbachol (carb), acting on muscarinic receptors, has 4 distinct actions. At low concentrations (1 μM) it depresses EPSPs by a presynaptic action, depolarizes the membrane potential, and blocks the afterhyperpolarization (AHP) which follows a series of action potentials; at 10-fold higher concentrations it blocks the K^+ current termed I_M. However, little is known concerning the coupling of the muscarinic receptors to these 4 responses. The goal of the present experiments was to differentiate these responses pharmacologically and to determine the nature of the coupling.

Conventional intracellular current-clamp or single electrode voltage-clamp recordings were obtained from CA1 pyramidal cells in rat hippocampal slices. Cholinergic drugs were added to the superfusing medium. For intracellular administration the recording electrode was filled with 3M KCl plus inositol trisphosphate (IP_3) (60 mM), EGTA (200 mM), or BAPTA (200 mM).

Cholinergic agonists have been classified according to their ability to stimulate PI turnover in the brain (Fisher et al., J. Biol. Chem., 258:7358, '83). We therefore tested the ability of these agonists to elicit the muscarinic responses. Carb (n=23) and oxotremorine-M (oxo-M) (n=13), which are full agonists for stimulating PI turnover, completely blocked I_M at 20 μM and also completely blocked the AHP, depressed EPSPs, and depolarized the membrane potential at 1 μM. On the other hand, although arecoline (n=5), pilocarpine (n=9) and oxotremorine (oxo) (n=8), which are weak agonists for stimulating PI turnover, had effects comparable to carb on the membrane potential, AHP, and EPSP, they had little effect on I_M. In fact, in agreement with the biochemical studies, oxo antagonized the action of carb and oxo-M on I_M.

The activation of PI turnover leads to the formation of diacylglycerol (DG), which activates C-kinase, and IP_3 which can release Ca^{++} from intracellular pools. In accord with previous experiments (Malenka et al., J. Neurosci., 6:475, '86), activation of C-kinase with phorbol esters failed to block I_M. On the other hand intracellularly applied IP_3 caused a dramatic and reproducible depression of I_M (n=22), but failed to affect I_Q, an anomalous rectifier current. Finally muscarinic blockade of I_M was unaffected by chelating intracellular Ca^{++} with EGTA or BAPTA, indicating that a rise in intracellular Ca^{++} is not required for this response.

In conclusion, these results strongly suggest that stimulation of PI turnover is involved in the muscarinic suppression of I_M, but may not be required for the other actions of carb seen at lower agonist concentrations. Furthermore, we suggest that IP_3 or one of its metabolites mediates the muscarinic blockade of I_M.

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44.11 CONTROL OF M-CURRENT IN DIALYSED SYMPATHETIC GANGLION NEURONS. P.J. Pfaffinger. Physiol & Biophys, U Wash, Seattle, WA (Spon: B Hille).

Muscarinic and t-LHRH control of M-current were studied in isolated frog sympathetic ganglion neurons using a whole-cell voltage clamp. M-current was recorded for long periods of time in cells dialysed with solutions containing Mg-ATP. Using no ATP or using ATP derivatives APP(NH)P or ATPγS caused spontaneous loss of M-current, suggesting that a functional M-current channel is a phosphoprotein. M-current was shut off reversibly with muscarine or t-LHRH added to the bath; the protease inhibitor leupeptin added to the intracellular solution enhanced the sensitivity and stability of this response. The role of a GTP-binding protein was tested. GTP was not a required ingredient in the internal solution; nevertheless, addition of the known G-protein activators GTPγS (at 50 μM) or fluoride (10 mM NaF or KF) spontaneously and irreversibly shut off the M-current. At lower GTPγS concentrations with added GTP (1 μM:100 μM; 5 μM:100 μM), muscarine or t-LHRH were required to produce an irreversible shutoff of the M-current. These experiments strongly suggest the involvement of a G-protein. The irreversible action of GTPγS was not prevented by 5 mM BAPTA, thus a long term Ca rise is not obligatory for G-protein action. This G-protein is not of the G_o or G_i type: First, activation by GPP(NH)P is poor. Dialysis with 100 μM GPP(NH)P did not shut off the M-current spontaneously. Addition of muscarine or t-LHRH did shut off M-current more than usual, but on washout the response reversed about 75%. Finally, overnight applications of Pertussis toxin (IAP at 0.1 μg/ml) had no effect on muscarinic or t-LHRH shutoff of M-current. In addition, no labeled substrate could be seen after ^{32}P-NAD/IAP labeling of solubilized membranes, suggesting that little to no IAP-sensitive G-proteins exist in these cells. Other experiments using the putative G-protein antagonist GDPβS produced paradoxical results. GDPβS can both block the response and give irreversible responses. This might be explained by variable enzymatic phosphorylation of the GDPβS inside the cell creating an irreversible GTP analogue. Unlike microelectrode studies, with long applications of muscarine or t-LHRH the shutoff of M-current was not sustained (7% of the M-current returned per min). This rate seems too slow to be explained by Ca buffering (1 mM EGTA or 5 mM BAPTA). Such desensitization occurred whether the cell had been dialysed only 5 min or 25 min; perhaps the agonist liberates some component that dialyses away. Finally, we tested the possible role of Protein Kinase C. The phorbol ester PMA (1 μM) produced an irreversible 50% loss of M-current when applied to the bath. PMA was slightly more effective with 200 nM Ca_i solutions. Subsequent addition of 1 μM t-LHRH on top of the PMA completely shut off the M-current. When t-LHRH was washed out, the M-current returned to the 50% level. These results suggest that the G-protein activated pathway cannot be entirely mimicked by PMA. Supported by NIH #NS08174 and the ARCS Foundation.

44.12 INOSITOL TRISPHOSPHATE ACTIVATES K^+ CHANNELS THROUGH ELEVATION OF INTRACELLULAR CALCIUM IN PEPTIDERGIC NEURONS OF APLYSIA. L. Fink*, J.A. Connor and L.K. Kaczmarek. Depts. Pharmacology and Physiology, Yale Univ. Sch. Med., New Haven, CT 06510 and Bell Labs, Murray Hill, NJ 07974.

Stimulation of a 30 minute discharge in the bag cell neurons of Aplysia is accompanied by increased production of water soluble inositol phosphates. We investigated the effect of inositol trisphosphate (IP3) on the intracellular calcium concentration of these neurons in Ca^{2+}-containing and Ca^{2+}-free media, and have characterized the effects of IP3 on their excitability.

Isolated bag cell neurons growing on glass coverslips were loaded with the calcium indicator fura-2 by pressure injection. Fluoresence (475-525 nm) of single neurons was measured with a CCD-based imaging system. Localized calcium concentrations were estimated from the ratio of fluorescence images taken at 340 and 380 nm excitation. Pressure injection of IP3 (0.8mM in 0.6M KCl) through a microelectrode for 1-15 seconds produced a significant increase in the calcium signal which recovered 1-2 minutes after injection. IP3-induced elevation of cytosolic calcium was also observed when cells were bathed in Ca^{2+}-free medium, indicating that the rise in calcium occurred through release from intracellular stores rather than entry across the plasma membrane. Injection of 0.6M KCl had no effect. The localization of calcium released by IP3 differed from that evoked by trains of action potentials. The rise in calcium produced by IP3 injection was localized to the soma, whereas action potentials produced dramatically increased calcium levels primarily in the neurites.

We also examined the electrophysiological consequences of IP3 injection. We reported previously that brief injection of IP3 hyperpolarizes the bag cell neurons, and that this response is accompanied by a conductance increase (Fink et al., Soc. Neurosci. Abstr. 11:854, 1985). This effect is mimicked by direct injection of calcium ions and is blocked by TEA. To investigate the basis of this IP3-induced hyperpolarization at the single channel level, bag cell neurons in culture were impaled with an IP3 containing microelectrode and also sealed to a patch pipette for cell-attached patch recording. IP3 injection produced a dramatic increase in the probability of opening of a channel passing outward current at rest and at more positive potentials. This channel had a slope conductance of ~50 ps and an extrapolated reversal potential close to E_K. Channel activity increased 2-5 seconds after a 1 second injection and returned to baseline within approximately 1 minute. Injection of calcium elicited similar increases in channel activity. In cell-free patches, exposing the cytoplasmic side of the membrane to IP3 did not affect the opening of this channel, but channel openings did increase with application of calcium. Our data is consistent with the hypothesis that IP3 activates a Ca^{2+}-dependent K^+ channel through an elevation of intracellular calcium.

Preliminary work shows that at least one additional channel is also modulated by IP3. In 4 experiments, IP3 injection led to the increased opening of a channel passing inward current. It is not yet known if this channel is modulated directly by IP3 or by elevated intracellular calcium.

44.13 **TRANSLOCATION AND ACTIVATION OF PROTEIN KINASE C IN STRIATAL NEURONS IN PRIMARY CULTURE.** S. Weiss, J. Ellis, D. Hendley*, and R.H. Lenox. Neuroscience Research Unit, Department of Psychiatry, University of Vermont, Burlington, VT 05405.

Reports from many laboratories have indicated that diacylycerol produced during phosphoinositide (PI) metabolism activates protein kinase C (PKC). Tumor-promoting phorbol esters are also able to bind to and activate PKC. The increase in PKC activity may provide negative feedback to receptors coupled to PI metabolism. We have been studying these processes in striatal neurons in primary culture. The preparation is maintained as a monolayer that is >93% neuronal; thereby, diffusional barriers and complexities due to contributions from non-neuronal cells are minimized. In these cells, both carbachol (EC_{50}, 80 μM) and epinephrine (EC_{50}, 200 nM) cause a marked increase (5- to 10-fold) in PI hydrolysis. However, treatment of the cells with the active phorbol ester PDBu reduces the efficacy of both agonists; in a 20 min incubation, half-maximal reduction is achieved at 100 nM PDBu. These observations have prompted us to evaluate the actions of several agents on translocation and activation of PKC in these neurons.

For studies of PKC activity, striatal neurons (maintained for 7-10 days in vitro) are incubated with appropriate agents for 20 min, rinsed, harvested, homogenized, and centrifuged to yield membrane and cytosolic fractions. The activity of PKC in the two fractions is measured as the ability to transfer the terminal phosphate of γ-[^{32}P]ATP to histone, by a filter-binding assay. A centrifugation method is used to measure the binding of [^{3}H]PDBu to the membrane fraction. We have found 12 μM 1-stearoyl-2-arachidonoyl-glycerol to be more effective than 100 nM PDBu in activating PKC in the cytosolic fraction, although these respective concentrations of the two substances are equally effective at stimulating membrane-bound PKC. Pretreatment of cells with 1 μM PDBu for 20 min results in a shift of PKC activity from the cytosol to the membrane, such that the activity in the membranes is increased by 240%; the binding of [^{3}H]PDBu to the same membranes is also increased (by 250%). Pretreatments with 1 mM carbachol or 100 μM 1-oleoyl-2-acetyl-glycerol lead to smaller increases in the binding of [^{3}H]PDBu to the membrane fraction (32% and 37%, respectively).

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44.14 **CHRONIC LITHIUM ALTERS THE PKC-MEDIATED PHOSPHORYLATION OF AN 83kDa PHOSPHOPROTEIN IN THE RAT HIPPOCAMPUS.** R.H. Lenox, D.D. Hendley,* J. Ellis and Y. Ehrlich. Neuroscience Research Unit, Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405.

Protein Kinase C (PKC) is one of two major classes of calcium dependent protein kinases known to exist in a variety of cell systems including the brain. This protein phosphorylation system has been shown to be involved in multiple cellular functions including cellular proliferation and regulation of neurotransmitter release and receptor activation. Receptor mediated hydrolysis of phosphoinositides liberates diacylglycerol, known to be a potent activator of PKC. Since chronic lithium has been shown to significantly alter the phosphoinositide metabolites in brain, our laboratory has initiated a series of studies to examine the effect of chronic lithium administration on PKC activity in rat hippocampus.

Male Sprague Dawley rats (150-175g) were administered a lithium diet (40mM kg/60mM kg) for three weeks and were matched to animals receiving a control diet. Following sacrifice by decapitation the brain was frozen in liquid nitrogen and hippocampus was dissected free. Plasma and brain concentrations of lithium were determined using atomic absorbtion spectrophotometry (emission mode). Cytosolic and membrane fractions were isolated from hippocampus by centrifugation. PKC activity was determined as the incorporation of [^{32}P] from the terminal phosphate of γ-[^{32}P]ATP into endogenous proteins of each fraction in the presence and absence of phosphatidylserine (PS) and a phorbol ester (PDBU) at 1μM free calcium. Quantitation was carried out using scanning laser densitometry of autoradiograms following SDS-PAGE.

Brain and plasma concentrations of lithium were 1.23±.05 meq/kg (n=5) and 1.33±.09 meq/l (n=5) respectively. Studies in our laboratory under similar conditions demonstrated no evidence for an alteration in the distribution of PKC activity between cytosolic and membrane fraction in the hippocampus of animals receiving chronic lithium. However, we now report a significant reduction in the in-vitro phosphorylation in the presence of PS/PDBU of a protein band with an apparent MW 83kDa in the cytosolic fraction of hippocampus from chronic lithium animals. Our data indicate this phosphoprotein band to be one of the most active substrates for PKC in the cytosolic fraction of the hippocampus. This phosphoprotein appears to be similar to that reported by Wu et al. (PNAS 79:5249, 1982) which has been shown to be a substrate for PKC, is phosphorylated in response to depolarization induced calcium influx, and may be related to neurotransmitter release.

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44.15 CONTINUATION OF Ca^{2+}/CALMODULIN-DEPENDENT PHOSPHORYLATION IN THE SYNAPTIC JUNCTION AFTER Ca^{2+} ELIMINATION. T. Suzuki[1*], T. Fujii[2*], and R. Tanaka[1*] (SPON: R. S. Aronstam). 1, Dept. of Biochemistry, Nagoya City Univ. Med. Sch., Nagoya 467, Japan; and 2, Dept. of Functional Polymer Science, Fuculty of Science and Technol., Shinshu Univ., Ueda 386, Japan

Synaptic junction (SJ) isolated from rat cerebrum contained independent protein kinase activity: SJ phosphorylated intrinsic proteins even in the absence of cAMP or Ca^{2+} plus calmodulin (CaM) exogenously added. The activity was affected neither by Ca^{2+} concentrations in the physiological range nor by the addition of specific ligands such as glutamate, aspartate, acetylcholine, and concanavalin A. The activity was not due to cAMP-dependent protein kinase in SJ, since the activity was not inhibited by an inhibitor protein for cAMP-dependent protein kinase, and since synapsin I was not specifically phosphorylated, whereas cAMP-dependent kinase appeared to phosphorylate selectively the protein in SJ. The apparent K_m for ATP was estimated to be 700 µM. Proteins of 16K Mr and 117K Mr were specifically phosphorylated under the basic condition (in the absence of the substances known to activate specifically protein kinases), and the other 6 proteins, both under the condition and in the presence of Ca^{2+} plus CaM. The phosphorylation of 150K Mr, 60K Mr, 51K Mr, and 16K Mr SJ proteins was enhanced after pre-phosphorylation of SJ proteins by intrinsic kinase in the presence of Ca^{2+}plus CaM. The fact suggest that a part of the independent kinase activity is attributable to the autonomous form of Ca^{2+}/CaM-dependent kinase which is derived from the autophosphorylation of the kinase, and that the Ca^{2+}/CaM -dependent autophosphorylation continues even after the elimination of Ca^{2+} from the postsynaptic region and affects the microtubules in the postsynaptic region inasmuch as 16K Mr protein occurs in both the brain cytosol and microtubule protein fraction prepared from rat brain. The continuation of Ca^{2+}/CaM-dependent type phosphorylation in SJ (or postsynaptic density, PSD) may represent a Ca^{2+}-triggered molecular switch (Miller, S. G. and Kennedy, M. B., Cell, 44, 861, 1986) and may modulate neuronal transmission, especially in posttetanic potentiation, long-term potentiation, and memory and learning.

44.16 EFFECT OF ALUMINUM FLUORIDE ON INOSITOL PHOSPHATE GENERATION IN GH_3 CELLS: COMPARISON WITH THE EFFECT OF TRH. L. Grandison and D. Bruno*. UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J. 08854-5635.

Aluminum fluoride (AlF_4) acts on Gs, a guanine nucleotide binding protein with the resultant activation of adenylate cyclase. A similar interaction between fluoride and the guanine nucleotide binding protein that associates with phopholipase C has also been reported in several tissues and indicates that AlF_4 increases polyphosphatidylinositol hydrolysis. The effect of fluoride on inositol phosphate generation was characterized in GH_3 cells and its action was compared to that of TRH. Cultures of GH_3 cells were incubated with ^{3}H myo-inositol for 36 hrs. The cells were then suspended, centrifuge, incubated in a balanced salt solution with lithium chloride (10 mM) for 15 mins., centrifuged, and incubated with treatments in the presence of lithium chloride for varying times. The incubation was terminated by addition of perchloric acid and the samples were neutralized and fractionated by Dowex 1-X8 resin prior to scintillation spectrometry. Sodium fluoride induced a dose dependent (1-10mM), time related (1-30 mins.) increase in inositol phosphates (IP_1, IP_2, and IP_3). This response was not blocked by pertussis toxin pretreatment but was diminished by prior treatment of cells with PMA, a phorbol ester. TRH and bombesin also induced inositol phosphate generation in GH_3 cells. The combination of TRH and bombesin at maximal doses (1 µM) produce greater (but not additive) stimulation than that produced by either receptor ligand alone. AlF_4 induced a 2 to 4 fold greater increase in inositol phosphates than that produced by TRH, bombesin or the combination of TRH plus bombesin. Addition of TRH or bombesin plus AlF_4 produced no greater stimulation than that produced by AlF_4 alone. In summary AlF_4 produces maximal stimulation of polyphophatidylinositol hydrolysis in GH_3 cells unlike its reported action in several other cell types where plasma membrane receptor ligands are found to be more effective. Consequently, AlF_4 may be a useful stimulus for studying inositol phosphate generation and metabolism in GH_3 cells.

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44.17 EFFECTS OF HYPOXIA ON BASAL AND CARBACHOL-STIMULATED PHOSPHOINOSITIDE TURNOVER IN RAT HIPPOCAMPAL SLICES. S. Leventer*, J. Corey*, E. Wulfert* and I. Hanin (Spon.: P. Rowell). Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153 and UCB s.a. Pharmaceutical Sector, Brussels, Belgium.

The receptor-stimulated hydrolysis of phosphoinositides is thought to be an important event mediating neuronal signal transduction. Hypoxia, in vivo and in vitro, has been shown to alter phospholipid metabolism. The purpose of the present investigation was to study the effects of hypoxia on phosphoinositide turnover in rat hippocampal slices in vitro.

Rat hippocampal slices (0.5 mm) were preincubated (60 min, 37°C) in Krebs-Ringer bicarbonate buffer (KRB) bubbled with 95% O_2/5% CO_2. The slices were then washed, and placed in KRB containing 0.3 μM [^{3}H]inositol. After 30 min of incubation, the slices were again washed and placed in KRB containing 10 mM LiCl to block the hydrolysis of [^{3}H]inositol-1-phosphate (IP). This buffer was bubbled either with 95% O_2/5% CO_2 or 95% N_2/5% CO_2, and, in some experiments, contained carbachol (10^{-5}M-10^{-3}M). After 60 min of incubation, as an index of phosphoinositide turnover, accumulated [^{3}H]IP was determined, with only slight modifications, as described by Berridge et al. (Berridge M.J., Downes C.P. and Hanley M.R., Biochem. J. 206:587-595, 1982). In addition, in some experiments, in order to investigate the effects of prior hypoxia on subsequent phosphoinositide turnover, the 60 min preincubation period was split into two 30 min periods. During the second 30 min preincubation period, slices were bubbled with 95% N_2/5% CO_2 or 95% O_2/5% CO_2.

In the absence of carbachol, 0.56% of the accumulated [^{3}H] was present as [^{3}H]IP. Direct hypoxia had no effect on basal [^{3}H]IP levels. Carbachol (10^{-5}, 10^{-4}, and 10^{-3}M) increased the amount of [^{3}H] present as [^{3}H]IP to 154%, 336%, and 554% of control, respectively. In the presence of 95% N_2/5% O_2, these percentages decreased to 136%, 177%, and 234% of control, respectively. Prior hypoxic exposure greatly decreased the total amount of [^{3}H] subsequently accumulated by the slices, but had no effect on the percentage of [^{3}H] present as [^{3}H]IP under basal or carbachol-stimulated conditions.

In summary, prior exposure to hypoxia may reduce subsequent [^{3}H]inositol uptake by hippocampal slices. In addition, direct hypoxia has little effect on basal phosphoinositide turnover. In contrast, carbachol-stimulated phosphoinositide turnover is greatly reduced by direct hypoxia. These changes in phospholipid metabolism may be involved in the mediation of hypoxia-induced tissue damage. Supported by UCB s.a. Pharmaceutical Sector, Brussels, Belgium.

44.18 CEREBRAL INOSITOL-1-PHOSPHATE AND EXTRACELLULAR AMINO ACIDS DURING CONVULSIONS INDUCED BY MICRODIALYSIS PERFUSION OF THE RAT DEEP PIRIFORM CORTEX WITH CARBACHOL. K.M. Savolainen[1]*, J.V. Wade[1]*, S.R. Nelson[2], F.E. Samson[3], and T.L. Pazdernik[1], Department of Pharmacology, Toxicology and Ther.[1], Department of Anatomy[2], and Ralph L. Smith Research Center[3], University of Kansas Medical Center, Kansas City, Kansas 66103, U.S.A.

In order to study the relationship of cholinergic convulsions to brain phosphoinositide (PI) turnover and amino acid transmitters, the extracellular amino acids within the rat deep piriform cortex (DPC) were measured during microdialysis perfusion of carbachol with subsequent determination of brain IP_1 concentrations. Rats were pretreatd with saline or LiCl (5 mEq/kg) 24 hr prior to carbachol perfusion. Dialysis fibers were perfused (1 ul/min) with Krebs-Ringer bicarbonate (KRB) for a 2 hr baseline period, then switched to carbachol (1 or 5 mM) or maintained on KRB (control) for an additional 2 hr. Effluent perfusate samples were analyzed for amino acids with HPLC. Gas chromatography was used to analyze brain IP_1 in bilateral DPC, frontal cortex, piriform cortex, and midline cerebellum. In saline pretreated rats, the low dose of carbachol (1 mM) induced mild clonic convulsions in 1/4 rats, whereas 4/4 rats had clonic convulsions with the high dose of carbachol (5 mM). With LiCl pretreatment, all rats (5/5) had clonic convulsions after the low dose of carbachol. In saline pretreated rats, no changes in regional IP_1 levels (range 0.32-0.42 mmole/kg dry weight) or amino acid concentrations occurred after the low dose of carbachol. However, the high dose of carbachol induced a 7-fold increase of IP_1 levels in the ipsilateral DPC, and a 4-fold increase in extracellular aspartate levels. With LiCl pretreatment, basal IP_1 levels increased to 3.5-4.2 mmole/kg dry weight and the low dose of carbachol produced a further 1.85-fold elevation of IP_1 in the ipsilateral DPC. Extracellular aspartate concentrations increased slightly in these LiCl pretreated rats. However, no major changes occurred in the other amino acids, or in the concentrations of brain IP_1 in other brain regions after carbachol in saline or LiCl pretreated rats. Thus, local application of carbachol into the rat DPC, unilaterally, induces generalized clonic convulsions and this response is potentiated by LiCl pretreatment. Moreover, increased PI turnover, as indicated by IP_1 levels in the ipsilateral DPC, and increased extracellular aspartate concentrations, are associated with carbachol-induced clonic convulsions elicited from the rat DPC. Therefore, increased PI turnover and release of aspartate into extracellular fluid may be involved in carbachol-induced clonic convulsions. Supported by Fogarty International Research Fellowship F05 TWO 3632 and U.S. Army DAMD 17-83-C-3242.

POSTSYNAPTIC MECHANISMS II

45.1 A FAST INHIBITORY POSTSYNAPTIC POTENTIAL IN CAT BLADDER PARASYMPATHETIC GANGLIA. E. Kumamoto*, M. Nohmi* and P. Shinnick-Gallagher (SPON: H. Rudenberg). Dept. of Pharmacology and Toxicology, Univ. of Texas Medical Branch, TX 77550.

In parasympathetic ganglia of the cat urinary bladder neurons respond to a single orthodromic pulse with a fast excitatory postsynaptic potential (f-EPSP) mediated through nicotinic receptor activation. Recently, we have observed two types of responses to orthodromic stimulation: one having only a f-EPSP (type SI, n=20) and the other, a f-EPSP followed by fast inhibitory postsynaptic potential (f-IPSP; type SII, n=41).

The f-IPSP had a duration comparable to that of the afterhyperpolarization (AHP) following an orthodromic spike. In type SII cells, the half-maximum duration (HMD) of the AHP following an orthodromic spike was longer than that of a direct spike (elicited with a depolarizing current injected through the recording electrode); in type SI cells, these durations were comparable. It appears that the f-IPSP contributes to the time course of the AHP following an orthodromic spike.

The f-EPSP - f-IPSP sequence was blocked completely and reversibly by nicotinic receptor antagonists (hexamethonium and d-tubocurarine). Antagonists of muscarinic, alpha-2 noradrenergic, and purinergic receptors, had no effect on the f-IPSP. Thus, the f-EPSP and the f-IPSP are nicotinic responses.

There was no correlation between the amplitudes of the f-EPSP and the f-IPSP recorded consecutively in type SII cells. The appearance of the f-IPSP was not dependent on the presence of a f-EPSP. In type SII cells, spontaneous EPSPs were not followed by a hyperpolarization. Depolarizing the membrane (by passing cathodal current through the recording electrode) to an amplitude comparable to that of a f-EPSP also did not elicit a membrane hyperpolarization in type SII cells. In addition, stimulating one nerve trunk induced a f-IPSP, but activating another nerve trunk innervating the same neuron did not.

Increasing the stimulus strength applied to the presynaptic nerve trunk potentiated the amplitudes of the f-EPSP and the f-IPSP. The f-IPSP was not associated with appreciable changes in input resistance. When the membrane was hyperpolarized, the amplitude of the f-EPSP increased, but that of the f-IPSP decreased. This decrease in f-IPSP amplitude was attributed in part to anomalous rectification at hyperpolarized membrane potentials. A reversal potential for the f-IPSP was not observed.

These results are consistent with the hypothesis that the f-IPSP is due to passive decay of the AHP following an action potential generated by acetylcholine released from nerve terminals in a region remote from the soma, probably the distal dendrites. Supported by NS16228.

45.2 SYNERGISTIC INTERACTIONS AMONG IDENTIFIED CENTRAL SYNAPSES. D.S. Faber and H. Korn, Dept. Physiology, SUNY, Buffalo, NY 14214 and INSERM U261, Institut Pasteur, Paris.

We previously suggested, on the basis of a pharmacological study, that the conductance change produced in the teleost Mauthner (M-) cell by synchronous activation of a pool of inhibitory interneurons is greater than the sum of this cell's responses to impulses in the individual presynaptic units (Korn, H. and Faber, D.S., Soc. Neurosci. Abst., 9:456, 1983). To further test this hypothesis we have paired inhibitory postsynaptic responses due to stimulation of 1) a single presynaptic interneuron and 2) the contralateral eighth nerve at a weak stimulus strength which excited neurons in the inhibitory pool other than the one activated intracellularly. The conductance changes (g_{ipsp}) were quantified, according to equations described earlier (Faber, D.S. and Korn, H., J. Neurophysiol., 48:654, 1982), on the basis of either the reduction of the M-cell's antidromic action potential, or, in Cl^--loaded cells, from measurements of depolarizing inhibitory postsynaptic potentials (IPSPs), The unitary response was timed to occur at, or one to two msec later than, the peak of the eighth nerve evoked inhibition. In approximately half of the experiments, the inhibition evoked when the two stimuli were paired was greater than the sum of their individual effects (i.e. $g_{VIII+unit} > g_{VIII} + g_{unit}$). The enhancement, defined as the percent increase in inhibitory conductance beyond that predicted on the basis of linear summation, ranged from 11 to 46% (m=29%, SD=13%, n=7). This effect occurred when only a few cells were activated by the eighth nerve, since the inhibitory conductance produced by stimulating this nerve alone was at most 7 times that due to direct activation of a single interneuron.

Arguments against a presynaptic mechanism for this synergism include: i) the absence of axo-axonic contacts on terminals of these interneurons, ii) its short latency, and iii) previous findings that field effects do not alter the magnitude of transmitter release. However, these observations are consistent with a postsynaptic mechanism. For example, adjacent boutons may have overlapping postsynaptic receptor domains (Faber et al., PNAS, 82:3504, 1985), with the enchancement occurring as the transmitter, glycine, spreads to the fringes of neighboring synaptic regions. If so, quantal size would be increased under these conditions and the hypothesis that its invariance is due to receptor saturation at some central synapses (Jack, et al., J. Physiol., 321:65, 1981) would be ruled out for this system.

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45.3 SYNAPTIC ACTIVATION OF CA1 PYRAMIDAL CELLS DURING BLOCKADE OF VOLTAGE-SENSITIVE SODIUM AND POTASSIUM CHANNELS. M.D. Mauk & J.D. Kocsis. Depts. of Neurology, Yale Medical School and VA Medical Center, Palo Alto, CA 94303.

Current pulses delivered to the stratum radiatum of the hippocampus (CA1) activate presynaptic fibers which in turn synaptically activate the dendrites of pyramidal cells. In hippocampal slice preparations, bath application of tetrodotoxin (TTX;0.5 uM) abolishes these responses by blocking voltage-sensitive sodium channels. We have found that when 1) voltage-sensitive sodium channels are blocked by TTX, 2) potassium channels are also pharmacologically blocked (with TEA & 4-AP, 5.0 & 0.5 mM) and 3) relatively long current pulses are used (0.1-0.4 msec), responses are elicited with two distinct components. Both components involve local sink currents as revealed by extracellular field potentials, and a large (20-30 mV) depolarization of pyramidal cells as revealed by intrasomatic recordings. The initial component of the field potential has a relatively long latency to onset, a duration of 15-25 msec, and an amplitude of 0.5 to 1.5 mV. This is followed by a second component of similar amplitude but longer duration (150-500 msec).

Subsequent experiments suggest that both components are produced by synaptic activation of pyramidal cells. The responses are abolished by the addition of calcium channel blockers (Co^{++}, Mn^{++},Cd^{++}). They are absent in slices in which the pyramidal cells have been killed by isolation of the stratum radiatum, indicating no contribution from presynaptic activity (e.g. presynaptic calcium). The postsynaptic depolarizations are sodium-dependant in that they are reversibly abolished by application of zero-sodium solutions. Finally, direct activation of pyramidal cells may be excluded by the long latency of the responses which increases linearly with conduction distance with a propagation rate of 0.05 m/sec. Thus, these responses appear to involve sodium-dependant, excitatory postsynaptic potentials in the pyramidal cells.

The initial component is insensitive to the excitatory amino acid receptor antagonist kynurenic acid and to the NMDA receptor antagonist APV. However, the second component is greatly attenuated by kynurenic acid, moderately attenuated by APV and completely abolished by both, indicating glutamate is the neurotransmitter mediating the second component.

These data indicate that when voltage-sensitive sodium and potassium channels are pharmacologically blocked in hippocampus, presynaptic fibers may be activated in a manner that promotes transmitter release and occasions postsynaptic depolarization of pyramidal cells. Furthermore, this activation appears to propagate slowly down the length of the presynaptic fibers. This situation may permit the study of hippocampal synaptic physiology in the absence of voltage-sensitive sodium and potassium conductances.

45.4 IDENTIFICATION OF COMPONENTS OF EVOKED COMPOSITE EPSPs in CA1 HIPPOCAMPAL PYRAMIDAL NEURONS. D.A. Turner, Dept. of Neurosurgery, VAMC and University of Minnesota, Minneapolis, MN 55417.

Dendritic afferents onto CA1 pyramidal cells were stimulated at low intensity (< 10 μA) during intracellular recording in vitro. The resulting composite EPSPs were digitized at high resolution and amplitude histograms of the EPSP peak and noise were calculated. A number of these histograms demonstrated clearly separated peaks (by more than 1.5-2.0 noise standard deviation (nsd) units), often including failures. The EPSP component of each histogram peak was identified by a subgroup average of only those responses falling within a certain voltage range. The variance, time course and probability of each component was compared to other components and to the entire composite EPSP ensemble. The quantal content was calculated under several assumptions (variance, failures and co-efficient of variation (CV), to assess the applicability of Poisson statistics.

Thirty-four EPSP ensembles (out of 84 cells) demonstrated clearly separable peaks, failures and components with a CV less than 0.2. Fourteen ensembles showed only failures and a single component, 12 had 2 components and failures and 8 had 3 or more components. The separation between components was highly statistically significant due to the small standard deviation around each component. Most components within an ensemble showed similar EPSP waveform characteristics but some components were clearly different in terms of risetime or halfwidth values. The spacing between components averaged 0.46±0.19 mV (n=69), compared to the average nsd value of 0.22±0.08 mV. In a few instances the components were stable on increased afferent stimulation, but the probability of components clearly shifted to larger amplitudes and fewer failures of response. The quantal content values for the smallest components ranged from 1 to 9 by the variance method, but these values were much lower than the quantal content calculated by either failures or CV (assuming Poisson statistics).

These results show that components can be identified in small EPSPs in CA1 pyramidal cells, with a number of EPSPs fluctuating only between failures and 1 or 2 states. However, the spacing between components was uneven, the statistics clearly did not fit standard Poisson assumptions and in many instances the components appeared to originate from different dendritic sites. Thus, each component or histogram peak may represent a single synaptic site or cluster of nearby sites, with its own characteristic voltage at the soma and a certain probability of occurrence. The identification of components may be helpful in pinpointing dendritic origin and in searching for changes at an individual site or cluster of sites.

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45.5 A COMPARISON OF THE CURRENT SOURCE DENSITY (CSD) PROFILES THROUGH IN VITRO SLICES OF RAT HIPPOCAMPUS AND VISUAL CORTEX IN RESPONSE TO SYNAPTIC ACTIVATION OF DISTAL APICAL DENDRITES. A.T.Perkins,IV*, L.J. Cauller* and T.J. Teyler. Dept. of Neurobiology, N.E. Ohio Univs' College of Medicine, Rootstown, OH.

In vitro slices of rat hippocampus and visual cortex were prepared as previously described (Shaw and Teyler, Br. Res., 243:35, 1982). Extracellular evoked field potentials were recorded at regularly spaced points (50 or 100um separation) by sequential penetrations across a vertical tract, perpendicular to pyramidal cell body layers. Afferent pathways were activated by discrete electrical impulse stimulation (75um, gold-sputtered, concentric bipolar electrodes; 100us biphasic pulses).

In hippocampal slices, stimulation was applied at least 0.5mm horizontal to the recording tract to either stratum oriens to activate basal dendrites, or distal stratum radiatum to activate distal apical dendrites. As reported previously (Cauller, et al., Neurosci. Abst. 11:505, 1985), synaptic input did not cross cell body layers into other dendritic branches (ie. from basal to apical) unless stimulation was sufficiently strong to evoke a somatic population spike (SPS). Such suprathreshold activation generated a somatofugal sink that ascended from the SPS into apical dendrites at a velocity of approximately 0.1 m/s. Distal stimulation generated a similar somatopetal travelling sink that descended from the level of synaptic input to distal apical dendrite toward the cell body layer at about the same velocity. These travelling sinks are thought to reflect active spikes propagating along the apical dendrites of pyramidal neurons.

Somatofugal sinks were observed to ascend from middle to superficial layers in cortical slices following stimulation of subcortical white matter at a velocity of approximately 0.05 m/s. Such ascending sinks are a common finding of cortical CSD studies whether evoked by peripheral or intracranial stimulation (Mitzdorf, Physiol. Rev 65:37, 1985; Vaknin, et al., Neurosci. Abst. 11:505, 1985). We have observed somatopetal sinks, travelling at about the same velocity, that descend from superficial to middle layers following stimulation of layer I at least 0.5 mm horizontally.

These observations of somatopetal and somatofugal sinks have been incorporated into a general model of cortical sensory processing. The possibility that cortical processing in general involves active dendritic spikes that transmit information between the cell body and distal dendrites is considered and methods to test this hypothesis will be discussed.

Supported by grants from HIH (DA03755) and EPA (CR813394).

45.6 **PHYSIOLOGICAL RECORDINGS FROM HUMAN HIPPOCAMPAL SLICES.** H.L. Haas*, R.W. Greene, M.G. Yasargil* and V. Chan-Palay. Depts. of Neurosurgery and Neurology, Univ. Hosp., Zurich 8091, Switzerland.

We have recorded from in vitro slices prepared from hippocampi immediately after removal, by unilateral hippocampamygdalatomy in patients with intractible temporal lobe epilepsy. The collection and use of these tissues in this research are consistent with NIH guidelines for the use of human tissues and approved by the Ethics Committee. Slices were studied in perfusion chambers for periods up to 15 hours. Field potentials, in particular the population spikes following electrical stimulation of afferent fibers to the CA1 and area dentata were recorded. Paired pulses revealed excitation and, more prominently, inhibition of the test pulse. This may be attributable to the anti-epileptic treatment of the patient from which the hippocampi were taken. Spontaneous epileptiform discharges were not observed. Tetanization (100-400 Hz for 40 to 1000 ms) was followed by a comparatively small post-tetanic potentiation and occasionally by long-term potentiation (LTP) or a long lasting depression of the synaptically evoked population spikes. Intracellular recordings of high quality were obtained from 11 CA1 and 3 dentate neurones. With potassium chloride filled recording electrodes, epsp-ipsp sequences consisted of an early large depolarizing and a late hyperpolarizing component. LTP of this potential was demonstrated in two cells. In the presence of TTX, slow (calcium) spikes were observed and enhanced by the addition of 10mM TEA to the medium. The following neuroactive substances were tested: D,L-homocysteic acid (1mM bolus application) depolarized 2 cells by 15 mV and increased the synaptic potential. Carbachol (1uM) depolarized one cell by 3 mV, decreased the conductance by 12.5 %, blocked the accommodation of firing during depolarizing current injection, and reduced the synaptic potential. Serotonin (10 uM) hyperpolarized one cell by 6 mV and increased the membrane conductance by 50 %. This action was followed by a rebound excitation and block of accommodation. Baclofen (5uM) hyperpolarized one cell by 6 mV and increased conductance by 25 % (measured at the resting voltage, -69 mV). Adenosine (10-100uM) hyperpolarized 3 cells by 6,7,10 mV and caused an outward current of 150 pA (1 cell, 10 uM). Long lasting afterhyperpolarizations, accommodation of firing and an outward tail current after depolarizing voltage jumps were enhanced. Neurons were identified and their precise hippocampal locations verified in each slice by histological methods. Neurons in CA1 of the human hippocampus and dentate area display properties typical for hippocampal neurons as previously described by recordings from slices of non-human species. The results are remarkable in showing the consistency in basic physiological cell function in similar brain regions in phylogeny, despite the inherent increase in hippocampal network complexity that is evident in humans. This emphasizes the validity of animal models in cellular neurophysiology. Supp. in part by US AFOSR 86-0176 and SNF 3002.0.84.

45.7 PROPERTIES OF SUBTHRESHOLD EPSPs IN RAT NEOCORTICAL NEURONS. B. Sutor and J. J. Hablitz. Sect. of Neurophysiol., Dept. of Neurol., Baylor Col. of Med., Houston, TX 77030

Cortical neurons have been reported to possess excitatory postsynaptic potentials (EPSPs) that are mediated in part by receptors for the excitatory amino acid agonist N-Methyl-D-aspartate (NMDA). The evidence for an NMDA-mediated EPSP was an unusual voltage sensitivity and blockade by magnesium and APV. However, little attention was given to the influence of the rectifying properties of cortical neurons. We have further examined properties of EPSPs in neocortical neurons.

Neocortical slices were prepared from rats using standard techniques. Intracellular recordings were made from layer II-III neurons using K-acetate filled pipets. Bipolar stimulating electrodes were placed in layer IV-V and stimuli were applied at 0.1-0.4 times the value needed to evoke an action potential. Hyperpolarizing current steps were used to measure input resistance (Rin) as the membrane potential (MP) was changed with DC current.

Subthreshold stimulation evoked an EPSP which was free of IPSP contamination as determined from analysis of effects of changing MP. EPSP amplitude was 2-4 mV and was unchanged by varying frequency from 0.1-2.0 Hz. EPSP amplitude and duration decreased with hyperpolarization (RMP = 78 +/-4 mV) due to an accompanying decrease in Rin and membrane time constant. Depolarization by 10-15 mV caused in increase in Rin and a slight increase in EPSP amplitude and duration. Further depolarization, below threshold for action potentials, led to decreases in EPSP amplitude. Concentrations of D-APV (10-20 µM) which blocked direct NMDA responses did not affect the unusual voltage dependence of the EPSP. Slight increases in stimulus strength evoked a late depolarizing potential occurring on the falling phase of the initial EPSP. This second EPSP disappeared at stimulus frequencies greater than .4 Hz and was sensitive to D-APV. This component partially resembles an NMDA-mediated synaptic potential but did not have the same voltage dependence as direct NMDA responses. Bursts of high frequency stimulation produced sustained increases in the late synaptic potential which resembled long-term potentiation in other brain regions. APV blocked the induction of this increase and, when applied after induction, reduced enhanced EPSPs.

These results indicate that a variety of subthreshold EPSPs can be evoked in neocortical neurons and display use-dependent plasticity. It also appears that NMDA-mediated contributions to EPSPs are present in voltage ranges where they are often assumed to be blocked by magnesium. Since apparent changes in EPSP amplitude and time course can result from changes in intrinsic membrane properties, we are currently using voltage clamp techniques to study cortical EPSPs. (Supported by NS-22373.)

45.8 PAIRED SHOCKS INDUCE A VERY HIGH AMPLITUDE FACILITATION OF LATE EPSPs IN DEEP NEURONS IN PIRIFORM CORTEX. G.-F. Tseng and L. B. Haberly. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

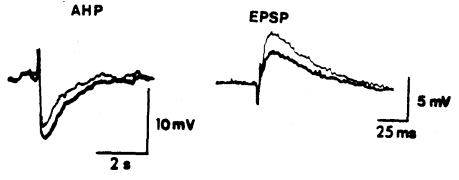
Cells in layer III of the rat piriform (olfactory) cortex and the underlying endopiriform nucleus have been studied with combined physiological-anatomical methods in 500µm thick, transverse slices maintained in vitro. Physiological properties of neurons were studied by intracellular recording with micropipettes containing either 4M KAcetate or 8% Lucifer Yellow in 0.5M Li_2SO_4. The morphology of cells was studied after iontophoresis of Lucifer Yellow either by fluorescence microscopy or by conventional light microscopy after processing with an antibody against Lucifer Yellow. Approximately 1/2 of the dye injected neurons (n=37) were pyramidal cells with a single apical dendritic trunk extending to the cortical surface. The remaining cells were multipolar with spiny, varicose dendrites confined to layer III. Axons from both types of cells arborized extensively in layer III. Stimulation of afferent or association fibers typically evoked variable, multicomponent EPSPs in deep pyramidal cells in contrast to a single component EPSP in layer II pyramidal cells. Deep multipolar cells either failed to respond to afferent fiber stimulation or responded at relatively long latency, but consistently displayed high amplitude, multicomponent EPSPs in response to association fiber stimulation. Spike thresholds were consistently lower for deep pyramidal and multipolar cells than for layer II pyramidal cells. An apparent Ca^{2+} activated K^+ conductance was observed in approximately 25% of deep pyramidal cells (compared with 5% or less of layer II pyramidal cells) and in approximately 50% of multipolar cells. Both cell types displayed early and late IPSPs as observed in layer II pyramidal cells (Tseng & Haberly, Neur. Abs. 12:667).

The most striking finding was an increase in amplitude of the late, multicomponent EPSPs evoked by the second of a pair of shocks by a factor of up to 30X in both types of cells. The facilitated long latency components (but not a brief latency component, when present) were blocked in 1.3mM Ca^{2+}/4 mM Mg^{2+} medium, suggesting that the facilitation is di- or multisynaptically mediated. We postulate that this extreme degree of facilitation results from an amplification of the effects of conventional monosynaptic facilitation (Bower & Haberly, PNAS 83:1115) via convergent excitatory input from superficial cells onto deep cells and positive feedback between deep cells by way of direct excitatory interconnections. The high amplitude facilitation of late EPSP components could play a role in epileptiform activity generated either by kindling (see Hoffman & Haberly, this vol.) or by injection of convulsant drugs into the deep layers of piriform cortex (Piredda & Gale, Br. Res. 377:205). Supported by NINCDS grant NS19865 to LBH.

45.9 REPEATED TETANI OF PERFORANT PATHWAY REVERSIBLY REDUCES SLOW AHPs IN DENTATE GRANULE NEURONS IN VITRO. A. Baskys and P. L. Carlen. Playfair Neurosci. Unit, Toronto Western Hospital, Addiction Research Foundation, and Departments of Physiology and Medicine, Univ. of Toronto, Toronto, Ont. M5T 2S8, Canada.

Pressure-ejected serotonin (5-HT) reversibly blocks AHPs in hippocampal dentate granule (DG) cells in vitro by reducing gK(Ca) [Baskys et al., Brain Res, 1987, in press]. The diffuse distribution of 5-HT fibres in the hippocampus suggests that high frequency tetanic stimulation used to evoke long-term potentiation (LTP) may also release 5-HT from serotonergic terminals and possibly block the gK(Ca). We examined AHPs in DG neurons before and after tetanic stimulation was applied to the perforant path 1-2 mm away from the recorded neuron. Intracellular recordings with 3 M CH3COOK filled electrodes were made in 8 neurons in slices taken from Fischer 344 rats (age 4-7 mos.). Three to 10 mV AHPs followed the train of 4-8 action potentials induced by an intracellular depolarizing current pulse of 100 ms. Concentric bipolar electrodes were used to deliver 400 Hz stimuli (.1 ms, 50-150 uA; 8 trains of 8 pulses/train). Following the tetanus there was a significant decrease in the AHP amplitude (mean decrease 24.6± 6.3%, P<.01). AHPs returned to the control level in 1 to 10 min. In 4/6 cells the AHP suppression was associated with an increase in EPSP amplitude. In a DG cell (figure below) the thick lines represent control responses, thin lines-posttetanic responses. The EPSP is an average of 20 responses at .1 Hz. These data show that tetanic stimulation normally used to evoke LTP can reduce AHPs in a way similar to externally applied 5-HT or norepinephrine.

Supported by the Ontario Mental Health Foundation and the Medical Research Council Of Canada.



45.10 A TRANSIENT CA-DEPENDENT K POTENTIAL FOLLOWS A BRIEF SPIKE TRAIN IN HIPPOCAMPUS. A. Williamson and B. E. Alger. Dept. Physiol., Univ. of Maryland Sch. of Medicine, Baltimore, MD 21201.

Brief trains of action potentials induced by direct depolarizing current steps in rat hippocampal CA1 pyramidal cells maintained in the in vitro slice preparation are followed by a complex afterhyperpolarization (AHP). The latter phase of the AHP is due to a slow calcium-dependent potassium current, I_{AHP}, which can be blocked by a number of compounds including NE, ACh, histamine, and active phorbol esters. When I_{AHP} is blocked, a fast component of the AHP is clearly visible. This potential has been called the middle AHP or mAHP to distinguish it from the first AHP following individual APS and the slow AHP mediated by I_{AHP}. The mAHP is poorly understood.

Our data indicate that the mAHP is a composite potential, part of which is Ca dependent. First, the amplitude of the mAHP varies directly with the extracellular Ca concentration. It increased from 3 mV in control saline (2.5 mM Ca) to 6 mV in 5 mM Ca and decreased to 1 mV in 1.5 mM Ca. These effects are reversible. Second, there is a distinct reduction of the mAHP amplitude in the presence of Ca channel antagonists such as Co and Cd. Third, similar reductions in mAHP amplitude can be obtained by bath application of 5 mM TEA and charybdotoxin, a component of scorpion toxin, both of which block a fast Ca-dependent K current known as I_C. The mAHP is resistant to block by phorbol esters, as is I_C. The mAHP is either all or partly mediated by K as it is seen in cells impaled with KCl-filled electrodes. None of the compounds tested completely abolished the mAHP, indicating that there is also a Ca-independent potential.

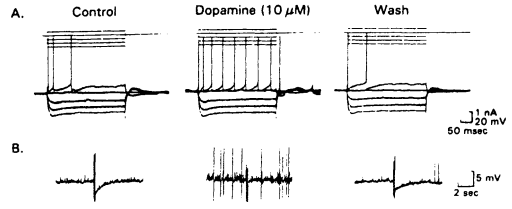
Thus, I_C, which is activated after individual action potentials and is involved in action potential repolarization, can also be seen at the end of a direct depolarizing pulse.

The AHP following direct activation of these cells is, therefore, made up of at least three distinct K-mediated potentials: a slow Ca-dependent potential, a fast Ca-dependent potential and a fast Ca-independent potential. We have previously presented evidence that the initial component of the AHP following epileptiform burst discharges is also mediated by I_C (Williamson and Alger, 1986, Soc. Neurosci. Abst. 12, 728), suggesting that this current plays a role in terminating burst potential discharges. (Supported by NIDA F31 DA05312 (A.W.) and NIH grant NS22010 (B.E.A.))

45.11 DOPAMINE BLOCKS SPIKE ACCOMMODATION AND AFTERHYPERPOLARIZATION IN NEOCORTICAL PYRAMIDAL NEURONS *IN VITRO*. N. Traverse Slater and Linda J. Larson-Prior. Departments of Physiology and Psychiatry, Northwestern University Medical School, Chicago, IL 60611

Dopamine (DA) has excitatory effects on neocortical neurons *in vivo* [1] mediated via the activation of receptors of the D-2 subtype [2]. Despite a wealth of information on the electrophysiologic actions of biogenic amines in other areas of brain, little data is available regarding the actions of biogenic amines in the cerebral cortex. Intracellular recordings were made with KCl or KAc-filled microelectrodes (30-50 MΩ) from 'regular spiking' pyramidal neurons in Layers III-IV in coronal slices of guinea-pig somatosensory cortex maintained completely submerged at 30-33°C. 'Regular spiking' pyramidal neurons were identified by their response to white matter stimulation and their rapid accommodation of spike discharge to depolarizing current injection [3].

DA (1-10 μM) applied by bath perfusion produced a reversible reduction of spike accommodation (Fig 1A) and afterhyperpolarizations (AHPs) (Fig 1B). In the presence of TTX-containing solution, both slow outward rectification during depolarizing current pulses and AHPs were reduced. Calcium spikes recorded in the presence of TTX and TEA were unaffected by DA. These effects of dopamine were also associated with a slow depolarization (3-8 mV) and an increase in the apparent frequency of spontaneous, depolarizing ipsps recorded with KCl-filled microelectrodes, suggesting an excitatory effect on recurrent interneurons. No significant effect of DA was observed on membrane resistance or inward rectification to hyperpolarizing current pulses.



These results provide evidence that the excitatory effects of DA on neocortical neurons observed *in vivo* [1,2] may result from the blockade of a calcium-activated potassium conductance which underlies spike accommodation and AHPs. Pharmacologic evidence for the mediation of these effects *in vitro* by a specific DA receptor subtype is lacking, and the contribution of adrenergic receptors which mediate similar effects in the hippocampus [4] cannot be excluded.

[1] Bevan et al. (1978) *Neuropharmacology*, 17:611. [2] Bradshaw et al. (1985) *Br. J. Pharmac.*, 86:483. [3] McCormick et al. (1986) *J. Neurophysiol.*, 54:782. [4] Malenka & Nicoll (1986) *Brain Res.*, 379:210.

45.12 RELATION BETWEEN THE SHAPE OF DEPOLARIZING PULSE POTENTIALS AND FIRING PROBABILITY OF CAT NEOCORTICAL NEURONS A.D. Reyes*, E.E. Fetz, & P.C. Schwindt, (SPON.: A.J. Berger) Dept. of Physiology & Biophysics and Primate Ctr., Univ. of Washington, Seattle, WA 98195

Excitatory post-synaptic potentials (EPSP's) produce a change in the firing probability of neurons that is given by the cross-correlation histogram of neuron firing aligned with the onset of the EPSP's. The relation between the time course of the EPSP and the correlogram has been studied directly only in rhythmically firing motoneurons (1,2). In the present study, we investigated how parameters of brief depolarizing potentials affect features of correlogram peaks in neocortical neurons.

Intracellular recordings were obtained from layer V pyramidal cells in slices of cat sensorimotor cortex (3). The neurons were impaled with either KCl or K-MeSO4 electrodes with resistances of 10-20 megohms. To mimic synaptic EPSP's, depolarizing membrane potentials were evoked by injecting brief rectangular current pulses into the cell. The resultant voltage deflections were characterized by a near linear rise of membrane potential during the pulse, followed by an exponential decay. The size and shapes of the "pulse potentials" (PP) were controlled by varying the amplitude and duration of the current pulses. After measuring the average PP with the cortical neuron at rest, rhythmic discharge was induced by injecting constant depolarizing current through the microelectrode. The effect of the PP on firing probability was determined by cross-correlating the stimulus train with the neuron's action potentials. Preliminary observations suggest that the effects of PP's on firing probability are similar to those of postsynaptic potentials evoked in these neurons by electrical stimulation of white matter.

To investigate the relation between PP's and correlograms, we systematically varied the amplitudes and rise times of the pulse potentials. Preliminary results indicate that the correlogram peak height was most strongly related to the rising slope of the PP (r = 0.76) and the correlogram peak area to the PP amplitude (r = 0.76). These relations were very similar to the relations between single-fiber Ia EPSP's and their corresponding correlograms in cat motoneurons (2).

1. Fetz & Gustafsson, J Physiol 341:87; 2. Cope, Fetz & Matsumura, J Physiol in press; 3. Stafstrom, Flatman, Schwindt & Crill, J Neurophys 52:244.

45.13 DESYNCHRONIZATION AND REDUCTION OF EPILEPTIFORM ACTIVITY WITH ELECTRICAL STIMULATION. D. Durand. Applied Neural Control Laboratory, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Recent studies have shown that electrical stimulation can reduce the amplitude of population spikes of synchronized neuroelectric activity resulting form epileptogenic drugs. A current pulse synchronized with the epileptiform activity can decrease the amplitude of the population spikes by 80 to 100% (see fig.1). The mechanisms responsible for the large decrease are unclear, but a possible explanation could involve the stimulation of another pool of neurons firing in opposite phase from the original set of neurons. This mechanism would then produce a desynchronization of neuronal firing leading to a reduction in extracellular potential amplitudes. Intracellular recordings were then used to test this hypothesis.

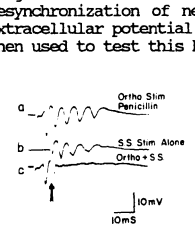


Fig. 1

Transverse hippocampal slices were prepared from Sprague-Dawley rats (150-200g). Repetitive firing was induced by adding penicillin (7000 units/ml) to the bathing medium. Orthodromic responses were triggered with a stimulation electrode in the stratum radiatum (fig.1a). Intracellular and extracellular activity were recorded simultaneously with standard glass microelectrode techniques. A 50um diameter stainless steel (ss) electrode located close to the recording electrode was then activated (arrow) with a current generator delivering pulses of various amplitudes and widths. The current pulse was applied following orthodromic stimulation with a variable delay.

Experiments on 10 CA1 cells showed that 5 cells were not affected by the stimulation even in the presence of a large decrease in the amplitude of the extracellular spikes. For the 5 cells that did show an effect, 3 cells were firing in opposite phase from the extracellular potential (synchronized with positive peaks), and 3 cells showed a marked depression in the amplitude of the action potentials (1 cell showed both effects).

These preliminary experiments confirm our hypothesis that the delayed stimulation pulse activates a pool of neurons firing in opposite phase from the first neurons and also suggest another mechanism by which the amplitude of the action potentials are reduced following depolarization. Both mechanisms are under investigation with further intracellular recordings, current-source density analysis and finite difference modelling.

Supported by NSF grant #ECS-8406861, NSF Presidential Young Investigator Award to the author and the Whitaker Foundation.

45.14 **FURTHER STUDIES ON FEED-FORWARD AND RECURRENT INHIBITION IN THE CA1 REGION OF THE HIPPOCAMPUS.** **H.V. Wheal, S. Phelps & S. Radpour. Department of Neurophysiology, University of Southampton, Southampton SO9 3TU, U.K.** (SPON: J. Chad)

There is evidence for both feed-forward and recurrent i.p.s.p.s in CA1 pyramidal cells in the in vitro hippocampal slice preparation (Dingledine et al. J. Physiol. 305: 297-313, 1980). Furthermore putatative inhibitory interneurones and feed-forward i.p.s.p.s can be evoked by low threshold stimulation of the Schaffer/commissural afferents (Ashwood et al. Brain Res. 293: 279-291, 1984; Turner, Neurosci. Lett. 22: S511, 1985). We have used a modified heterosynaptic paired-pulse protocol (Lynch et al. Exp. Neurol. 71: 527-540, 1981) to study functional feed-forward inhibition. Also, following reports that the ventral hippocampus is more prone to epileptiform activity (Lothman et al. Brain Res. 218: 299-318, 1981) we have measured synaptic inhibition at the septal and temporal poles of the hippocampus.

Feed-forward inhibition was demonstrated using heterosynaptic paired-pulse stimuli applied to the stratum radiatum of the CA1 region. In order to prevent any contamination of the inhibition with a recurrent component, two extracellular recording electrodes were carefully positioned in the stratum pyramidale of CA1. The second recording electrode was used to ensure that the orthodromic conditioning stimulus intensity was subthreshold for activation of all pyramidal cells, including those closest to the site of the stimulus. With these precautions and a C-T interval of 22msec, the test population spike was significantly inhibited (n=10), thus demonstrating a functional component of feed-forward inhibition in the hippocampal slice.

To compare the levels of synaptic inhibition in the septal and temporal poles of the hippocampus the tissue was either sliced transversely or longitudinally along its septo-temporal axis. Recordings were made from a single extracellular electrode placed in the CA1 stratum pyramidale. Suprathreshold paired-pulse stimuli were applied via a single bipolar electrode to the stratum radiatum. The data from 20 pairs of septal and temporal slices showed a significant difference in the percentage inhibition of the test response. Very little inhibition was seen in the temporal hippocampal slices and no advantage was found in cutting the slices longitudinally. This observation may explain why the temporal or ventral hippocampus has been reported to be more prone to epileptiform activity. We are presently investigating regional differences in inhibition in a chronic model of temporal lobe epilepsy.

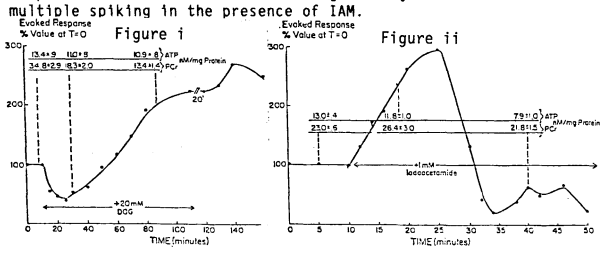
Funded by the Wellcome Trust. H.V.W. is a Wellcome Senior Lecturer.

45.15 SOME NOVEL EFFECTS OF GLYCOLYTIC INHIBITORS ON SYNAPTIC TRANSMISSION IN GUINEA PIG HIPPOCAMPAL SLICES. J. Waalen* and P. Lipton (SPON: H. Karavolas). Dept. Physiol., U. Wisc., Madison, WI 53706

In the course of studies to determine the role of glycolysis in synaptic transmission two classical blockers of glucose utilization were added to the buffer perfusing guinea pig hippocampal slices. Their effects were quite surprising and are shown in the figures below where the ordinate represents the height of the population spike recorded in dentate gyrus following stimulation of the perforant path.

Figure (i): 20 mM deoxyglucose was added to slices incubated 10 mM glucose/10 mM pyruvate-containing buffer. After an early depression in the population spike there is a slow and profound increase over the course of the next 60-90 minutes. Deoxyglucose is incorporated into glycoproteins and this may be a mechanism for its enhancement of transmission. Figure (ii): 1 mM iodoacetamide (IAM) was added to slices incubated in 10 mM glucose-containing buffer. There is a rapid profound enhancement of the population spike and a more slowly developing inhibition of glycolysis which leads to the abolition of the population spike. All evidence indicates these are two independent actions.

We assume the IAM-enhancement is a -SH effect; it may indicate a way in which synaptic transmission is affected in physiological situations. We are studying the effect of IAM on release of endogenous transmitters; both glutamate/aspartate and serotonin. In preliminary results IAM enhances basal release of serotonin and depresses K-stimulated release. IAM appears not to stimulate basal release of glutamate and profoundly depresses release evoked by 50 mM K. Thus, the enhanced population spike is probably not mediated by an increase in release of neurotransmitter. This suggests either a post-synaptic site of action or a depression of GABA release as mechanisms for the enhanced response. The latter is militated against by the absence of multiple spiking in the presence of IAM.



45.16 DECREASE OF EXCITATORY SYNAPTIC TRANSMISSION IN CAT SPINAL CORD BY NH_4^+ IS ACCOMPANIED BY NEURONAL DEPOLARIZATION. W. Raabe. Neurology, VA Med. Ctr. and Univ. of MN, Minneapolis, MN 55417.

A systemic ammonia intoxication decreases excitatory synaptic transmission in cat spinal cord (Neurosci. Abstr. 12:825, 1986). Possible mechanisms underlying this effect of NH_4^+ are i) a decrease of the utilization of glutamine as a precursor for transmitter-glutamate because of the inhibition of glutaminase by NH_4^+ (J. Neurochem. 33:1295, 1979), and ii) a depletion of transmitter-glutamate in nerve endings because of inhibition of Na^+-dependent uptake of glutamate into synaptosomes by NH_4^+ (Exp. Neurol. 89, 1985). To further investigate the mechanisms of action of NH_4^+ this study investigates the effects of NH_4^+ on presumed glutamatergic synaptic transmission in cat spinal cord in relation to cholinergic synaptic transmission.

Cats, deeply anesthetized with pentobarbital and paralyzed with gallamine triethiodide, were used. The PBST nerve was stimulated (1.5 x T) and the motoneuron pool EPSP (VR-EPSP) recorded from the S1 ventral root. The L7 ventral root was stimulated and the Renshaw cell discharge was recorded in the spinal cord by an extracellular electrode. Ammonium acetate (NH_4Ac), 20% solution in H_2O adjusted to pH 7.4, was given i.v.

NH_4Ac i.v. reversibly decreased the VR-EPSP. During NH_4Ac i.v., VR-EPSPs of decreased amplitude suddenly triggered discharges of motoneurons. Discharges ceased when the VR-EPSP was less than 10% of control. During recovery from NH_4Ac i.v., decreased VR-EPSPs also transiently triggered discharges. Before NH_4Ac i.v., stimulation of L7 ventral root readily evoked repetitive discharges of Renshaw cells. When the VR-EPSP decreased to less than 10% of control, stimulation of the L7 ventral root did no longer evoke Renshaw cell discharges. The negative phase of the antidromic motoneuron population potential evoked by stimulation of the L7 ventral root markedly increased when NH_4Ac i.v. began to decrease the amplitude of the VR-EPSP. When the VR-EPSP was maximally decreased, the negative phase of the antidromic motoneuron potential suddenly decreased below control values. With recovery of the VR-EPSP, the negative phase of the antidromic motoneuron potential recovered to control values.

These observations suggest that NH_4^+ does not selectively affect presumed glutamatergic excitatory synaptic transmission in cat spinal cord. The experimental data indicate that NH_4^+ depolarizes neurons and eventually produces a depolarization block of neuronal excitation. This may be due to a direct effect of NH_4^+ on the neuronal membrane. Alternatively, inhibition of Na^+-dependent glutamate reuptake into synaptosomes and extracellular accumulation of glutamate may decrease the VR-EPSP and depolarize neurons, respectively.

45.17 ARE RETINAL GANGLION CELLS ISOPOTENTIAL? R.F. Miller and P.A. Coleman* Dept. of Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110.

We have obtained electrophysiological evidence which suggests that retinal ganglion cells are isopotential as determined by a current pulse injected into the soma. The results which support this conclusion were obtained by a newly developed whole cell recording (WCR) technique applied to ganglion cells in the superfused retina-eyecup preparation of mudpuppy or tiger salamander. In this preparation, retinal neurons retain their synaptic connections and maintain normal light evoked activity. Because a "giga-seal" is formed between the cell membrane and the tip (1-2 μm) of the patch electrode, high quality, long duration recording are possible. With this approach, we measured the input resistance of more than 20 different ganglion cells with values ranging from a few hundred megohms to more than two gigaohms. The mean time constant in these cells was 70 msec. Assuming a value of 1 uF/cm^2 for the membrane capacitance, the time constant measurements suggest that the average specific membrane resistance (R_m) for retinal ganglion cells is 70,000 ohms cm^2. In addition, the rising phase of the voltage transient, produced by a current pulsed injected into the soma was usually well described by a single exponentional function. These findings suggest that the electrotonic lengths of ganglion cell dendrites are short, due to a high value of R_m.

Anatomical measurements from retrogradely labelled, horseradish peroxidase filled ganglion cells, coupled with our physiological measurements, were used in computer simulations to determine the average electrotonic length of the dendritic processes. For a ganglion cell with 5 primary dendrites, each approximately 200 μM long, the dendritic to somatic conductance ratio was approximately 2, with an average electrotonic length of 0.3λ, using our experimentally obtained R_m value of 70,000 ohm cm^2. These findings support the idea that synaptic inputs along the dendritic tree are nearly equipotential in their ability to charge the soma and hence modulate the excitability of the cell. Thus, we believe that previous models of retinal ganglion cells may have considerably underestimated R_m.

Supported by NEI grant EY03014 and NSF grant BNS-85-12385.

45.18 **THREE-FOLD OVERESTIMATION OF DENDRITIC ELECTROTONIC LENGTH BY SINGLE EQUIVALENT CYLINDER MODELS.** Loyd L. Glenn and Brad G. Samojla. *Department of Physiology, Ohio College of Podiatric Medicine, University Circle, Cleveland, Ohio 44106-3082.*

The electrotonic length of dendrites have been estimated by analysis of the voltage response to a pulse or step of current, where the neuron is assumed to be represented by a single, equivalent cylinder. It has been shown that this model describes dendritic trees under the condition that dendritic branch point meet the $d^{3/2}$ constraint of Rall, and that dendritic lengths are equal. The $d^{3/2}$ constraint is met in most neurons, but the equal-lengths constraint is not met. In order to test the effects of nonuniform dendritic lengths on estimates of the average electrotonic length, mathematical models were developed that describe neurons with multiple dendrites of unequal length.

Three boundary conditions were applied to the general solution for the cable equation: (1) a sealed end on the distal dendrites, (2) conservation of current between dendrites, and (3) equality of voltage at the proximal ends of all dendrites. The resulting equation was:

$$\tan \alpha_1 L_1 + \tan \alpha_1 L_2 + \tan \alpha_1 L_3 + \ \ldots \ + \tan \alpha_1 L_n = 0$$

$$\tau_1/\tau_0 = 1 / (1 + \alpha_1^2)$$

where $L_1, L_2, \ldots, L_n$ are the electrotonic lengths for the dendrites of a neuron with n dendrites, and α_1 is the lowest, non-zero positve root of the transcendental equation. Once α_1 is determined, the ratio of the membrane tim constant (τ_0) to the first equalizing time constant (τ_1) can be claculated. When the dendrites are equal in length ($L_1 = L_2 = \ldots = L_n$), the first equation reduces to that for a single cylinder (sin $\alpha_1 L = 0$).

The τ_1/τ_0 ratio was related to the average length of dendrites in the special case of uniform dendrites. However, contrary to our expectations, this was not true when dendrites of the multipolar model differed in length. When lengths differed, the τ_1/τ_0 ratio reflected the electrotonic distance from the tip of the longest dendrite in a model multipolar neuron to the tip of the second longest dendrite. Inasmuch as natural dendrites do not meet an equal-lengths constraint, we conclude that the values presently reported for the electrotonic length of neurons are actually the maximal tip-to-tip electrotonic distance for a neuron.

The consequence is that dendritic lengths have been overestimated in the literature by a factor of 3. The factor of 3 is simply the maximal tip-to-tip electrotonic length divided by the average dendritic electrotonic length of a neuron, as ascertained from published intracellular HRP labeling studies. For spinal motoneurons, the dendrites are not 1.5 λ in length, but actually 0.5 λ. For hippocampal neurons, such as CA1-CA3 pyramidal cells and granule cells, the electrotonic length is 0.3 λ rather than 0.9 λ. This shortening has the effect of reducing the synaptic propagation losses for the most distal synapses on dendrites from 50% (presently accepted) to only 5%. [Supported by Ohio Board of Regents Research Challenge Program]

45.19 NEURON SIMULATIONS USING SABER.

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Hypotheses of neuronal function that are closely linked to anatomical and biophysical data require realistic quantitative simulations for verification. Some groups have used commercially available programs such as SPICE (1,2) for these simulations. Others have developed their own programs for reasons including efficiency and user interface (3,4). We now report our experience using SABER, a new general purpose simulator. This program uses algorithms (5) that make it faster and more general than SPICE, so it may be especially well suited for modeling individual and interconnected neurons.

Most simulation programs were designed specifically for electronic circuits and need special dodges to deal with voltage-, ion-, or neurotransmitter-dependent conductances. For example, spike currents have been implemented under SPICE (2) by incorporating what are effectively analog computer models for gNa(v,t) and gK(v,t).

SABER is not restricted to a predefined set of components, but allows modeling of biophysical properties directly from the empirically determined functions and differential equations that describe their kinetics. Because this program can handle non-electrical elements, models can readily incorporate fluxes and local concentrations of ions and neurotransmitters. SABER also allows formulation of models in terms of user-defined subunits, which would facilitate the construction of complex models of individual neurons, and in turn, the assembly of circuits of interconnected cells.

We have used SABER to simulate anatomically detailed neuron models as well as simplified models that were derived from extensive anatomical and biophysical data using: our previously described empirical approach to the optimization of neuron models (6); our recently developed methods for the automatic optimization of simplified neuron models (7). SABER was particularly helpful for exploring the sensitivity of simulations to changes of model parameters. We also examined the effects of voltage-dependent membrane conductances on the accuracy with which simplified models could reproduce the properties of anatomically detailed models.

SABER is a trademark of Analogy, Inc.

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TRANSPLANTATION I

46.1 **REGENERATIVE RESPONSE OF AMPUTATED LIMBS AFTER IMPLANTATION OF CULTURED CNS AND PNS EXPLANTS.** Betty F. Sisken. Center for Biomedical Engineering and Dept. of Anatomy, University of Kentucky, Lexington, KY 40506.

Previous studies have described the responses of four day chick embryo limbs after amputation and implantation with freshly-dissected neural tube (Fowler and Sisken, 1982; Sisken et al, 1983-6). These responses include: induction of complete regeneration (formation of middle and distal segments) in approximately 1/3 of the operated animals, restoration of the ratio of the area of nerve:area of the amputated limb, and preservation of sensory and motor components of the amputated stump.

We have reported that only neural tissue taken from 2 to 4 day donor embryos is capable of inducing a regenerative response. Although central nervous tissue (CNS), or peripheral nervous tissue (PNS) freshly-dissected from older donor embryos are ineffective, we asked if culturing these tissues in vitro for periods of 3-10 days restores the inductive properties. Pieces of cerebral cortex, spinal cord, dorsal root ganglia, or heart obtained from 6-8 day chick embryos were cultured in separate 60 mm culture dishes. The cultures were washed 2X and single explants from these cultures were implanted into the stumps of 4 day chick embryos using our standard procedure (Fowler and Sisken, 1982). The eggs were incubated for an additional nine days, stained in Alcian Blue and the limbs examined for a regenerative response. From the results of the studies presented in Table 1, we conclude that: (1) culture for a minimum of three days of CNS and PNS tissue obtained from older embryos restores the limb-inducing potential and, (2) culture of non-nervous tissue does not promote this capability.

Table 1

Tissue	Regenerative Response	% Successful Regeneration
Dorsal root ganglia, 8 day embryo		
Fresh	0	0
Culture 3-10 days	+	45%
Spinal Cord, 2-4 day embryo		
Fresh	+	32%
8 day embryo		
Fresh	0	0
Culture 3-10 days	+	36%
Cerebral Cortex, 4 day embryo		
Fresh	+	30%
Culture 3-10 days	+	50%
Heart, 1 day		
Fresh	0	0
Culture 3-10 days	0	0

46.2 EXPRESSION OF L2/HNK-1 FAMILY OF CELL ADHESION MOLECULES AND GFA ANTIGENS IN CEREBELLAR ALLOGRAFTS IN MICE. M. Poltorak*, W.J. Freed and M. Schachner+. Preclinical Neurosciences Section, Neuropsychiatry Branch, NIMH, Washington, D.C. 20032 and +Department of Neurobiology, University of Heidelberg, Federal Republic of Germany.

Cell-cell interactions are of primary importance in the morphogenesis of the nervous system. Cell surface molecules have been implicated in molecular mechanisms of cerebellar development. Since embryonic cerebellar fragments deprived of input fibers can survive and develop as grafts when transplanted to the host adult brain, we were interested in the expression of L1, BSP-2/N-CAM, J1, MAG (myelin-associated glycoprotein) and GFA (glial fibrillary acid protein) antigens as well as the L2 epitope in cerebellar tissue transplanted into the lateral ventricle.

The donor cerebella were taken from C57/Bl mouse embyros at gestational day E14-E15 and implanted into the lateral ventricles of adult host mice. Indirect immunofluorescence staining was performed on fresh frozen sections after 2 to 6 weeks survival. Routine cresyl violet staining revealed that the implanted cerebellar neurons had developed a normal trilaminar organization. Immunoreactivity with antibodies against cell adhesion molecules (L1, BSP-2/N-CAM, J1, MAG) showed patterns generally similar to those observed during normal development of the cerebellum. Since the cytoarchitecture of transplanted tissue was maintained and migration of granule cells occurred it is likely that the postulated molecular basis of granular cell migration also occurs in transplanted cerebellar tissue. The characteristic GFA positive elongated Bergmann glia were not detected even though the migration of granule cells from their germating zone to the internal granular layer was observed. These data confirm the importance of cell adhesion molecules during cerebellar morphogenesis, but suggest that the behaviour of migrating granule cells has multiple mechanisms and does not depend entirely on interactions between granule cells and GFA positive Bergmann glia.

46.3 FETAL BRAIN TRANSPLANTS INDUCE FUNCTIONAL RECUPERATION OF TASTE AVERSION LEARNING AND NORMAL CONNECTIVITY IN CORTICAL LESIONED RATS. J. Fernández*, M.L. Escobar*, R. Guevara and F. Bermúdez-Rattoni. Instituto de Fisiología Celular and Facultad de Medicina. Universidad Nacional Autónoma de México. Apdo. Postal 70-600. 04510 México, D.F.

Recently, it has been reported that fetal brain transplants have been used to study recovery of brain damaged regions of adult rats. Thus, it has been shown that fetal transplants establish functional and anatomical connections with the host. On the other hand, it is well-established that the gustatory neocortex (GN) mediates conditioned taste aversion (CTA). The involvement of this area in CTA learning has been demonstrated by the observation that lesion of GN disrupts both acquisition and retention of a learned taste aversion response. In this study we are demonstrating that fetal cortical transplants can induce recovery of a previously lost acquired CTA, and the reestablishment of normal connectivity.

Rats were divided into an unoperated control group, and a group who sustained large electrolytic lesions of the GN. Following postoperative recovery each group received CTA acquisition; saccharin (.1%) was added into the drinking solution, followed by lithium chloride (190 mg/kg). After 2 days, taste solutions were presented again and those GN lesioned rats which showed disrupted taste aversions were divided in two groups; one group (GGN; n=8) was transplanted with homologous GN tissue, the other group (TGN n=7) received heterogeneous brain stem tissue, both obtained from 17 old fetuses. Eight weeks later all the animals were given an acquisition trial and two tests trials again. We compared the taste aversion scores before and after the graft for the GN groups and for the unoperated group. At the end of the experiment all grafted animals were anesthetized and the brain removed for Histological verification; of graft characteristics. The behavioral results showed a significant aversive recuperation of the GN group after brain transplants, since the aversive scores became similar to those of unoperated control group. In contrast, those animals which received brain stem transplants did not recuperated the taste aversion and were significantly different from the control group ($p<0.01$). In addition, horseradish peroxidase histochemistry revealed that HRP injections in the homologous cortical transplant produced retrograde neuronal labeling to the VPM nucleus of the thalamus. This suggests that there were establishment of connections between graft and host. These results support the hypothesis that homologous but not heterogeneous fetal brain transplants can restore the association of taste with its visceral consequences and restablished normal connectivity between graft and host.

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46.4 TRANSPLANTATION OF OLFACTORY BULBS INTO OLFACTORY AREAS AFTER BULBECTOMIES. J. N. Kott[1], M. H. Hankin[2], L. E. Westrum[3], and R. D. Lund[2]. (SPON: D.F. Farrell). Depts of Neurosurg.[3], Biol. Structure[3], and Psychology[1], Univ. of Wash., Seattle, WA 98195 and Dept. of Neurobiol., Anat. and Cell Sci.[2], Univ. of Pittsburgh, Pittsburgh, PA 15261.

Factors influencing successful grafting of olfactory bulb (OB) into other olfactory areas are being studied in neonatal and adult rats. The effect of transplantation at differing times after OB removal on the survival of the grafted tissue is of especial interest at present. OB ablations are performed in newborns, postnatal (PN) 0-3 days, or young adults of approximately PN 100 days. Post-ablation times include acute (same day), intermediate (3-4 days) or chronic (2-4 weeks). Then OBs from fetal rats of known gestational age are either used whole or dissociated in trypsin and used in suspension. Implantations are done with a cannula manually placing the donor tissue/cells into the olfactory peduncle or rostral prepiriform area on the ablated side. The host subjects are sacrificed 2-3 months later. The forebrains are either dissected and removed from the calvarium with all obvious bulbar tissue intact and subsequently frozen sectioned or processed in situ by decalcification and paraffin embedment. Cell body stains and silver stains (unsuppressed Nauta and Loots) for fibers are done on alternate sections. The preparations show clearly successful grafts, especially in those with intermediate and chronic OB ablations. Same day implants may be less successful. Neuronal clusters often are glomerulus-like in appearance. Extensive fiber patterns occur within the graft and sometimes between the implant and adjacent host tissue. A particularly impressive situation is the grafts in or onto the anterior olfactory nucleus (AON), an area normally innervated by the OB. In these cases numerous fibers pass between the grafted OB and host AON suggestive of connectivity between the two tissues. The findings emphasize the importance of a deafferenting lesion and that either a few days' or weeks' recovery may be used in preference to lesion and graft on the same day. Also in this system the AON, after ipsilateral OB removal, is a promising host site in which to study some of the morphological events of fetal OB development.

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46.5 ELECTROPHYSIOLOGY OF MORPHOLOGICALLY IDENTIFIED SEPTAL NEURONS GRAFTED INTO RAT HIPPOCAMPUS. L.J. Reece and P.A. Schwartzkroin. Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195.

Fetal neurons grafted into the brains of animals with previous lesions have been shown to survive and mature in the new location in the host and to ameliorate both lesion-induced enzyme alterations and behavioral deficits. We have been interested in the viability of septal neurons transplanted into the hippocampal formation. While septal grafts into the hippocampus have been shown to be functional by biochemical, histological, and behavioral techniques, little information is available on the physiological characteristics of morphologically identified grafted neurons.

We have previously reported data from recordings of putative grafted cells, including functional synaptic connections from the host onto the graft. The present study combines intracellular recording with subsequent dye injection in order to positively identify the grafted nature of the neuron and to further characterize cell morphology and host-graft interactions. Here we report recordings from cells which are morphologically identified as non-hippocampal cells by intra-cellular injection of lucifer yellow.

Adult female Sprague-Dawley rats were given unilateral lesions of the fimbria-fornix. One week later, the animals were given 2 injections of dissociated fetal basal forebrain neurons in the dorsal hippocampus. These cells were prepared from embryonic day 13-15 rat fetuses (crown rump length approximately 16 mm.), incubated in trypsin, dissociated by trituration, and resuspended with a fluorescent marker. The marker, rhodamine-labelled microspheres, is taken up by the cells and allows subsequent localization of the graft in the host parenchyma. Animals were sacrificed at 1-12 weeks after surgery and cell physiology was studied in the in vitro hippocampal slice preparation. Intracellular recordings were made with lucifer yellow-filled micropipettes (3%); grafted neurons were dye filled following physiologic characterization.

The morphology of the grafted cells is consistent with that of cells in the medial septum-diagonal band area as determined by our studies in normal animals. Labelled cells are distinct from those cells normally found in the hippocampus. They exhibit either a bipolar or multipolar soma with extensive processes. The graft could be stimulated from a variety of sites in the host, depending upon location of the graft within the host. Intracellular recordings were consistent with our previous report.

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46.6 ADRENAL MEDULLARY TRANSPLANTS: I. VIABILITY OF HUMAN ORGAN DONOR ADRENALS. D.M. Gash, M.F.D. Notter, J.T. Hansen and S.H. Okawara*. Dept.'s of Neurobiology and Anatomy and Surgery, Div. of Neurosurgery, Univ. of Rochester Sch. of Med., Rochester, NY 14642

This study was conducted to determine the factors important for maintaining viable human organ donor adrenal chromaffin cells which might later be used for transplantation. The present report summarizes the results from 11 donors, 6 males and 5 females, ranging in age from 3 years to 51 years old. In each instance, adrenals were obtained through the Rochester Region Organ Donor Program after receiving informed consent for use of the organs for research. Upon removal from the donor, the adrenals were placed in ice cold lactate-Ringer's solution. Under a dissecting microscope, the adrenal was cut into cross-sections approximately 1 cm thick and the medulla carefully dissected free from the cortex and minced. The small tissue blocks, approximately .5 mm in their largest dimension were then stored in sterile cold calcium, magnesium-free buffer. Samples from six donors were taken within time periods ranging from 30 minutes to several hours following cessation of circulation and processed for light and electron microscope examination. Other samples were prepared for tissue culture using differential plating procedures slightly modified from those described by others (Lillien and Claude, Exp. Cell Res. 161:255, 1985). Essentially the cortical cells attach at a faster rate than chromaffin cells to a collagen substrate which allows enriched populations of chromaffin cells to be obtained from the supernatant. The time from cessation of donor circulation to complete dissection of the adrenal medulla was approximately 2 hours for six of the cases and processing the tissue for culture required another 1 1/2 hours. In a seventh case, the adrenal tissue was maintained in ice cold lactate-Ringer's solution for 10 hours following death before cell cultures were prepared.

Fine-structure analysis of the dissected blocks revealed that great caution had to be exerted to obtain pure medullary tissue. Only blocks dissected from the center of the medulla were completely free from cortical contaminants which implies that those extensive portions of the medulla close to or in direct contact with the cortex should not be used for transplantation. However the differential plating procedures seemed to be very effective for obtaining relatively pure populations of viable chromaffin cells. High yields of viable chromaffin cells were found when the adrenals were obtained from the donors within two hours of death. In the one instance where 10 hours intervened between cessation of circulation and preparation for cell culture, only 5 to 10 viable chromaffin cells could be identified in vitro. The human chromaffin cells could be maintained in culture for periods of up to at least eight weeks. The age of the donor did not appear to be a factor in chromaffin cell survival.

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46.7 ADRENAL MEDULLARY TRANSPLANTS: II. FINE STRUCTURE OF HUMAN ORGAN DONOR ADRENALS. J. T. Hansen, M. F. D. Notter, S. Okawara and D. M. Gash. Dept. Neurobiology and Anatomy, and Dept. Surgery, Div. Neurosurgery, Univ. Rochester School of Medicine, Rochester, NY 14642.

The chromaffin cells of the human adrenal medulla obtained from our organ donor program were examined ultrastructurally to characterize their general morphology and assess their potential for use as CNS grafts in the treatment of Parkinson's disease. Samples were obtained from 3 females (ages 12, 48, and 49) and 3 males (ages 3, 22, and 24). Entire adrenal glands were received from surgery in cold media (4° C), the medulla quickly and carefully dissected free from the cortex, and then immersion fixed in cold 3% glutaraldehyde, 2% paraformaldehyde in 0.1M cacodylate buffer. The chromaffin cells were post-fixed in osmium tetroxide and routinely processed for electron microscopy. The fine structure of the chromaffin cells was directly related to the time span required for removal of the organ until immersion in the primary fixative. Nevertheless, the overall morphology of the chromaffin cells was good, and if the cells were placed in culture for several days, their fine structure improved to the point that the cells appeared quite robust. Although adrenalin and noradrenalin secreting cells can be identified in lower mammals based upon the appearance of the chromaffin cell vesicles, this distinction was not clear in the human medulla. Chromaffin cell vesicles often were quite uniform in size and density, and significant departures from this pattern correlated directly with a delay in fixation. The cells interdigitated with one another and were innervated by small unmyelinated nerve fibers whose terminals contained many small electron-lucent synaptic vesicles and several larger dense-core vesicles. Omega figures indicative of exocytosis were observed along the plasma membrane of the chromaffin cells. In addition to chromaffin cells, the human adrenal medulla also contained Schwann cells, fibroblasts, vascular elements such as endothelial cells and pericytes, and blood-borne cells which were trapped in the capillaries. Autografts of the medulla would naturally contain at least all of these different cell types as well as small groups of isolated cortical cells which occasionally penetrated into the medulla. However, even though several hours may pass between the time at which the medulla is harvested and then cultured, the chromaffin cells are viable as evidenced by their survival and robust appearance in vitro.

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46.8 ADRENAL MEDULLARY TRANSPLANTS: III. PHENOTYPIC PLASTICITY IN VITRO. M.F.D Notter, J.T. Hansen, S.H. Okawara* and D.M. Gash. Dept. of Neurobiology and Anatomy and Dept. of Surgery, Div. of Neurosurgery, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Adrenal medulla from several species of mature animals was examined for phenotypic plasticity when exposed to different growth factors in vitro to establish the potential of this tissue for transplantation in to the central nervous system. Chromaffin cells from adult rat, monkey and human glands were obtained by a trypsin-collagenase procedure and subjected to differential plating on collagen-coated surfaces. With both human and monkey tissue, initial attachment of non-chromaffin cells was obtained followed by replating of an enriched chromaffin cell population. Adult rat adrenal medulla cells survived very poorly in vitro and could not be enriched in this procedure. Cultured human and monkey chromaffin cells survived in vitro as epithelial cells; however, treatment with nerve growth factor (NGF) (100 ng/ml) induced neuritic outgrowth after eight days. These cells showed strong catecholamine histofluorescence, tyrosine hydroxylase (TH) and dopamine beta hydroxylase staining. Electron microscopic observations of these cultured cells indicated that neurites from NGF treated cells contained granules of variable density and that cell bodies exhibited well preserved organelles. Electron microscopy also confirmed that all differentially plated medulla cells examined were chromaffin cells. Cultures were routinely maintained for at least eight weeks in the neuronal phenotype. In contrast, less than ten percent of adult rat chromaffin cells survived in culture. These cells could be rescued by treatment with NGF in which thirty percent of the cells survived, extended neurites after one week in vitro and stained positively for TH.

In order to provide a continuous supply of growth factor(s) for these medulla cells in vitro, chromaffin cells were cocultured with C_6 glioma cells. These astrocytic cells secrete a non-NGF factor(s) which sustains sympathetic neurons in vitro. Glioma cells were treated with mitomycin C bromodeoxyuridine to inhibit mitosis and were plated with the various medulla cells in a one to one ratio. Both human and monkey chromaffin cells expressed extensive neuritic arborization within eight days of coculture and appeared to have contact with the glioma cells as seen at the ultrastructural level. Importantly, survival of adult rat adrenal medulla cells was enhanced by 50% or more and chromaffin cells extended neurites when cocultured with glioma cells for seven days. Chromaffin cells from all three species stained for TH in co-culture and were histofluorescent. These data indicate that primate adrenal medulla can be maintained in vitro for long periods of time as the neuronal phenotype and that adult rat chromaffin cells can survive preferentially in vitro in the presence of different growth factors.

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46.9 **ADRENAL MEDULLARY TRANSPLANTS IV. COGRAFTS WITH GROWTH FACTOR PRODUCING CELLS.** G. Bing*, M.F.D. Notter, J. T. Hansen, C. Kellogg, J. H. Kordower, and D.M. Gash.(Spon: M.Blair)
Department of Neurobiology and Anatomy, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642

Transplantation of adrenal medullary cells into the striatum of rats with unilateral 6-OHDA lesions can attenuate the drug induced asymmetric rotational behavior. Recently, attempts at ameliorating the symptoms of Parkinson's disease by implanting autografts of adrenal medullary cells into the striatal system have been tried clinically. Since nerve growth factor is very low in the striatum, the survival and phenotypic differentiation of grafted adrenal medullary cells needs to be enhanced. In the present study, we have cografted amitotic C6 glioma cells (which are known to produce neurotrophic factor(s) which promote the survival of dissociated adrenal medullary cells) in an attempt to improve chromaffin cell survival and phenotypic differentiation.

Thirty-seven young adult, male, Long Evans rats were used as recipients. The host animals, all with unilateral 6-OHDA nigrostiatal lesions, were divided into four groups: (1) Adrenal medullary cells co-transplanted with C6 glioma cells (n=11); (2) adrenal cells alone (n=8); (3) glioma cells alone (n=8) and (4) vehicle control (n=10). All rats were sacrified one month after transplantation. Electron microscopic, immunohistochemical and autoradiographic methods were used to identify and characterized the grafted cells. Tyrosine hydroxylase-like (TH) immunoreactive cells were found in all animals which received grafts of the adrenal alone or received adrenal grafts co-transplanted with C6 glioma cells. The co-transplanted hosts had more cells and more intense TH immunoactive staining than the hosts receiving the adrenal grafts. Additionally, more cells formed processes in the former group. All three groups (adrenal medullary , C6 glioma cell, and cografted animals) had a significant reduction ($p<0.05$) in ipsilateral rotations after amphetamine (0.5 mg/kg) injection compared to the control group. Morever, the reduction in rotation was more robust in cografted hosts than in the other two implanted groups ($p<0.05$). Reduction in the rotational behavior in rats receiving the C6 glioma graft alone suggests that trophic factor(s) produced by the C6 glioma cells could promote regeneration of damaged host catecholamine nerve fibers or sprouting from the surviving intact fibers. Our studies demonstrate that intraparenchymal transplantation of both adrenal medullary and C6 glioma cells can significantly reduce the rotation induced by amphetamine in 6-OHDA lesioned rats, and the combination of these two results in a greater reduction than adrenal or C6 grafts alone. Analysis of catecholamine content with HPLC in the striatum of the rats in all four experimental groups is in progress. **Support: PHS S7RR05403-25 (JTH). and a grant from the American Health Assistance Association(DMG).**

46.10 NEURAL GRAFTS ENTRAPED IN A COLLAGEN HYDROGEL: A POTENTIAL TOOL TO REPAIR REGIONS OF THE CNS. R. Marchand, S. Woerly and L. Bertrand, Lab. Neurobiol., Hôp. Enfant-Jésus, Qué., Can. G1J 1Z4

The adult mammalian CNS tissue is lacking in extracellular matrices (E.C.M.). In the peripheral nervous system and other organs, ECM are present and play an important role to channel and to pattern cell movements in developing, remodeling and regenerating events. This led us to formulate the following hypothesis: would the presence of a three-dimensionally organized ECM-like structure containing young immature neurons injected into a transplantation site of the CNS favor integration and differentiation of the embryonic material? Artificially made cavities in the neostriatum and in the cortex of adult rat brains were therefore infiltrated with an ice-cold neutral prepolymerised type I collagen solution (concentration 2.4 mg/ml, Vitrogen, Collagen Corp.) containing, respectively, a dopaminergic-rich cell suspension from E14 rat embryos and cortical implants from E15 rat embryos. Cryosections of brains harvested 1 week to 4 months post-implantation were examined for the presence of collagen, for axons growing in the matrix and for the response of the host tissue to the presence of the bioimplants. Methods were used to visualize catecholamine, cholinesterase, collagen and cell bodies. Bioimplants were also examined using scanning electron microscopy. Our results demonstrate (1) that the gelling behavior of the collagen dispersion is initiated in situ at the temperature of the brain parenchyma and results in a highly porous sponge-like gel composed of a meshworks of fibrils and wide interfibrillar spaces (2) the gelled collagen completely fills the space of premade cavities and fuses intimately with the adjacent host tissue making with it a tight interface (3) after collagen fibrillogenesis, the neural grafts become immobilized within the structure of the gels and spatially positioned inside the cavities (4) the implanted dopaminergic nerve cells survive and elaborate processes that extend along the fibrilar elements inside the gel. The cortical implants survive and undergo cytoarchitectural differentiation (5) the host tissue responds to the presence of the gel by sending processes into the bioimplant. Although the gels were partially resorbed by macrophages, these observations reveal 3 features on which can be based strategies to repair a local brain injury with an artificial three-dimensionally organized collagen derived E.C.M.:(1) by its capacity to swell in a restricted volume, the hydrogel restores a physical continuity between the edges of the healthy tissue (2) it provides a physical scaffolding for cell attachment and growth (3) by its high void volume it provides (i) an extracellular environment of least resistance mechanically favorable for extensive growth of nerve fibers, (ii) an extracellular compartment for the diffusion of nutrients. (Supported by the MRC and FRSQ).

46.11 THE EFFECT OF FETAL SUBSTANTIA NIGRA GRAFTS ON DOPAMINE RECEPTORS IN THE CORPUS STRIATUM. E. Korfali* (Sponsor: J.P. Conomy). Department of Neurosurgery, School of Medicine, Uludag University, Bursa, Turkey.

Much research has been devoted to transplanting fetal brain tissue into adult host animals. One of the most commonly studied systems is the dopamine (DA) containing substantia nigra. Most of these studies utilized fetal substantia nigra tissue grafted into the DA innervated caudate nucleus of adult recipient animals. These grafts have been shown to be capable of re-establishing DA innervation of a host caudate, forming ultrastructurally normal synapses within a previously denervated corpus striatum. In addition, graft derived catecholamine axons appear to release DA within the host CNS. These grafts have also been shown to restore a variety of motor and sensorimotor deficits associated with destruction of the DA pathway. However, only one previous study has investigated the effects of nigral grafts on DA receptors (Freed, W.J. et al., Science 222: 937-939, 1983). In this study fetal substantia nigra grafts were found to restore DA receptor densities to normal levels in adjacent areas of the corpus striatum, demonstrated by light microscopic autoradiography with [^{3}H] spiroperidol.

The present study utilized [^{3}H] spiroperidol homogenate binding in 34 randomly bred Norvegicus albino rats (Bursa, weight 180-250 grams) to evaluate the effects of nigral grafts on DA receptors. Four weeks after the intraventricular injection of 6-hydroxydopamine (6-OHDA, 250 µg/ 25 µl), a 2 mm X 3mm cavity was made by suction unilaterally on the surface of the caudate nucleus. Four to six weeks later, after a new vessel rich pia had grown over the surface of the cavity, the cavity was re-opened, and in 20 rats fetal grafts were inserted. These monoamine containing ventral mesencephalon grafts were obtained from rat embryos of 17-18 days gestation (crown to rump length 19-22 mm). The remaining rats (n = 14) served as sham operated controls and did not receive grafts. Two months after grafting, 81% of the grafts appeared grossly to have survived. In the sham operated control rats, [^{3}H] spiroperidol binding to the caudate was reduced 53.7% ($p < 0.01$). In contrast, in grafted rats there was a 25% decrease ($p > 0.05$) in binding to the graft-bearing caudate, and a 15% decrease ($p > 0.05$) in binding on the non-grafted side. These findings suggest that fetal substantia nigra grafts may cause an increase toward normal levels of binding in corpus striatum DA receptors previously depleted by 6-OHDA.

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46.12 ELONGATION AND ALIGNMENT OF ASTROGLIA PROCESSES IN INTRACRANIAL PC12 CELL GRAFTS. C. B. Jaeger, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Radial glia may guide axons and migrating neurons to distant locations during development of the CNS. Such glia have the shape of long processed ependymal cells, stretching between the ventricular lining and the pia. Radial glia give rise to astroglia with short processes, subsequent to migration of neurons and axons. This study sought to answer the question whether short astroglia could give rise to long processed astroglia similar to radial glia. This is an important issue because glia with such morphologies may be necessary for neo-pathway formation of regenerating neurons and neural transplants. It was hypothesized that short processes of glia cells could grow longer in response to mechanical stretch of the surrounding environment, as neurites do, in response to a tensile force (Bray D., Devel Biol 102: 379, 1984). Grafts consisting of PC12 cells were used because they grow extensively within the brain of Sprague Dawley rats and do not themselves contain astroglia, but host brain astroglia migrate into such grafts. Moreover, it has been shown that Millipore filters provide a good attachment substrate for cultured astroglia. Therefore, in addition to PC12 cell grafts, such "inert" nitrocellulose filters were implanted into the cerebral cortex either by themselves or together with clusters of PC12 cells. It was thought that the astroglia attached to a filter and their processes could elongate as the surrounding PC12 cell graft grew in size (Fig. 1).

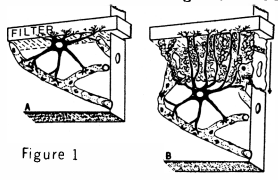

Figure 1

Schematic drawing of glia process elongation as a result of growth in surrounding tissue. A. Glia cell attached to filter (top) and capillary. B. Growing PC12 cell graft leads to lengthening of glia processes.

It was found that short astroglia in different brain regions extended furry filopodia into filters at variable densities. Filters became suspended within PC12 cell grafts but they were connected at different points by bands of long astroglia that were continuous with the host brain interface. Processes of glia in such bands were parallel oriented, they stained with antisera to glia filament protein and attached to the filter edge, thus confirming the notion of glia process elongation in response to a mechanical 'stretch' mechanism. Supported by the American Parkinson Disease Assoc. and BRS grant S07 RR-5399.

46.13 TRANSPLANTS OF FETAL NEOCORTICAL TISSUE FAIL TO IMPROVE BEHAVIOR OF MICRENCEPHALIC RATS. A. Rabe, M.H. Lee, P.C.M. Wang* and R. Recce*. Dept. of Psychobiology, NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10304.

The rat with transplacentally induced cerebral hypoplasia (micrencephaly) and the associated behavioral deficits provides a reasonable animal model of several developmental brain abnormalities. We are investigating whether transplants of tissue from normal fetal neocortex into infant micrencephalic brains could alleviate the behavioral deficits characteristic of micrencephalic rats.

Micrencephaly was induced by an injection into the pregnant Long-Evans rat of 30 mg/kg of methylazoxymethanol acetate on day 15 of gestation. On postnatal day 10 ± 2, some of the micrencephalic rats received 10 mm^3 of neocortical tissue from normal E18 fetuses. The donor tissue was injected into the posterior aspect of the host neocortex. Such transplants are partially intraparenchymal, grow very large, and appear to be permanent. At 2 months and 1 year, normal (N), micrencephalic (MAM), and transplant-bearing micrencephalic (MAM+TR) rats of both sexes were trained to escape from a water-filled Lashley maze (3 daily trials for 5 days) and tested for ambulatory activity during a 30-min session in an open field. After the tests, the presence of a grossly acceptable transplant has been confirmed in a substantial sample of brains, but a microscopic analysis of the transplant and transplant-host interface still remains to be done.

In the maze, at both ages the MAM rats made significantly more errors than the N rats, 123 (n=15) vs 43 (n=15) average errors at 2 months, and 107 (n=11) vs 30 (n=9) errors at 1 year. The MAM+TR rats were similarly inferior to the N rats as they made essentially the same number of errors as the MAM rats, 136 (n=29) at 2 months and 81 (n=11) at 1 year. In the open field, at both ages the pattern of results was similar: both the MAM and the MAM+TR rats (while not differing from each other statistically) ambulated significantly more than the N animals. The average number of infrared beams crossed at 2 months were 1361 (n=17) for N, 1915 (n=26) for MAM, and 2297 (n=46) for MAM+TR groups. At 1 year the respective values were, 976 (n=24), 1626 (n=24), and 1672 (n=30).

These results indicate that the presence of a large transplant of normal fetal neocortical tissue did not reduce the maze learning deficit and hyperactivity of the micrencepahlic rat.

46.14 NEUROBLASTOMA CELLS AS A POSSIBLE DONOR FOR TRANSPLANTATION IN ANIMAL MODELS OF PARKINSON'S DISEASE. M.A. Mena, J.G. de Yebenes, A. Dwork, N. Latov, S. Fahn, J. Herbert. The Neurological Institute, Psychiatric Institute, Columbia University, New York, NY 10032.

We studied the pharmacologic, immunohistochemical and immunological features of human neuroblastoma cell lines as possible candidates for transplant in animal models of PD. Subcultures of neuroblastoma cell lines (CHP100, CHP126, IMR32, LAN1, LAN5, NB69 and SKNSH) were used. Endogenous levels of DA were high in CHP126 (9.9±1.6ng/mg prot), LAN5 (8.8±1.5) and NB69 (14±2.8) and low (<1ng/mg prot) in other cell lines. Maximal values of NE were found in NB69 (27.8±3.9ng/mg prot) and much lower levels (<6ng/mg) in the other lines. Pharmacological responses to DA receptor blockade were tested measuring DA metabolism in the presence of sulpiride (10^{-3}M). Extracellular DOPAC levels increased to 180% of baseline in the NB69 cells. DOPAC and HVA rose to around 200% of baseline in the LAN5 cells. The response to NGF was evaluated by measuring protein content, monoamine levels and rate of division according to ^{3}-H-thymidine uptake in the presence and absence of NGF. Protein content was not significantly changed by NGF in any of the cell lines examined. NGF induced a very mild increase in DA (10%) and NE (20%) in respect to the baseline in NB69 cells but the small changes were apparently due to a decrease in firing rate since an opposite effect of the same magnitude was found in the levels of HVA and MHPG. In LAN5 cells NGF induced a 50% increase in the levels of DA ($p=0.017$), almost a 100% increase in the levels of DOPAC ($p\leq0.001$), and a smaller increase in the levels of HVA, suggesting that LAN5 respond to NGF. The rate of cell division, measured as ^{3}-H-thymidine uptake was unchanged by NGF in LAN5 cells and slightly decreased (22%, $p\leq0.05$) in NB69. Treatment of the catecholamine-rich cells with prostaglandins and cAMP induced a dose-dependent elevation of the DA levels (up to 5-fold in LAN5 and 2-fold in NB69) and a dose-dependent decrease in the division rate, as measured by the uptake of ^{3}-H-thymidine (up to 55% of values found in untreated LAN5 cells and 16% of values in untreated NB69 cells.) Immunohistochemical studies on paraffin-embedded cell blocks, using the avidin-biotin-peroxidase technique with a primary antibody to tyrosine hydroxylase, provided qualitative confirmation of the relative catacholaminergic activities of the native cell lines. The presence of cell surface MHC Class I and Class II (Ia) molecules was tested by flow cytometry using monoclonal antibodies to MHC determinants. Class I antigens were present in LAN1 and Ia in IMR32. Other lines lacked MHC antigens. LAN5 are best for transplant.

46.15 POSSIBLE ROLE OF TERMINAL CONNECTIVITY IN THE EXPRESSION OF PERIKARYAL IMMUNOREACTIVITY TO THE MONOCLONAL ANTIBODY RT97 IN BASAL FOREBRAIN NEURAL GRAFTS. L.C. Doering, O.G. Nilsson, A. Björklund, and A.J. Aguayo. Neuroscience Unit, Montreal General Hospital and McGill University, Montreal, Quebec, Canada and the Department of Histology, University of Lund, Lund, Sweden.

Cytoskeletal abnormalities develop in embryonic telencephalon grafts when isolated for 6-12 months in peripheral nerve (Doering, L.C. & Aguayo, A.J., Brain Res. 401:178, 1987). In these neurons, filamentous inclusions known as Hirano bodies form in dendrites and there is an intense immunoreactivity for the monoclonal antibody (MA) RT97 in the perikaryon. The MA RT97 normally recognizes the phosphorylated 200 kD neurofilament subunit in axons. Although the underlying mechanisms responsible for these changes in long term grafts are unknown, inappropriate or lack of connections with target tissues may play a role in the expression of these abnormalities.

To determine if neuronal interactions with a target influence the formation of these cytoskeletal alterations we examined long term CNS transplants that make connections with the host brain. For these studies, a series of adult rats with bilateral fimbria-fornix transections were implanted with embryonic day 13 basal forebrain tissue. This type of transplant develops connections with the host hippocampus (Björklund, A. et al., Brain Res. 173:57, 1979). The animals were prepared for histological and immunocytochemical studies after 12-14 months of survival.

Networks of axons were defined by immunoreactivity to the MA RT97 in the 6 grafts examined. When sectioned in the appropriate plane, long axonal tracts were seen to extend from neurons in the grafts to the host hippocampus. These neurons showed no perikaryal immunoreactivity to the MA RT97. However, in 5 of the grafts, isolated groups of neurons that did not appear to have extended their processes to the hippocampus displayed a strong perikaryal affinity for the MA RT97.

These observations provide additional evidence that neurons in basal forebrain transplants can project axons into the host hippocampus for prolonged time periods. Neurons that do not appear to connect with their hippocampal targets show cell body immunoreactivity to the MA RT97, a finding that suggests that terminal interactions may be important for the regulation of the phosphorylated neurofilament proteins and other phosphoproteins possibly recognized by the MA RT97.

46.16 INJURY INDUCED SPROUTING INTO THE CAUDATE NUCLEUS, AFTER SOLID TISSUE IMPLANTATION IN MPTP-INDUCED PARKINSONIAN MONKEYS.

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MPTP administered i.v. into primates damages nigrostriatal neurones and causes a parkinsonian syndrome. When given into the internal carotid artery, the ipsilateral nigrostriatal dopaminergic (DA) neurones are destroyed and the animal becomes HP affecting the contralateral extremites. The HP animals circle toward the damaged side; the direction of the circling is reversed after treatment with drugs which stimulate DA receptors. We used these two primate models to quantify objectively motor deficits and their response to treatment and to assess changes in function, histology, and chemistry of the basal ganglia after implantation of DA-containing tissue. The caudate nuclei (CN) were exposed via a transcallosal approach, biopsies were obtained from the CN for assay of DA by HPLC, and the cavities were filled with trypan blue-stained gelfoam. Implantation was performed 1-6 weeks later. The full parkinsonian animal received a unilateral solid rhesus fetal mesencephalic (FM) graft; 4 HP animals received bilateral implants and 1 received a suspension of FM cells. The other 4 HP monkeys received bilateral adrenal medulla allografts. Another group of HP (n=3) animals was implanted with sural nerve autografts bridging the left and right CN. Control HP animals received adrenal cortex (n=2) or fetal cerebellum (n=2); 2 control monkeys were untreated. Before and after implantation, each animal was tested for spontaneous, apomorphine, and amphetamine-induced turning. HP monkeys which received fetal DA tissue recovered from motor deficits whereas animals which received adrenal medulla showed only transient behavioral recovery. The full parkinsonian animal that received a unilateral FM graft recovered to normal motor function with 1 week. One month after surgery this animal consistently turned away from the implant side during spontaneous activity. This increased markedly after amphetamine and was reversed (towards the implant) after apomorphine. Seven months after implantation, TH-immunohistochemistry showed large numbers of TH-positive cell bodies and many fluorescent varicose fibers growing from the implant into the surrounding CN. On the contralateral side, there were many TH-positive fibers that eminated from the ventral striatum towards the site of the surgical cavities in the CN. The density of TH fibers in the n.accumbens were normal bilaterally. The DA cell bodies in the ventral tegmental area (A10, VTA) which innervate the n.accumbens were normal, whereas the substantia nigra cells (A9) were markedly depleted. The HP animals that received nerve graft recovered from apomorphine-induced turning. Six months after the nerve graft there were many catecholamine glyoxyic acid stained fibers within the MPTP treated CN. In MPTP-induced parkinsonian primates, sprouting occurs from DA nerves which probably emanate from the remaining A10 mesolimbic cells. This sprouting may be induced by creation of a cavity in the caudate or implantation of tissue in the caudate.

46.17 **SOME FACTORS AFFECTING THE INFLUENCE OF CORTICAL GRAFTS ON THE BEHAVIOURAL RECOVERY OF RATS WITH MEDIAL FRONTAL CORTEX LESIONS.** B.D.Fantie, B.Reynolds*, D.Di Lullo*, R.Anchan*, and B.Kolb, Psychology Department, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4

We have shown that cortical grafts in rats with medial frontal cortex lesions can produce a greater disruption of behaviour than that caused by the lesion alone (Fantie & Kolb, 1985).

Since this result is not consistent with previous studies (e.g., Labbe, Furl, Mufson, & Stein, 1983), we examined several variables that might resolve the discrepancy including: 1) the size of the lesion, 2) the age of the donor tissue, 3) postoperative recovery time, and 4) the behaviour tested. In addition, we looked at the morphology of the grafts using various techniques, especially Golgi-Cox staining and electron microscopic analysis.

Adult rats were given bilateral medial frontal cortex lesions and 1-2 wks later most received either embryonic (E17 or E19) or perinatal (<24 hr or 48-72 hr after birth) cortical tissue implants. The animals were later tested on a variety of measures including spatial navigation in the Morris water task, spontaneous alternation, running wheel activity, noxious prod burying, grooming, and nail clipping.

The performance of some tasks by rats with very small or very large lesions was better if they had received grafts. Generally, rats with grafts tested one month after implantation were impaired. If, however, testing occurred within the first postoperative week, rats with grafts showed a modest improvement although this improvement was less than that shown by rats with similar lesions and no grafts after at least one month recovery from surgery. Age of the donor tissue appeared to be important since tissue taken at E19 attenuated the behavioral deficits whereas tissue taken at other ages potentiated it. Overall, the grafts produced greater behavioural impairments.

Anatomically, the Golgi studies showed that graft tissue was organized differently than normal tissue. The dendrites of pyramidal cells failed to orient in any consistent direction, often appearing similar to the dendrites of stellate cells. Dendrites and axons were never observed to invade the host. EM analysis showed no physical barrier between host and graft tissue. Preliminary examination suggests a higher density of synapses in the graft compared to the surrounding host tissue.

Our findings lead us to be conclude that the implanted tissue probably has a dual effect with the components influencing recovery in opposite directions. The net behavioural result of these opposing events is determined by factors such as lesion size, the type of tissue implanted, the behaviour tested, and the nature of the relationship between the structures involved.

46.18 MOUSE NIGRAL TRANSPLANTS INTO THE BRAINS OF DOPAMINE-DEPLETED NEONATAL RATS. R.K. Carder*, A.M. Snyder-Keller, R. D. Lund, (SPON: M. Ontell), Dept. of Neurobiology, Anatomy and Cell Science and Center for Neuroscience, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261

When embryonic CNS tissue from CD-1 mice is transplanted into the brain of neonatal Sprague-Dawley rats, the transplant frequently survives for long periods, sending projections to the host brain. However, the xenograft is still susceptible to rejection by the host immune system. Using a mouse skin graft to induce an immune response, one can obtain a gradual rejection of the neural xenograft. This approach offers a useful tool for investigating the function of nigral transplants placed into the striatum of dopamine (DA)-depleted neonatal rats.

Bilateral injections of 6-hydroxydopamine were administered to rat pups 2-3 days postnatally. Cell suspensions of E13 mouse substantia nigra were then placed unilaterally into the rostral striatum 5-6 days postnatally. These animals were tested for amphetamine-induced (2.0 mg/kg, i.p.) and stress-induced (tail pinch) turning at approximately one month of age. In response to both types of stimuli, animals made tight contraversive turns (1-40 turns per minute). A group of animals was perfused and prepared for histological examination at this time. Transplants and their projections were identified using tyrosine hydroxylase immunocytochemistry and antibodies specific for mouse neural tissue. The transplants appeared viable and well-integrated with the host brain. After the initial behavioral tests, a second group of animals received a mouse skin graft on their flank. These animals were then retested at various post-grafting intervals. The rotation behaviors were completely abolished with longer survival periods (13 days post-grafting). Histological examination of these animals revealed no evidence of viable transplanted neurons.

These results show that a temporal coincidence exists between the immunological rejection of the xenograft and the disappearance of these DA-mediated behaviors, thus emphasizing the importance of the xenograft in eliciting the behavioral response.

Supported by NIH grants EY05283 and NS19608.

46.19 ANATOMICAL CORRELATES OF THE DEVELOPMENT OF AMPHETAMINE- AND STRESS-INDUCED TURNING IN DOPAMINE-DEPLETED RAT PUPS WITH UNILATERAL NIGRAL TRANSPLANTS. A.M. Snyder-Keller, R.K. Carder, M.J. Zigmond, and R.D. Lund. Dept. of Neurobiology, Anatomy and Cell Science, Dept. of Behavioral Neuroscience, and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh PA

We have previously demonstrated that embryonic mesencephalic tissue produces an extensive innervation of striatum by dopamine (DA)-containing fibers when transplanted into the striatum of infant rats pretreated with 6-hydroxydopamine (6-HDA). Furthermore, we have reported behavioral evidence suggesting that these terminals can respond to both pharmacological and environmental challenges (Carder et al., Dev. Brain Res., 1987). In the present experiments we have examined the anatomical basis for such behavioral responses. Rats were given intraventricular injections of 6-HDA (110 ug) at 3 days of age and 3 days later given unilateral striatal injections of a cell suspension consisting of ventral mesencephalon from 13 day rat embryos. At intervals thereafter, rats were challenged with amphetamine (2 mg/kg, i.p.) or the stress of a brief tail pinch. Animals were found to turn away from the transplanted side in response to amphetamine by 15 days post-transplantation at a rate of 5-55 turns per minute. In cases where animals were retested at a later time, a higher rate of turning was observed. Contralateral turning also was observed in response to stress, but this turning did not occur until 25 days post-transplantation. An immunohistochemical analysis of DA fiber outgrowth was carried out by visualizing terminals containing tyrosine hydroxylase. At 15 days post-transplantation a correlation was observed between the extent of outgrowth of DA fibers coming from the transplant and the number of amphetamine-induced turns. Fibers grew primarily into the medial striatum, even when the transplant was located centrally, and the innervation frequently assumed a patchy appearance. At 25 days post-transplantation the DA innervation still was varied in its extent and density, and often was no greater than that seen in animals killed at 15 days. The hyperinnervation of serotonergic projections from raphe that occurs after neonatal 6-HDA treatment (Snyder et al., J. Comp. Neurol., 1986) was unaffected by the transplant. No obvious difference in the extent or topography of DA innervation was noted between those animals that were turning at high rates to both stress and amphetamine and those turning less well to stress than to amphetamine. These observations suggest that something in addition to the dopaminergic outgrowth underlies the development of the behavioral response to stress. (Supported in part by USPHS grants NS19608 and EY05283.)

NEURAL PLASTICITY IN ADULT ANIMALS: SPINAL CORD

47.1 MORPHOLOGICAL STUDIES OF THE INTRAMEDULLARY AXON COLLATERAL SYSTEMS OF AXOTOMIZED α-MOTONEURONS. L. Havton* and J-O. Kellerth* (SPON: G.Grant). Dept. of Anatomy, Univ. of Umeå, S-901 87 Umeå, Sweden.

Intact axon collaterals have been suggested to exert a general protection against retrograde degeneration in axotomized neurons. However, little is known about the reactions of these axon collaterals in the axotomized cells. Therefore, the aim of this investigation was to study the effects of peripheral motor nerve transection on the intramedullary axon collateral systems of axotomized α-motoneurons in the cat spinal cord.

The medial gastrocnemius (MG) nerve was transected and prevented from reinnervation. Three, six and twelve weeks later axotomized MG α-motoneurons were labeled intracellularly with horseradish peroxidase (HRP) through microelectrodes. Intravascular perfusion and processing of the spinal cord tissue permitted subsequent morphological analysis of the HRP-labeled cells.

Compared with normal gastrocnemius α-motoneurons (Cullheim and Kellerth, 1978) there was a successive reduction of the number of intramedullary axon collateral trees in the axotomized MG α-motoneurons. Twelve weeks postoperatively approximately 40% of the axon collateral trees had been eliminated ($p<0.02$). The size and projection areas of the remaining axon collateral systems in the axotomized cells appeared unaffected by the injury. Ultrastructurally, however, some terminals of the latter systems contained dense-cored spheroid vesicles or amorphous material instead of the clear spheroid synaptic vesicles found in the motoraxon collateral boutons of normal unoperated cats (Lagerbäck et. al. 1981).

We conclude that following transection of a peripheral motor nerve the axotomized α-motoneurons may exhibit a partial loss of axon collateral systems together with ultrastructural changes in the terminals of the remaining axon collateral trees.

This work was supported by grants from the Swedish Medical Research Council (Project 02886).

47.2 COLLATERAL SPROUTING OF SAPHENOUS AFFERENTS IN THE ADULT RAT DORSAL HORN FOLLOWING PRONASE INJECTION OF THE SCIATIC NERVE. S.E. Kapadia* C.M. Kocol* and C.C. LaMotte. (Spon E. Manuelidis) Sec. of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510

A past study (Fitzgerald, JCN 240:407) has reported sprouting of saphenous nerve terminals in the rat spinal cord following early postnatal sciatic nerve section in the rat; however there was a failure to sprout if the lesion was made after day 6. Similarly, thigh nerves failed to sprout collaterals in the dorsal horn following section of sciatic and saphenous nerves in the adult rat (Seltzer and Devor, Br. Res. 306:31). One explanation may be that while peripheral nerve section induces severe ganglion cell death and central terminal atrophy in the neonate (Bondok & Sansone, Exp Neurol. 86:322; Yip et al., J. Neurosci. 4:2986), in older animals more ganglion cells may survive (Aldskogius and Arvidsson, J. Neurocytol. 7:229; Sugimoto and Gobel, Br. Res. 248:377) and fewer primary afferent terminals degenerate; hence the stimuli for sprouting would be reduced. In order to determine if a profound loss of sciatic afferents can produce collateral sprouting in the adult rat, we injected Pronase, which produces death of ganglion cells and degeneration of their central terminal fields (LaMotte and Kapadia, JCN in press), into the sciatic nerve of adult rats, and examined the terminal fields of the saphenous with HRP labelling.

Adult Sprague Dawley rats were anesthetized with Nembutal. Then a unilateral injection of Pronase (Calbiochem, San Diego, CA) was injected into the sciatic nerve. After 4 months, the saphenous nerves on both the lesioned and control side were cut and the proximal end dipped in a mixture of HRP (7.5 mg) and WGA-HRP (.5mg) in PO4 buffer. Two days later the animals were perfused, and vibratome sections of L2-L6 reacted using the TMB technique.

On the control side of each animal, HRP/WGA-HRP labelled terminals followed the map of normal distribution of the saphenous in L2-L3. The saphenous terminal field on the lesioned side was identical to the control side at L2; however, beginning at the L2-L3 border, the saphenous terminal field of the lesioned side expanded laterally and medially into sciatic areas. The expansion increased to a maximum in caudal L3 and tapered to a narrow area in rostral L4; no label was found beyond mid L4.

The results indicate the adult rat does have the capacity for dorsal root afferent collateral sprouting when the appropriate lesion is made. The stimulus needed to induce sprouting may require a critical balance between too little deafferentation (nerve section) and too much deafferentation [rhizotomy, which may induce post-synaptic dendritic atrophy and gliosis (Kapadia and LaMotte, JCN in press)]. (Supported by NIH grant NS10174)

47.3 ANDROGENIC ORGANIZATION OF SYNAPTIC INPUT TO SPINAL MOTONEURONS IN ADULT RATS. A. Matsumoto*, P.E. Micevych and A.P. Arnold (SUM: C.H. Sawyer), Depts. of Anatomy and Psychology, Brain Research Institute, University of California, Los Angeles, CA 90024.

The motoneurons in the spinal nucleus of the bulbocavernosus (SNB) in male rats innervate the muscles bulbocavernosus and levator ani which attach to the penis. Both the SNB motoneurons and their target muscles contain androgen receptors. Castration of adult male rats results in a significant decrease in both soma size and dendritic length of the SNB motoneurons, and androgen treatment reverses this effect. This androgenic regulation of soma and dendritic membrane area implies a concomitant regulation of synaptic inputs to these membranes. We studied androgenic influences on synaptic inputs to SNB motoneurons using quantitative electron microscopic analysis. Adult male rats (Sprague-Dawley) were castrated and implanted subcutaneously with Silastic capsules containing testosterone or nothing. Sham-castrated males served as controls. There were 5 animals per group. Four weeks following castration, 1 ul of 0.2 % cholera toxin-horseradish peroxidase (CT-HRP) was injected bilaterally into the bulbocavernosus muscle and animals were sacrificed 2 days later. The spinal cords containing the SNB were sectioned at 50 um, processed with a modified tetramethylbenzidine (TMB) method for visualization of retrogradely transported CT-HRP and examined ultrastructurally. Montages were made of 141 TMB-labeled SNB motoneurons. The synaptic covering of SNB cells was analyzed by measuring the percentage of soma and proximal dendritic membrane contacted by synapses with clear membrane specializations, by synapses without specializations, and by other kinds of contacts such as soma-soma, soma-dendrite or dendrite-dendrite. In general, castration profoundly reduced the percentage of soma and dendritic membranes covered by synapses, and androgen prevented this effect. For example, all synaptic types covered 46.7 $\pm$ 1.3 % (mean $\pm$ SE) of soma membrane in sham castrates, 49.7 $\pm$ 2.3 % in castrates given testosterone, and 17.7 $\pm$ 1.0 % in castrates given blank capsules ($p<.0001$, ANOVA). Proximal dendritic synapses were similarly influenced by androgen. Castration also reduced the size and number of synapses per unit length of soma and dendritic membrane. For example, the average length of somatic synapses with membrane specializations was 1.76 $\pm$ 0.03 um in sham castrates, 1.43 $\pm$ 0.04 um in castrates given blank implants, 1.78 $\pm$ 0.03 um in castrates given testosterone ($p<.0001$). The average frequency of synapses was 0.229 $\pm$ 0.011 per um of soma membrane, 0.098 $\pm$ 0.005, and 0.253 $\pm$ 0.012 for the same groups ($p<.0001$). These results indicate that androgen is critical for maintaining the organization of synaptic input to these spinal motoneurons in adult male rats. (Supported by NIH grants NS23468 and HD15021).

47.4 SPINAL LOCALIZATION OF A PRIMATE MEMORY SUBSTRATE: OPERANTLY CONDITIONED H-REFLEX ASYMMETRY SURVIVES CORD TRANSECTION. J.R. Wolpaw and C.L. Lee. Wadsworth Labs, NYS Dept Hlth, Albany, NY 12201; and Depts Neurol & Anat, Albany Med Coll, Albany, NY 12208.

Study of primate memory substrates requires an experimental model in which the persistent CNS alterations are accessible to study. Our recent work (J Neurophysiol 50:1296-1319, 1983 & 57:443-459, 1987; Cell Molec Neurobiol 5:147-165, 1985) shows that the wholly spinal, largely monosynaptic, spinal stretch reflex and its electrical analog, the H-reflex, can be operantly conditioned: monkeys can gradually change reflex amplitude if reward depends on amplitude.

To learn whether H-reflex conditioning produces persistent spinal alterations, we measured triceps surae (TS) H-reflexes in previously conditioned (HR↑ or HR↓) monkeys before and after thoracic (T9-10) cord transection. In each monkey, we compared the conditioned leg to the unconditioned leg to determine whether reflex asymmetry persisted after removal of supraspinal control by transection.

Twelve previously conditioned monkeys (Macaca nemestrina) were deeply anesthetized throughout study and sacrificed by overdose. Recording cuffs were placed around TS nerve branches in both legs. L6-S1 dorsal roots (DR) were cut, and the proximal DR bundle on each side was placed in a stimulation (stim) cuff. A stim cuff was also placed around the intact L6-S1 ventral roots (VR) of both sides. The reflex response to 0.5 Hz supramaximal DR stim was measured in terms of the response to supramaximal VR stim which was the maximum possible response. Data from both legs were collected repeatedly before and for up to 72 h after transection.

In 7 HR↑ monkeys prior to transection, the response to DR stim in the HR↑ leg averaged 227% ($\pm$64%SE) of that in the other leg. Conversely, in 5 HR↓ monkeys prior to transection, the response to DR stim in the HR↓ leg averaged 67% ($\pm$10%SE) of that in the other leg. These asymmetries were still present after transection and persisted through the three days of study.

The results indicate that H-reflex operant conditioning causes persistent spinal cord alteration, a potentially accessible memory substrate. Current studies are further defining these physiologic data and beginning to seek anatomic correlates
(Supported by NIH 22189 and by United Cerebral Palsy)

47.5 RAPID NEURONAL AND GLIAL CHANGES IN THE PHRENIC NEUROPIL FOLLOWING SPINAL CORD INJURY: QUALITATIVE AND QUANTITATIVE RESULTS. H. G. Goshgarian and J. A. Rafols. Dept. of Anatomy and Cell Biology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

It is generally believed that injury induced morphological changes of the spinal cord take at least a few days to develop, especially in regions which are far removed from the initial site of injury. We have, however, observed, at the C4 segment of the spinal cord, specific morphological alterations of the normal ultrastructure of the rat phrenic neuropil occurring within 4 hours after spinal cord hemisection at C1. The phrenic neuropil is defined as the area of the spinal cord within the phrenic nucleus and immediately adjacent to phrenic cell body profiles. Phrenic neurons and their processes were identified at EM levels by retrograde HRP labeling. Several morphological features of the phrenic neuropil in uninjured animals and at 4 hours, 1, 2 and 4 days after injury were qualitatively analyzed and then quantitated with a computerized Bioquant morphometric system. Data was obtained from 100 micrographs of the phrenic neuropil taken from 4 animals in each group. Our results demonstrate: 1) astroglial process retraction away from their normal position in between adjacent phrenic dendritic profiles, 2) increases in direct membrane apposition among phrenic dendrites, and 3) an increase in the number of multiple synapses contacting phrenic motoneuron profiles. Multiple synapses are defined as contacts established between a terminal and more than one postsynaptic profile in the same plane of section. In the uninjured group, 80 $\pm$ 8.9 (S.E.M) multiple synapses were counted. The number of multiple synapses increased significantly (118 $\pm$ 8.9, $P < .0005$) as early as 4 hours after spinal cord injury and this high number persisted for as long as 4 days after injury (122 $\pm$ 9.3 at 1 day, 121 $\pm$ 10.1 at 2 days, and 117 $\pm$ 11.0 at 4 days). The percentage of phrenic dendrodendritic membrane appositions in the uninjured group was 35.4 $\pm$ 6.0. The increase in dendrite membrane appositions took at least 2 days after injury to reach significant levels (54.6 $\pm$ 8.4 at 2 days and 65.6 $\pm$ 10.0 at 4 days, $P < .005$).

It is possible that the above injury induced neuronal and glial changes may have a functional significance. In previous studies (Exp. Neurol. 66: 547-55, 1979 and 72: 211-25, 1981), we have associated functionally ineffective synapses to a delayed expression of a respiratory reflex in spinal cord injured rats. In this model, the conversion of the ineffective synapses to effective ones results in the functional recovery a portion of the animal's diaphragm which had been paralyzed by spinal cord injury. Evidence exists which suggests that the above neuronal and glial alterations could represent the morphological substrate for the unmasking of functionally ineffective synapses in our spinal cord injury model. Supported by U.S. Public Health Service Grant NS-14705.

47.6 EFFECTS OF SPINAL CORD TRANSECTION ON ONUF'S NUCLEUS MOTONEURONS OF CATS. J.C. Bresnahan, M.G. Leedy, M.S. Beattie. Depts. of Anatomy and Surgery, The Ohio State University, Columbus, OH 43210.

Urination and defecation reflexes are affected by spinal cord transection. Such changes may reflect alterations of the neural circuitry occurring within the sacral spinal cord (Thor,K. et al., Development and Plasticity of the Mammalian Spinal Cord, ed. M.E.Goldberger, A. Gorio, M. Murray, 1986). The present study sought to determine whether spinal cord transection results in changes in the organization of presynaptic elements to those motoneurons, located within Onuf's nucleus of the S1 spinal cord, which innervate the sphincters, Three groups of female cats were used: spinally intact, acute spinal transection (survival time of 4 days), and long term spinal transection (survival time of 10-11 weeks). Motoneurons in Onuf's nucleus were labelled by applying HRP to the pudendal nerve. Following a survival time of four days, all animals were perfused and the S1 spinal cord segments were prepared for electron microscopy. Onuf's nuclei from four animals per group were chosen for ultrastructural examination in which micrographs were obtained through the perimeter of five labelled cells per animal. From the resulting montages, the numbers and types of terminals contacting each labelled neuron, as well as the percentage of the perimeter contacted by terminals, with or without active zones, were determined. We found a tendency towards a decrease in the percentage of the motoneuron perimeter directly apposed by terminals for both spinal cord transection groups, compared to the spinally intact animals. When the percent of the neuronal perimeter contacted by synaptic active zones was examined, this decrease was more apparent for the short term transection animals, with some recovery seen for the long term animals. No changes were seen when the number of terminals per 100 um of perimeter were compared, although the long term transection group tended to have a larger number of terminals containing dense cored vesicles than did the other two groups. We conclude that changes in synaptic input to Onuf's nucleus motoneurons do occur following spinal cord transection, including changes in the amount of synaptic interaction, and, in the case of the long term transection group, shifts in terminal types. Such changes may reflect reorganization of the reflexes involved in urination and defecation following spinal cord transection, and may be indicative of both an initial decrease in synaptic efficacy, and in later reorganization of the reflex pathway. (Supported by NIH grants NS-10165 and NS-07747)

47.7 TESTOSTERONE DEPENDENT ULTRASTRUCTURAL ALTERATIONS IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS (SNB) IN MALE RATS. M.G. Leedy, J.C. Bresnahan and M.S. Beattie. Dept. Anat., Div. Neurosurg., and Neurosci. Res. Program, Ohio State Univ., Columbus, OH 43210.

The size of the motoneurons innervating the bulbocavernosus muscle (BC) in adult male rats have been shown to be sensitive to tesosterone (T) levels (e.g. Breedlove and Arnold, Science, 1981). While hormone dependent ultrastructural changes have been reported in hypothalamic neurons, no such analysis has been performed in the spinal cord. In the current study, 12 male rats were castrated and randomly assigned to the following groups; No T replacement; 48 hr T replacement initiated 6 weeks after castration; Long term T replacement initiated immediately after castration. SNB motoneurons were labelled by injection of HRP into the BC muscle. L6 spinal cords segments were processed for sequential light and electron microscopy (EM).

The SNB is characterized by dense bundles of rostrocaudally oriented dendrites. EM montages of the perimeter of 5 labelled neurons per animal indicated that approximately 4.6% of the somatic and attached proximal dendritic surfaces of the labelled cells in the nucleus was apposed by other somatic and dendritic elements, and frequently these appositions exhibited puncta adherentia (0.5% of the neuronal surface). Overall, 67% of the terminals in apposition to the labelled motoneurons contained round vesicles, 32% contained pleomorphic vesicles, and less than 1% contained flat vesicles; 23% of all terminals contained at least one dense cored vesicle.

Light microscopic analysis of labelled neurons in the SNB indicated that somatic ($p<0.05$) and nuclear size ($p<0.001$) decrease following castration without T replacement, with no reversal seen in the 48 hr group. Differences in nucleolar size were also seen (Long term T= 22um2, 48 hr T=20um2, and No T= 21um2, $p<.005$). The proportion of the somatic and proximal dendritic surface of the labelled motoneurons that was contacted by synaptic terminals was significantly increased by T replacement, and this effect was observed within 48 hours ($p<.005$). Similar results were found for the number of terminals per 100 um of membrane perimeter. An inverse response was observed in glial apposition ($p<.005$).

These results suggest that testosterone can not only affect neuronal morphology but also synaptic connections in the spinal cord. (Supported by NS-10165 and NS-07747)

47.8 CLARKE'S NUCLEUS IN THE ADULT RAT: CELL SURVIVAL AFTER AXOTOMY AND ITS RELATIONSHIP WITH RETROGRADE TRANSPORT. E. LOWENGER*, M.E. GOLDBERGER, and M. MURRAY. (SPON: L.L. Ross) Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129

Axotomy of intrinsic CNS neurons results in structural and metabolic changes culminating either in cell death, or survival without full functional recovery. The factors determining survival are uncertain. We have therefore studied the response of neurons within Clarke's nucleus (CN) in the rat to axotomy. CN is a continuous cell column within lamina V of Rexed in thoracic and upper lumbar spinal cord segments, their axons forming the DSCT within the ipsilateral lateral funiculus.

In adult female Long Evans rats (250-350g body wt) CN neurons from segments T12-L1 were studied. Cell morphology, number and size were studied using cresyl violet staining of 15μm paraffin sections. Gelfoam soaked in 40% HRP was placed bilaterally into the lateral funiculi at T5 48hrs prior to sacrifice to investigate retrograde transport. The tissue was then frozen-sectioned at 35μm and processed for TMB histochemistry.

In the normal animal CN from T12-L1 is bilaterally asymmetrical with 5.043±0.203 cells/section ($\bar{x}$±s.d.) on the right side and 4.432±0.543 cells on the left, but with equivalent cell sizes. About 70% of these cells label retrogradely with HRP symmetrically on both sides, and are thus projection neurons, indicating that about 30% of CN neurons do not contribute axons to the DSCT at T5 and may be interneurons. In experimental animals the right DSCT was lesioned by lateral funiculotomy at T1. One week after axotomy ipsilateral cell loss is about 9%. The ability to transport HRP is impaired with both number and intensity of labeled cells being greatly reduced, and many cells are chromatolytic. By two weeks, cell loss has increased to about 34%. However, the ability of surviving cells to transport HRP has recovered and the intensity of HRP labeling has returned. Cell survival was not limited to a specific cell size class within CN, and the average nuclear diameter and somal area of the axotomized neurons was comparable to those of controls.

CN neurons respond to axotomy initially with a decrease in retrograde transport, followed either by cell death or by a recovery of the transport. Liu ('55) described a similar dichotomy in cat CN, where axotomy led to two distinct forms of chromatolysis, only one of which led to cell death. This would indicate that the metabolic response to axotomy within CN is not uniform, and that CN projection cell survival is associated with a functional retrograde transport system.

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47.9 PLASTICITY OF INTRINSIC AND EXTRINSIC SYSTEMS AFTER RHIZOTOMY OF RAT SPINAL CORD. S.D. Wang*, T. Eckenrode*, M.E. Goldberger and M. Murray. Dept. of Anatomy, The Medical College of Pennsylvania, Phila., PA 19129.

Previous studies of intrinsic (Tessler et al., Brain Res. 191:459, '84) and extrinsic (Goldberger & Paige, Soc. Neurosci. Abstr. 12:512, '86) systems in cat spinal cord had shown anatomical plasticity in response to complete lumbosacral rhizotomy. In the present study, we used the same paradigm to examine the plasticity of intrinsic and extrinsic systems in rat spinal cord in which previous studies had suggested a lack of plasticity. Thiamine monophosphatase (TMP), substance P (SP) and serotonin (5HT) were examined after acute and chronic unilateral dorsal root deafferentation. TMP was found across lamina II_i of the dorsal horn and was bilaterally symmetrical in normal animals. Ipsilateral to rhizotomy, TMP disappeared completely by 4 days and did not recover by 8 months. The contralateral dorsal horn was unaffected. An image analysis system was used to measure density of SP and 5HT immunochemical reaction product in dorsal horn of rats. After deafferentation in adult rats, SP decreased 22.3% on the experimental side in lamina I and II_o by 4 days and the density of SP staining had decreased 72.8% by 10 days. After six weeks postoperative, SP staining had recovered to 64.5% of normal values. Therefore the density of SP doubles between 10 days and six weeks postoperative. After deafferentation in 5 day-old rats, SP decreased and partially recovered on the experimental side more rapidly than in adult rats. In contrast to SP, 5HT did not decrease after deafferentation. Rather, we found a several-fold increase in 5HT staining at 4 days, 10 days and after six weeks postoperative in laminae I and II. Therefore, these three markers exhibited different responses to rhizotomy. TMP, an extrinsic system marker disappeared and did not recover. SP, present in extrinsic and intrinsic systems, exhibited a decrease and then a partial recovery. 5HT, an extrinsic system did not decrease but increased in response to partial denervation of a shared terminal field. This plasticity, comparable to that seen in cats, may be a result of sprouting terminals by the undamaged intrinsic and extrinsic systems or may represent an increased production of their markers.

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47.10 MYELINATED FIBERS CONTRIBUTE TO DORSAL ROOT SPROUTING AFTER PARTIAL DEAFFERENTATION IN RATS. Dean POLISTINA*, Marion MURRAY and Michael E. GOLDBERGER. (SPON. T.J. Cunningham) Department of Anatomy, The Medical College of Pennsylvania.

Evidence for sprouting, following partial deafferentation, was obtained by comparing chronic and acute sides of the spinal cord (intra-animal) in cat. Inter-animal comparisons in the rat however, have provided no evidence for sprouting. The present study uses an intra-animal design in rats to demonstrate sprouting of the dorsal root terminals. Cholera-toxin HRP (CT-HRP) was used to see if myelinated fibers contribute to the dorsal root's response. For transganlionic labeling CT-HRP was injected into the sciatic nerve in rats with lumbosacral rhizotomies sparing L5. Controls included: 1.unoperated controls and 2.operated controls a) unilateral acute and b) bilateral acute rhizotomies. Experimental animals had a chronically spared root (>30 days) on one side and an acutely spared root on the other. In the unilateral controls labeling was ipsilateral except in S1 where some projections crossed the midline. There was no labeling in lamina II. Labeling in lamina I shifted from the lateral 2/3 in L6 to the medial 2/3 in L4 and was nearly absent in L3. From rostral L6 to rostral L4 the projection to laminae III and IV occupied the width of the dorsal horn, except the lateral 1/5. Operated controls unlike unoperated controls showed alternating bands of labeling density most noticeable in the more rostral projections to laminae III and IV. The absence of a banding pattern in these laminae in unoperated controls is presumably due to the overlapping terminal fields of the L4 and L6 dorsal roots. Projections to laminae V-VII were seen from S1 to mid L2 in the acute unilateral operated control, whereas in the unoperated controls labeling in these laminae was more dense and extended further caudally and rostrally. In rostral L2 only Clarke's nucleus and the fasciculus gracilis were labeled in either control preparation. Projections of the L5 dorsal root were variable; some animals had projections as caudal as S2, while some had projections to Clarke's nucleus as high as the T9-T8 border. These variations were always bilaterally symmetrical. In contrast, in animals with a chronic and acute spared root labeling was assymetric; the chronic side showed greater labeling than the acute side in laminae III-VII, Clarke's nucleus, and nucleus gracilis. Using an image analysis system, an increased density was shown quantitatively in lamina III and Clarke's nucleus. Cell measurements in the DRG showed that large cells were among the population labeled. The number of dorsal root ganglion cells labeled on the chronic and acute L5 ganglia was similar (± 7%) indicating that the increased projection on the chronic side was not due to diferences in CT-HRP application. Results suggest that the increased labeling may be due to collateral sprouting of the spared root or metabolic changes in the chronically isolated L5 DRG.

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48.1 REINITIATION OF OUTGROWTH FROM DORMANT NEURONS IN CELL CULTURE. D.S. Grega and S.B. Kater. Program in Neuronal Growth and Development and Dept. of Anatomy, Colorado State Univ., Ft. Collins, CO 80523 and Boehringer Mannheim Diagnostics, Indianapolis, IN 46250.

Individual adult Helisoma neurons placed in culture, grow for several days after which time, they attain a stable, non-changing morphology. Growing growth cones are flattened and phase-dark, with very active filapodial and lamellapodial processes. With cessation of outgrowth, the growth cones are distinctly different; they become rounded and phase-bright and show no motility. Once attaining stable morphology, neurons remain viable for at least 7 to 10 days. This stable morphological state previously has been considered a non-reversible, terminal phase for growing neurons.

We report here that these morphologically stable neurons are in fact in a **reversible, dormant state.** Neurons from the pedal (P5) and buccal (B5 & B19) ganglia were grown in cell culture until they reached a stable morphology. At that time Helisoma serum was added to the culture dishes to a final concentration of 10%. Within hours,the majority of the neurons responded with a change in their stable state growth cone morphology from rounded, phase-bright neurite ends to flattened, phase-dark endings with active filapodial and lamellapodial processes. By 24 hours after the addition of serum, 70% of the P5's (n =33), 48% of the B5's (n = 29) and 39% of the B19's (n = 23) had grown, adding more than a criterion level of 50 μm in total neurite length. In fact many P5's increased more than 40 to 150% of their initial total neurite length (increased 800 to 7,000 μm).

Although the predominant neuroplastic change in response to serum is the initiation of outgrowth, there were neurons that responded with a net retraction of neuritic processes. In response to serum addition, 70% of P5 neurons (n=33) increased their number of neurites and the total neurite length, one did not change and the remaining 30% exhibited a net retraction of neurites. Thus, serum can significantly alter existing neuronal architecture.

The effect of outgrowth-modifying factors is clearly dependent upon the timing of their appearance.Serum can reverse a transmitter effect: B19 neurons whose growth was prematurely stopped by the addition of serotonin (40μM) exhibit the reinitiation of outgrowth in response to serum. In fact, serum itself is not uniquely able to reevoke outgrowth. Dopamine,which inhibits particular growing neurons, can reinitiate outgrowth from particular dormant neurons. Dopamine (100 μM) mimics the effect of serum on P5's with an increase in neurite length. B19 and B5 were unaffected by dopamine.

From these results our view of neuronal architecture of Helisoma neurons in cell culture is significantly modified. Given the proper stimulus even previously "stable" neurons can reinitate outgrowth,therefore demonstrating greater potential for remodeling than previously thought.Supported by N.I.H., N.S. 18819-04 to S.B.K.

48.2 CYTOARCHITECTURE OF THE NEUROMUSCULAR JUNCTION OF TENOTOMIZED-REPAIRED MUSCLE FOLLOWING IMMOBILIZATION. B.R. Pachter and N. Spielholz* Dept. of Rehab. Med., New York Univ. Med. Ctr., New York, NY 10016.

Rupture of the Achilles tendon is a common sports injury. Conventional surgical treatment involves tendon suture and immobilization using plaster cast with the foot in plantar flexion. Tenotomy as well as limb immobilization are known to have multifold effects on the muscle. Limb immobilization, especially in a shortened position, has been shown to produce denervation-like changes at the neuromuscular junctions which lead to terminal sprouting and an ultrastructural remodelling (Pachter, B.R., and Eberstein, A., J. Neurocytol.,13:1013,1984). The present study examined limb immobilized muscle in female Wistar rats (200-250 g) following tendon-repair. The Achilles tendons were cut and immediately suture repaired. These animals had their hind limbs immobilized in a shortened position by the application of a plaster cast; the knee was extended, the ankle fixed in plantar flexion, thereby shortening the soleus muscle. After two weeks, the soleus muscles were removed whole and prepared for electron microscopic examination. The innervation zone was localized and ultrathin sections were taken. Morphologically, the immobilized soleus muscle fibers appeared atrophic and their mitochondria and fibrillar size reduced. Autophagic vacuoles were observed and contained mitochondrial remnants and granular debris. Concentric membranous bodies were abundant. The majority of endplates of experimental animals contained one or more of these large concentric lamellated bodies juxtaposed to the postjunctional folds. In many endplates, the folds appeared highly-irregular shaped and attenuated. In addition, many of the synaptic clefts contained two or more axonal terminals separated by Schwann cell cytoplasm. Quantitatively, the mean postsynaptic area of junctional folds and clefts per nerve terminal in the tenotomized-repaired, immobilized endplates was significantly larger than those of controls. It would appear that Achilles tenotomy, tendon suture and immobilization in a shortened position results in an altered muscle morphology as well as a disrupted neuromuscular junction. Such damage to the muscle and endplate appear to be factors in the observed decreased strength following such a surgical approach. Supported in part by NIHR Grant G008300071.

48.3 EFFECTS OF DENERVATION AND REINNERVATION ON DENTATE GRANULE CELL FIRING. T.M. Reeves and O. Steward. Dept. of Neuroscience, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

There is extensive evidence that following unilateral entorhinal cortex (EC) lesions, the ipsilateral dentate gyrus is reinnervated through sprouting of surviving afferents. One sprouting system, the crossed temporo-dentate (CTD), originates in the contralateral EC and shares an overlapping terminal field with the lesioned system. The time course of sprouting by the CTD is highly correlated with behavioral recovery after unilateral EC lesions (Reeves and Smith, 1987). However, the actual mechanism that sprouting plays in recovery remains unknown. A sprouting system may contribute to recovery by providing information normally conveyed by the lesioned system. Alternatively, sprouting may elevate the level of excitation in denervated cells, and thus restore processing regardless of information content. The present experiment approached this problem by examining the firing properties of dentate granule neurons before and after EC lesions.

Twenty-two adult male Sprague-Dawley rats (250-300 gm.) were prepared for single-unit electrophysiological recording under chloralose-urethane anesthesia. Twisted-wire stimulating electrodes were lowered into the right EC at 3, 4 and 5mm lateral to midline, with exposed tips at 2, 4 and 6mm below the cortical surface. A recording electrode (micropipette filled with 2M NaCl and 1% HRP, or glass-insulated tungsten) was lowered into the right dentate granule cell layer, and extracellular single-unit activity was amplified and stored on magnetic tape. Granule cells were identified by firing monosynaptically to EC stimulation, and in some cases by subsequent histological verification of HRP uptake. After 30 min. of baseline activity was obtained for each cell, the EC was lesioned by passing current (1 mA for 45 sec) through each wire used for stimulation. The mean spontaneous firing rate prior to the lesions was 6.48/s, but fell to 2.51/s immediately after the lesion. Firing rates remained significantly depressed (range of t values: 2.66-4.07;$p<.05$) even in cells held for up to 8 hr. Nine additional rats were given EC lesions and survived for 2, 4 or 6 days prior to recording. Mean firing rates were significantly depressed at 2 days (0.79/s) and at 4 days (2.3/s), but not at 6 days (4.94/s). Pattern of cell firing, as measure by joint interval histograms and coefficients of variation, was not altered by the lesions.

Following the EC lesions there was a clear recovery of dentate granule cell firing rate, but no such time-dependent change was observed for firing pattern. These results suggest that reinnervation may contribute to recovery by raising excitation in denervated cells, and not by supplying information normally conveyed by the lesioned system.

48.4 NEONATAL TRANSECTION OF THE INFRAORBITAL NERVE RESULTS IN TRIGEMINAL GANGLION CELLS WITH MULTIPLE PERIPHERAL AXONS. R.W. Rhoades, N.L. Chiaia, B.G. Klein, W.E. Renehan and M.F. Jacquin (SPON: S.E. Fish). Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Two different anatomical techniques were employed to demonstrate that neonatal transection of the infraorbital (IO) nerve in rats results in trigeminal (V) ganglion cells with multiple peripheral axons. In 19 neonatally nerve damaged, adult rats, horseradish peroxidase (HRP) was applied directly to the IO nerve proximally to the point of the initial lesion. In all animals, this procedure resulted in labelled V ganglion cells, and in 14, axons in mandibular V branches were also labelled. Such labelling was interpreted as an indication that some ganglion cells labelled by application of HRP to the IO nerve also sent axon collaterals into at least one other V branch. This phenomenon was never observed in identically treated, normal adults rats (N=4). In 28 additional, neonatally nerve damaged animals, multiple labelling techniques were used to verify the reorganization suggested by the HRP tracing. Here, diamidino yellow (DY) was injected directly into the regenerate IO nerve and true blue (TB) was deposited subcutaneously into non-IO peripheral territory. These experiments invariably produced a small number (46-401) of double labelled cells in the ophthalmic-maxillary portion of the ipsilateral V ganglion. Identical experiments in 9 normal adult rats never produced more than 6 double labelled cells per ganglion. Additional control experiments demonstrated further that this labelling could not be completely accounted for by peripheral sprouting of undamaged V primary afferents (see Chiaia, N.L. et al., this meeting).

Two additional series of sequential double labelling experiments showed further that multiply projecting V ganglion cells probably arose in two ways: 1. Development of non-IO projections by ganglion cells that contributed axons to the IO nerve at the time of the lesion, and 2. Elaboration of IO axon branches by primary afferents that had only non-IO projections at the time of the lesion.

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48.5 PERIPHERAL SPROUTING OF UNDAMAGED TRIGEMINAL AXONS AFTER NEONATAL INFRAORBITAL NERVE LESIONS IN THE RAT. H.L. Enfiejian*, N.L. Chiaia and R.W. Rhoades (SPON: F.J. Wilson). Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

We have used retrograde axonal tracing techniques to determine whether undamaged trigeminal (V) primary afferents sprout into denervated skin after transection of the infraorbital (IO) nerve in either newborn or adult rats. The IO nerve was transected behind the whisker pad either within 12 hours of birth or at about 60 days of age. After a 60 day survival period, the IO nerve was retransected and and 2 mm segment was removed. The second transection was carried out to insure that any labelled ganglion cell transported tracer from the periphery via a non-IO, V axon. Horseradish peroxidase conjugated to wheatgerm agglutinin (WGA-HRP) or diamidino yellow (DY) was then injected into the central portion of the ipsilateral whisker pad. Accomplishment of this experiment in otherwise normal, adult rats, normal neonates, or animals that sustained adult IO nerve transections produced no labelled cells in either the ipsilateral or contralateral V ganglia. Thus, the IO nerve provides the sole V innervaton of the whisker pad in both newborn and adult rats and IO nerve transection in adulthood does not result in peripheral V sprouting that is detectable by these methods.

Retrograde tracing in rats that sustained neonatal nerve transections invariably produced labelled cells in both the ipsilateral and contralateral V ganglia. Labelled cells on the contralateral side were restricted to the medial portion of the ophthalmic-maxillary part of the ganglion while those on the ipsilateral side were located more laterally. A few of these cells were in the mandibular part of the ganglion. In WGA-HRP labelled material, the average number of labelled cells in the contralateral ganglion was 19.5 $\pm$ 17.7 and their average diameter was 23.1 $\pm$ 4.0 um. The average number of labelled cells on the ipsilateral side was 84.5 $\pm$ 7.8 and their average diameter was 27.0 $\pm$ 6.6 um. The last value is somewhat greater than that for V ganglion cells labelled after injection of WGA-HRP into the whisker pad of normal rats (21.9 $\pm$ 6.1 um).

These data demonstrate that neonatal, but not adult, transection of the IO nerve results in both ipsilateral and contralateral peripheral sprouting of V axons. They indicate further that the primary afferents that sprout on the ipsilateral side arise from large V ganglion cells.

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48.6 ENTORHINAL LESIONS, SPROUTING AND BEHAVIORAL RECOVERY. W. L. Isaac*, A. J. Nonneman & S. W. Scheff. University of Kentucky, Lexington, KY 40506.

Lesions resulting in partial loss of neuronal inputs signal undamaged residual inputs to sprout and replace lost synaptic connections. Coupled with the initial denervation is a marked change in specific behaviors which appears to recover with a time course parallel to the axonal growth. Glucocorticoid treatment suppresses sprouting of some afferents in the hippocampus following partial lesions. Glucocorticoid suppression of sprouting was paired with behavioral recovery on a DRL-20 task to explore this relationship.

Sprague-Dawley rats (2 mos) learned a DRL-20 task prior to surgery. The animals were assigned to one of 4 surgical groups: 1) unilateral entorhinal cortex lesion (UE), 2) bilateral entorhinal cortex lesion (BE), 3) unilateral occipital lesion (UO), and 4) bilateral occipital lesion (BO). Half of each surgical group received daily subcutaneous injections of hydrocortisone (H) (2 mg/kg) or vehicle beginning with surgery and continuing throughout all DRL-20 testing. Subjects further had either a 3 or 30 day interval before retention testing. Number of bar presses, reinforcements obtained and efficiency of performance were evaluated. Animals with UE lesions and a 3 day recovery interval (UE+3) received a lesion of the contralateral E after completion of retention testing. Bilateral operates with a 3 day recovery interval (BE+3) were given sham operations following retention testing. Both of these groups were allowed 3 days recovery following the second operation, followed by a second retention test. Thirty day recovery rats were subjected to a transection of the dorsal psalterium, which eliminates the crossed entorhinal-hippocampus projection. The occipital lesions failed to disrupt retention testing. UE+3 show an initial deficit in retention testing but return to preoperative levels. UE+H+3 subjects increased their bar pressing throughout testing, with no change in reinforcements obtained. BE+3 subjects showed increases in bar pressing following the lesion, with the vehicle treated subjects returning to control levels while H subjects failed to decrease responding. UE+30 animals failed to demonstrate any deficit during first retention testing while UE+H+30 subjects were severely impaired throughout testing. BE+30 rats were impaired on all measures regardless of treatment group. The second lesion (UE or dorsal psalterium) did not alter the subjects' response rates regardless of lesion or treatment group. It appears that, while entorhinal cortex is not mediating behavioral recovery in this situation, glucocorticoids and experience can alter behavioral recovery in this system.

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48.7 PHORBOL ESTERS ALTER MORPHOLOGY AND N-myc EXPRESSION IN NCB-20 NEUROBLASTOMA. S.A. Berman, R.L. Kinnard,* W. Lu,* Neil Cashman and B.G.W. Arnason. Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637.

Expression of myc oncogenes has been reported in several tumors and cell lines. The amplification of N-myc has been shown to correlate with degree of malignancy in human neuroblastoma (Brodeur et al. Science, 224, 1121, 1984), yet oncogenes, including N-myc, have been found to show expression at specific times in normal neurodevelopment (Zimmerman et al. Nature, 319, 780, 1986). Previous work in our laboratory and in laboratories of others has shown correlations between oncogene levels, differentiation, and treatments with agents such as dibutyryl cyclic AMP (db-cAMP) which affect the protein kinase A system. In particular we can demonstrate a correlation between db-cAMP treatment, apparent differentiation, and N-myc mRNA levels in NCB-20 cells, a hybrid cell line made from mouse neuroblastoma and chinese hampster embryonic brain cells. It was of interest, therefore, to extend our studies to examine the effects of stimulating the protein kinase C system with agents such as phorbol myristate acetate (PMA).

When NCB-20 cells are treated with PMA the following morphological changes are apparent: 1) appearance of short, stubby outgrowths quite unlike the slender neurite-like extensions produced with db-cAMP, 2) a rounding of the cell bodies and 3) an increase in the size of the cell bodies. These changes become apparent after 6 hours and are more distinct after 24 hours. Three levels of PMA treatment were studied: 10^{-8}M, 10^{-7}M, and 10^{-6}M.

Levels of N-myc were monitored by hybridizing ^{32}P labelled N-myc cDNA to mRNA in situ, in cells grown upon plastic cover slips. (Lawrence and Singer, Nuc. Acids. Res. 13, 1777, 1985). Stimulation with PMA appeared to produce a three fold increase in N-myc production.

When cells are treated with a combination of 10^{-6}M PMA and 10^{-3}M db-cAMP they appear similar to cells treated with db-cAMP alone. However the db-cAMP treated cells frequently cluster together and form ganglion-like structures whereas the cells treated with both PMA and db-cAMP do not form these organized aggregates. This feature may related to an alteration in surface membrane properties. Cells receiving combined PMA and db-cAMP treatment exhibit N-myc levels not significantly different than cells receiving PMA alone.

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48.8 EFFECTS OF THE CALCIUM IONOPHORE A-23187 ON INJURED CENTRAL NEURONS OF THE LAMPREY. S. Clausse* and M.J. Cohen. Department of Biology, Yale University, New Haven, CT 06511.

In the lamprey, giant reticulospinal neurons sprout following axotomy. Moreover, shortly after axotomy, a large current has been measured entering the cut end of the axon. A large part of this current is due to an influx of calcium. In several in vitro preparation, calcium seems to be involved in regulation of growth cone elongation. The purpose of these experiments was to observe, in vivo, the effects of calcium on sprout elongation in regenerating vertebrate central neurons.

The axons of the giant reticulospinal cells of the larval sea lamprey were severed about 150 µm from their cell bodies. A 1 mm cube of gelfoam, soaked in 0.5-2 µM of the calcium ionophore A-23187, was placed in the fourth ventricle adjacent to the lesion. A-23187 increases the passive plasma membrane transport of calcium, leading to an increase in the intracellular concentration of calcium. Controls were treated in the same way, except that the gelfoam did not contain A-23187. Eight days after axotomy, the morphology of the cells was examined by injecting them with intracellular dyes.

We found that after axotomy, the sprouts from the soma-dendritic complex emerge earlier from cells treated with A-23187 than from control cells. These sprouts can reach a length of 60 µm by eight days. They differ from the thin sprouts usually seen in regenerating giant reticulospinal cells in that the entire length of these new processes are thick and swollen, rather than only the growing tip. The effect of A-23187 differs with the distance of the axotomy from the cell body. The sprouts appear less numerous and shorter when the axon is severed farther from the soma. With A-23187, when the axotomy is at an even more distant site, no soma dendritic sprouting is observed; however, there is a reduction in the size of the cell body.

Using an in vivo preparation, we have shown that the intracellular augmentation of calcium accelerates and increases the extension of new sprouts from injured neurons. The increased diameter of these new processes may be related to the abnormal level of the calcium in the cytoplasm of the treated cells. Thus calcium may act by affecting the incorporation of membrane or the rearrangement of cytoskeleton elements all along the length rather than only at the tip of the sprout.

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48.9 MICROTUBULE-NEUROFILAMENT SEGREGATION AND INHIBITION OF NEURITE GROWTH IN CULTURED CHICK EMBRYONIC DORSAL ROOT GANGLIA CAUSED BY B,B'-IMINODIPROPIONITRILE (IDPN). R.G. Nagele, K. Bush*, E. Hunter* and H. Lee*. Univ. Med. Dent. of New Jersey - Sch. of Osteopathic Med. and Rutgers University, Camden, New Jersey, 08103.

IDPN has been shown to induce a distinctive reorganization of the cytoskeleton of large diameter axons such as those in the sciatic nerves of adult rats in which microtubules (MTs) are completely segregated from neurofilaments (NFs) (Papasozomenos et al., J. Cell Biol., 95:672, 1982; Griffin et al., J. Neurosci., 3:557, 1983). Furthermore, slow axonal transport of NFs along these axons is inhibited with no apparent effect on the rate of fast axonal transport. In the present study, we have investigated the effects of short- and long-term exposure of IDPN on neurite initiation and elongation and on established neurites. Particular attention was given to the neurite cytoskeleton in an attempt to resolve the separate roles of MTs and NFs in neurite initiation and elongation.

Dorsal root ganglia (DRG) were isolated from day 8 chick embryos and grown as either organized or dissociated cultures on glass coverslips coated with carbon and poly-L-lysine. Cultures were treated with the following concentrations of IDPN (1, 2.5, 5, 10, 20, 50, 100 and 150 ug/ml). At intervals during incubation, cultures were examined to determine the effects of IDPN on the extent of neurite sprouting, rate of elongation, number of neurites, and length of neurites. Randomly selected cultures from each treatment group were processed for conventional transmission electron microscopy.

In organized DRG cultures, IDPN treatment caused a reduction in the number and length of neurites and the radial growth of non-neuronal (glial) cells in a dose-related manner. At doses up to and including 50 ug/ml, the effects of IDPN appeared to be reversible. In cultures with neuritic outgrowths established for 48 h before treatment, IDPN was found to induce segregation of MTs and NFs, which were organized into separate clusters. MT-NF couplers (Nagele and Roisen, Brain Res. 253: 31, 1982) were redistributed and remained associated preferentially with MTs. Quantitative evaluation of neurite sprouting in dissociated cell cultures revealed that IDPN-treated neurons were able to extend only fewer and shorter neurites which contained predominantly MTs. This finding provides further evidence for the hypothesis that MTs are more important for the initial sprouting of neurites, but NFs are probably required for the subsequent extension and maintenance of longer neurites.
[Supported by the UMDNJ Foundation and the NIH (NS21730)]

48.10 CORRELATION BETWEEN MOSSY FIBER SPROUTING AND SEIZURES IN KAINATE-TREATED RATS. J. Cronin and F. E. Dudek, Dept. of Psychology, Tulane University and Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

The neurotoxin, kainate, preferentially kills hippocampal pyramidal cells; the CA4/CA3 subfields are especially vulnerable. The CA4 forms associational and commissural fibers which project to the dentate granule cells, so their loss removes an input to the dentate granule cells. The mossy fiber (MF) axons of the granule cells sprout collaterals that appear to reinnervate the granule cell dendrites. Field potential recordings from dentate granule cells of kainate-treated rats have suggested these MF collaterals form functional connections (Tauck, D. L. and Nadler, J. D., J. Neurosci., 5:1016, 1986), which may then cause recurrent excitation. Since spontaneous seizures occur in kainate-treated rats, the present study was conducted to calculate the correlation between MF sprouting and spontaneous seizures.

Rats were systemically injected via the lateral tail vein with kainate at 14 mg/kg body weight. For 4 wk after the injection, subjects were observed daily (blind to experimental treatment), and the frequency and severity of any seizures was recorded. The daily scores were summed and divided by the number of observations to give a total behavior score. The animals were then sacrificed (n=36 kainate-treated, 29 vehicle-injected controls), brains removed, and hippocampi dissected out. The hippocampi were divided into thirds along the septo-temporal axis, and sections from each third were stained with a modified Timm's stain, which visualizes the MFs. The extent of MF sprouting in these tissues was scored subjectively as well as analyzed by densitometry using an image analysis computer.

When sprouting occurred, it was found almost exclusively in sections from the temporal third of the hippocampus; therefore, only data from the temporal sections were included in calculations of correlations. A significant correlation ($r=0.50$, $p<0.001$) was found between the subjective Timm's ratings and behavioral scores, and also between the densitometry-based Timm's score and the behavioral score ($r=0.24$, $p=0.05$). The two methods of quantifying the Timm's score also were highly correlated ($r=0.67$, $p<0.001$). No seizures were observed in the control animals, although 16 of the kainate-treated animals (44%) were observed experiencing seizures. Only one control animal showed MF sprouting (possibly miscoded), while 19 of the 36 (53%) kainate-treated animals had MF sprouting. These results indicate that kainate-treated animals showing spontaneous seizures are likely to have MF sprouting in the temporal hippocampus.
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48.11 INTRAVENTRICULAR INFUSION OF NGF IN THE RAT DOES NOT ELICIT SYMPATHETIC INGROWTH. B.N. Saffran*, W.C. Mobley' and K.A. Crutcher (SPON. G.C. Schoenwolf) Dept. of Anatomy, University of Utah School of Medicine, Salt Lake City, UT, 84132 and 'Dept. of Neurology, Univ. of Calif. San Francisco, CA, 94143.

Substantial evidence suggests that central neurons in the mature mammalian brain can grow long distances given the proper environment (e.g., Aguayo et al., 1981), and that "reactive synaptogenesis" occurs after lesions to specific tracts in the CNS. Axonal elongation within the mature brain, however, is rare. One particular example of directed axonal growth within the adult CNS is the sprouting of perivascular sympathetic axons into the hippocampal formation following damage to the septohippocampal projection. Current evidence suggests that sympathohippocampal sprouting is specific in that it only occurs in response to septal denervation and is topographically restricted, at least in the rat, to the dentate gyrus and CA3 region of the hippocampus.

Since this example of axonal elongation represents collateral sprouting, i.e., the responsive neurons are uninjured, the stimulus responsible for its initiation must arise external to the growing axons. NGF, or a factor very similar to NGF, has been implicated in this particular sprouting model. In fact, NGF is present, and synthesized, in the rat hippocampal formation and the total amount of hippocampal NGF protein increases following septal denervation. In addition, injection of NGF antiserum has been reported to block sympathetic sprouting in response to septohippocampal denervation.

To test whether elevation of NGF levels in the CNS would elicit sympathetic sprouting without septal denervation, rats received intraventricular injections of NGF (or a vehicle control) via an osmotic minipump for a period of two weeks. Bisbenzimide was added to the vehicle in order to be certain that the pump solution was ejected. The brains were removed and immediatly frozen for histological analysis. Cryostat sections were reacted for norepinepherine flouresence using the SPG method of de la Torre to detect sympathetic sprouting and acetylcholinesterase staining was used to monitor the integrity of the septohippocampal innervation.

In none of the cases where there was evidence of successful infusion of the pump solution into the ventricular system (as determined by the presence of bisbenzimide labeling in the choroid plexus) was there any evidence of sympathetic sprouting in the hippocampal formation or any other brain region. The absence of sympathetic sprouting suggests that intraventricular injection of NGF is insufficient, by itself, to elicit sympathetic sprouting. Possible explanations for these findings include: 1) that NGF is not involved in the sprouting response, 2) intraventricular NGF is not available to the perivascular sympathetic fibers, 3) a gradient of NGF is necessary for the elicitation of sympathetic sprouting and such a gradient is not established by intraventricular infusions of the protein during the time period we studied, 4) NGF is necessary but not sufficient for sympathetic sprouting to occur, suggesting that removal of septal fibers results in other changes in addition to effects on hippocampal NGF. (Supported by NIH grant #NS-17131)

49.1 SMOOTH PURSUIT EYE MOVEMENTS ARE NOT DRIVEN SIMPLY BY TARGET VELOCITY. R.J. Krauzlis* and S.G. Lisberger (SPON: S. Bodary). Dept. of Physiology, Div. of Neurobiology, University of California, San Francisco, CA 94143

The primate smooth pursuit system uses retinal image motion to generate tracking eye movements. Simple models of the pursuit system possess a fixed sensitivity to retinal velocity errors (RVEs). We have found, however, that the pursuit system has a separate response to retinal acceleration errors (RAEs) and that the sensitivity to RVEs changes dynamically during the 300-400 ms required to foveate a moving target.

Eye movements were monitored using the scleral search coil technique in two Rhesus monkeys. The monkey was seated with head immobilized 3.7 feet from a screen onto which targets were projected. Trials began when the monkey fixated a stationary spot at straight-ahead gaze. At a random time, this spot was extinguished and a second spot appeared at an eccentric position and moved horizontally with a specified velocity or acceleration. One set of experiments examined the initiation of pursuit. Known RVEs and RAEs were presented by taking advantage of the fact that target motion equals retinal image motion during the 90 ms prior to the initiation of pursuit. Other experiments imposed known errors in open loop conditions during pursuit. The target was driven with a signal composed of the current eye position plus the desired error signal.

Constant RAEs of 45-400 °/s^2 and constant RVEs of 2-25 °/s produced smooth eye accelerations. The averaged data were subjected to a quantitative analysis to determine whether the responses to constant RAEs were actually due to RVE sensitivity. Using observed eye accelerations to constant RVEs, we predicted on a millisecond time scale the eye velocity response expected from the sequence of velocity errors contained within a smooth acceleration. The actual eye velocity increased at an initial rate that was greater than that predicted from RVEs alone. We conclude that the pursuit system possesses a sensitivity to RAEs *per se*.

We examined changes in sensitivity to RVEs as a function of time by imposing RVEs under open loop conditions at various times, from just before to 600 ms after the onset of pursuit. The magnitude of eye acceleration for a given RVE depended on when the RVE was imposed and on whether the RVE was in the same (*onward*) or opposite (*backward*) direction relative to target velocity. Eye accelerations for onward RVEs were largest when presented within the first 50-150 ms of eye motion and then decreased. Eye accelerations for backward RVEs increased from initiation to 600 ms. For example, in one monkey, eye acceleration was 331 °/s^2 to an onward RVE of 25 °/s imposed at 100 ms after pursuit initiation, but 156 °/s^2 when imposed at 200 ms. Eye acceleration was 93 °/s^2 to a backward RVE of 25 °/s imposed at initiation, but was 235 °/s^2 when the error was imposed 200 ms later. Thus, the pursuit system is initially much more sensitive to onward RVEs. This bias reverses at 200-300 ms after initiation of pursuit, when the pursuit system becomes more sensitive to backward RVEs.

We conclude that pursuit eye movements are not driven exclusively by a neural analogue of target velocity and that sensitivity to visual feedback is dynamically regulated. Sensitivity to RAEs may improve the response characteristics of the pursuit system. Changes in RVE sensitivity may reflect a strategic gating of visual information that improves the ability of the pursuit system to extract relevant feedback signals. (Supported by NIH grant EY03878)

49.2 EVIDENCE THAT VISUAL INPUTS DRIVE OSCILLATIONS IN EYE VELOCITY DURING SMOOTH PURSUIT EYE MOVEMENTS IN THE MONKEY. D. Goldreich* and S.G. Lisberger (SPON: J. Bixby). Dept. of Physiology, Div. of Neurobiology, University of California, San Francisco, CA 94143

When primates use smooth pursuit eye movements to track constant-velocity target motion, their eye velocity oscillates about the target velocity. The oscillations are sinusoidal and undamped, with a peak-to-peak amplitude of about 5 deg/sec when the target moves at 30 deg/sec. The goal of our study was to determine whether the oscillation frequency changes when the delay around the visual feedback loop is altered. Variation of oscillation frequency as a function of delay would imply that the oscillations are visually driven. Invariance of the oscillation frequency would imply that the oscillations result from an internal oscillatory mechanism.

We monitored horizontal and vertical eye position using the magnetic search coil technique in two Rhesus monkeys. During experiments, the monkey was seated with head immobilized 3.7 ft from a screen onto which targets were projected. The monkey was required to fixate a stationary target at straight-ahead gaze. At a random time, this target was removed and a second, eccentric, target was moved horizontally at 30 deg/sec for 900 msec. The monkey was required to track the moving target in order to receive a water reward.

In one set of experiments, pursuit latencies ranging from 65 to 104 msec were obtained by varying the size and brightness of the projected target. The oscillation frequency decreased monotonically as the latency was increased. When the latency was 65 msec, the oscillation frequency was 6 Hz; when the latency was 104 msec, the oscillation frequency was 4 Hz. In another set of experiments, we artificially imposed extra delay in the visual feedback loop. The target was driven by a signal that was equal to desired target velocity (T = 30 deg/s) plus the difference between current eye velocity (E(t)) and eye velocity at a previous time (E(t - Δt)). At time t, this procedure produces a retinal velocity error of T minus E(t - Δt) instead of T minus E(t). The loop delay is therefore increased by time Δt. The oscillation frequency decreased monotonically as artificial delays up to 100 msec were imposed. In one experiment, for instance, as the imposed delay was increased from 0 to 100 msec the frequency fell from 6 to 2 Hz. Our two methods for increasing the delay - naturally by decreasing target size and brightness or artificially by delaying feedback - appear to be equivalent procedures. A frequency of approximately 4 Hz, for example, resulted from either (1) a 104 msec latency without added delay or (2) a 65 msec latency with 40 msec added delay. In both cases, the total delay around the loop, equal to the sum of latency and added delay, was approximately 105 msec.

Our results show that the frequency of pursuit oscillations depends on the total delay around the visual feedback loop. A delay-dependent oscillation frequency is incompatible with models in which the oscillations are driven solely by internal oscillatory mechanisms. We conclude that the oscillations in smooth pursuit eye movements are visually driven. (Supported by NIH grant EY03878).

49.3 ANTICIPATORY SLOW EYE MOVEMENTS DURING SMOOTH PURSUIT. Duane K. Boman and John R. Hotson. Institute for Medical Research-Santa Clara Valley Medical Center, San Jose, and Stanford University School of Medicine, Stanford, CA 94305.

Anticipatory slow eye movements are highly preditive, low velocity, smooth eye movements that precede both ramp and step target motions. The utility of these predictive responses is unclear. They are restricted by visual fixation targets and add little toward synchronizing smooth pursuit with the onset of target motions unless the fixation target is extinguished for a short period of time. Predictive, low velocity, smooth eye movements also precede terminations and direction changes of ramp stimuli. All of these predictive responses are similar in that they move the fovea away from the target. These observation make it attractive to suggest that these predictive responses share common mechanisms and are part of the predictive component of smooth pursuit. Therefore, a model is proposed in which the predictive responses produced during smooth pursuit are the sum of the anticipatory deceleration produced prior to the termination of ramp motion and the anticipatory acceleration produced prior to the initiation of ramp motion (when visual fixation is removed by a gap in the target presentation).

This model was tested by presenting subjects with highly predictable double ramp stimuli. The second ramp of the pair changed the direction of motion by either 90 or 180 degrees. With 180 degree direction changes, horizontal ramp speeds of 2, 6, and 10 deg/sec were presented, thus producing nine conditions. With 90 degree direction changes, the horizontal ramp speed was 6 deg/sec and the vertical ramp speed was either 6 or 10 deg/sec. Under each condition the stimulus was extinguished for 600 msec prior to the initiation of the first ramp. Eye movements were recorded from three subjects with a Dual Purkinje Image eyetracker. Average slow eye velocities were determined before and after the initiation of the first ramp, the termination of the second ramp, and the change in the direction of ramp motion.

Under each condition, eye velocity decreased prior to the change in ramp direction and was subsequently followed by a rapid acceleration that synchronized the eye and target velocities. The time course, velocities and direction of these 180 and 90 degree predictive turns in smooth pursuit were closely reproduced by summing the anticipatory velocities that were produced prior to the initiation and termination of unidirectional ramps of comparable speeds. Therefore, it is suggested that anticipatory slow eye movements are both active and useful during smooth pursuit, that they show directional specificity and are produced by the predictive component of smooth pursuit.

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49.4 PRIMATE OCULOMOTOR PURSUIT OF A HAND CONTROLLED VISUAL TARGET: SMOOTH PURSUIT LATENCY DECREASES TO ZERO DURING ACTIVE HAND MOVEMENTS.

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Initiation of foveal pursuit has a latency T of about T=120 ms between the onset of target movement and eye movement. This latency was used here as an indicator to study possible synchronization and coupling of the neural control of eye and hand movements in primates.

Two monkeys (Macaca fascicularis) sitting in an upright position with their heads firmly attached to the upper portion of a primate chair, had been trained to pursue a horizontally moving (on a semi-circle with 15 cm radius) visual target (0.8 deg diameter). One hand (invisible to the animal) held a pointer, which could be moved along the semicircular path of the visual target between 20 deg left and right. Target movements were generated under three conditions: a) external control unrelated to hand movement; b) target position coupled with hand-held pointer position during spontaneous hand movements; c) as in b) but target and hand move in opposite rather than same directions.

Results

1. Under condition a), statistical analysis of the latency T yielded mean values of T=120 ms in agreement with the literature.
2. Under condition b), T decreased within several days of training from initial mean values of T=120 ms to T=0 ms.
3. Under condition c), too, T decreased within several days of training this condition from initial values of T=120 ms to T=0 ms.

Conclusions

Motor control signals for active hand movements as under conditions b) and c) can be used to generate synchronized oculomotor control signals for initiation of foveal pursuit eye movements. This finding supports the hypothesis that the motor program generators for both hand movement and pursuit eye movement can be initiated by the same pre-motor signal.

The neural pathways connecting the two motor program generators for hand and eye movements (presumably by means of efference copy signals) are the topic of a subsequent study using chemical lesions.

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49.5 TORSIONAL EYE MOVEMENTS IN THE MONKEY DURING HORIZONTAL FOVEAL PURSUIT: BEHAVIORAL AND NEUROPHYSIOLOGICAL EVIDENCE.
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The existence of rapid and slow torsional eye movements (Collewijn et al., Exp.Brain Res. 59, 185-196, 1985; Fender, Brit.J.Ophthal. 39, 65-72, 1955) is ignored and not detectable by most oculomotor recording systems and seems particularly unlikely during foveal pursuit of horizontally moving small targets. A detailed analysis of the transformation of retinal events into pursuit eye movements, however, yielded two lines of evidence for additional torsional components.

Java monkeys (Macaca fascicularis) sitting in an upright position had been trained to pursue a small (8 min. of arc) target against a homogeneous background at 1.5 m distance in the lower photopic range. Sinusoidal target movement (0.3 - 1.2 Hz at 10 deg amplitude) was confined to a line on the screen in the horizontal plane of the eye balls. Movements of the fundus pattern of the left eye were monitored by means of TV-ophthalmoscopy (ophthalmoscope combined with a light-sensitive TV camera and video tape attached to the primate chair), while vertical and horizontal eye movements of the pursuing right eye were recorded with an infrared oculometer(Bouis).

Single unit recordings from ocular motoneurons were obtained in a related study by one of the authors (R.E.).

Results

1. During spontaneous fixations close to the primary position, small rapid torsional movements (below 1 deg of rotation about the line of gaze) often accompanied spontaneous saccades, although a systematic correlation between torsion and eye position could not be detected.
2. During horizontal foveal pursuit both rapid and smooth torsional movements (below 1 deg of rotation) were clearly superimposed on the horizontal (and occasionally small vertical) eye movements of the left eye. A systematic correlation between the smooth torsional components and horizontal eye position is presently being studied.
3. The instantaneous impulse rate of ocular motoneurons for control of vertical and/or torsional eye movements (musculus r.s., r.i., o.s., o.i.) was often found to be systematically slightly modulated (range of ± 5 Imp./s at 100 Imp./s) during horizontal pursuit but not during fixation along the pursuit track. While the line of gaze passed the primary position (with maximum eye velocity) during horizontal pursuit, the impulse rate typically reached its maximum or minimum in the absence of any measurable vertical movements.

It is concluded that: A) the observed torsional movements generate afferent visual signals of target rotation during horizontal pursuit and B) the emerging finding of a systematic correlation between torsion and eye position implies that the four vertical eye muscles are not kept in a 'fixation mode' during horizontal pursuit but receive small dynamic neural control signals.

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49.6 OCULAR PURSUIT DURING YAW ROTATION IN HUMANS. X.N. Sahyouni*, V. Matsuo, and B.W. Peterson. Dept. of Physiology, Northwestern Univ. Med. Sch. and Sensory Motor Performance Program, Rehab. Inst. of Chicago, Chicago, IL 60611.

While it has been shown that smooth pursuit and suppression of the vestibulo-ocular reflex (VOR) are not the same process (McKinley and Peterson, Exp. Brain Res. 60:454) the interaction between the two has not been carefully studied. We examined this interaction by requiring a subject (S) to pursue a horizontally moving target while he was rotating in the horizontal plane.

We used five normal Ss who sat in a servo-controlled rotating chair (Neurokinetics) with the head held in a chair-mounted head restraint. The pursuit target was a 0.25 degree spot produced by a servo-controlled laser projection system. Eye movements were recorded using D.C. electro-oculography. Vestibular stimuli consisted of 40 deg/sec ramps. Pursuit stimuli were ramp target motions that initially moved with the chair at 40 deg/sec and then either moved in the same direction at 80 deg/sec, or in the opposite direction at 40 deg/sec. We also measured smooth pursuit of 40 and 80 deg/sec targets with S stationary (SP40, SP80) and baseline VOR with S relaxed (VORr) or attempting to follow an earth-stationary or chair-fixed target (VORe, VORs).

In the condition where the target moved in the direction of chair rotation at 80 deg/sec (40 deg/sec relative to S), Ss' smooth eye velocities averaged 22 deg/sec, which was not different from the sum of VORr + SP40. It was lower than the sum of VORs + SP40, which averaged 32 deg/sec. Thus Ss cannot effectively combine suppression of the VOR and pursuit to enhance the tracking of a target moving with the chair.

In the condition where the target moved opposite to the chair at 40 deg/sec (80 deg/sec relative to S), Ss' smooth eye velocities averaged 52 deg/sec. This was lower than the sum of the predicted smooth pursuit contribution + either VORr (66 deg/sec) or VORe (72 deg/sec). Thus not only were Ss unable to combine pursuit and VOR enhancement, they also could not combine pursuit and baseline VOR when tracking the target. This suggests that Ss may suppress their VOR when pursuing moving targets while rotating.

These findings suggest that interactions occur between smooth pursuit and the VOR whenever both the subject and the target are in motion.

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49.7 SMOOTH PURSUIT AND SACCADIC EYE MOVEMENTS IN PATIENTS WITH ALZHEIMER'S DEMENTIA AND SCHIZOPHRENIA. Daniel Hommer, Trey Sunderland*, Thomas Clem*, GRECC, Rm. 182B, Seattle VAMC, 1660 S. Columbian Way, Seattle WA, 98108, Laboratory of Clinical Science, NIMH, Biomedical Engineering Branch, NIH

Eye movements of patients with schizophrenia, Alzheimer's dementia (DAT) and age matched controls were examined using an infra-red reflection technique. Eye position data was collected at 1000 hz and stored, displayed and analyzed using a specially developed microcomputer system. Recording was done in a quiet, darkened room. A small square on a video monitor was used as the target.

Patients with DAT showed a marked impairment in their smooth pursuit which correlated with the severity of their cognitive impairment. The more severely affected individuals with DAT had nearly complete replacement of smooth pursuit by saccadic eye movements. Patients with DAT also showed a prolonged saccadic latency and failure to learn a step-gap task which required the ability to predict a simple, repetitive pattern of target movements.

As a group schizophrenics also had worse smooth pursuit than cotrols but the magnitude of their dysfunction was much less than that of the patients with DAT. Over half of the schizophrenics had smooth pursuit which was indistinguishable from that of controls. Schizophrenics had normal saccadic latency, but patients receiving chronic neuroleptic treatment had marked dysmetria (undershot) on a step-gap task. This dysmetria was present to a much less extent in drug free schizophrenics. Dysmetric saccades to a remembered target may be secondary to chronic dopamine blockade. A small group of schizophrenics who also showed the worse smooth pursuit tracking failed to learn to predict target behavior in the step-gap task. These patients may represent a subpopulation of schizophrenics with cortical dysfunction.

49.8 EFFECTS OF KETAMINE ON OCULOMOTOR FUNCTION IN THE MONKEY. Charles J. Bruce and Gary S. Russo*. Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven CT 06510.

The effects of the dissociative tranquilizer ketamine hydrochloride on the oculomotor system of macaque monkeys were investigated using a search coil to monitor eye movements. The primary effect of ketamine, especially in the absence of sensory stimulation, was to suppress all manner of eye movements. A few min after giving the ketamine (5-10 mg/kg IM) the gaze came to rest near the primary position (PP) in the orbit and did not move appreciably for 15-30 min thereafter. This severely fixed gaze is in marked contrast to the spontaneous saccades characteristic of awake monkeys and the slow, wandering eye movements characteristic of light sleep and sedation. We have analyzed the effects of ketamine on the different classes of conjugate eye movements.

All types of slow eye movements were strongly affected by ketamine. Optokinetic nystagmus (OKN) was virtually abolished; large (~50° square) drifting gratings no longer elicited OKN even though the eyes remained open. Similarly, the vestibular ocular reflex (VOR) was effectively eliminated; during passive sinusoidal head rotation the eyes were fixed in the orbit, at least to the accuracy of our measurements in this situation. We further investigated the effects of ketamine on the VOR via caloric stimulation of the canals. Instead of nystagmus, the gaze quickly drifted 2-6° ipsilateral to the ear that was irrigated with cold water, stayed there, and then slowly drifted back to the PP shortly after the irrigation ceased. Two monkeys with pathological constant-velocity nystagmus were tested; one had an omnipresent nystagmus accidentally caused by an experimental surgery, the other had nystagmus in the dark, probably caused by antibiotic therapy. For both monkeys ketamine completely eliminated the nystagmus and their gaze quickly came to rest near the PP.

Ketamine also had several effects on saccadic eye movements. (1) The gaze immediately drifted exponentially back to the PP after saccades in any direction. (2) Very few saccades ocurred during the acute intoxication, especially in the absence of sensory inputs (e.g. in the dark) . (3) More frequent saccades, predominantly upwardly directed, ocurred during the recovery from ketamine, especially in an illuminated environment. Epochs of these rapid upward movements and slow downward drifts comprised a gaze-dependent vertical nystagmus. Nystagmus is a prominent clinical sign of intoxication by ketamine or by the closely-related substance phencyclidine (PCP). (4) Threshold currents for evoking saccades with electrical stimulation of the frontal eye fields were raised by ketamine, and oblique elicited saccades became more vertical.

An hypothesis that explains many of these effects is that ketamine temporarily disables the "neural integrators" in the brain stem that are postulated as the mechanism for converting eye velocity commands into tonic eye position signals for the oculomotor neurons. Thus movement caused by the saccadic "pulse" are not maintained because the saccadic "step" that usually accompanies the pulse is eliminated. Similarly, feed-through of a tonic head velocity signal from caloric stimulation of the canals can effect a small static shift via direct connections between the vestibular and oculomotor nuclei, but the absence of ongoing integration of that velocity signal precludes true ocular tracking. If this neural integration hypothesis proves correct, then ketamine should provide a usefull tool for dissecting the basic mechanisms of the oculomotor system.

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49.9 THE DEVELOPMENT OF NYSTAGMUS IN INFANT MONKEYS FOLLOWING VISUAL DEPRIVATION. R.J. Tusa, C.B. Smith and S.J. Herdman, Johns Hopkins University, Baltimore, Md. 21205, and Lab of Cerebral Metabolism and Lab of Neuropsychology, NIMH, Bethesda Md. 20892.

Monkeys monocularly deprived of vision by tarsorrhaphy at birth and then reverse sutured 25 days later (opening the closed eye and closing the other) developed nystagmus 2-3 days after the reversal. This nystagmus was not due to blindness; the monkeys visually-tracked small moving objects and their reflection in a moving mirror. In contrast, monkeys monocularly or binocularly deprived of vision for 25 days did not develop nystagmus that could be detected by visual observation.

In 2 monkeys eye movements were recorded with scleral search coils that were implanted at the time of the reverse suture. A conjugate jerk nystagmus with slow phases directed up and nasally with respect to the opened eye developed 2-3 days after the reversal. The slow phases appeared linear and the peak velocity reached a maximum of 10deg/sec. During the second week, the nystagmus waveform became more variable. Jerk nystagmus in all directions, pendular nystagmus, and combinations of pendular and jerk nystagmus were all observed. The slow phase components of the jerk nystagmus were often of increasing velocity peaking at 30deg/sec. By the third week, peak velocity of slow phases reached values as high as 100deg/sec. The nystagmus did not change when the monkeys were placed in the dark, although the nystagmus was markedly attenuated during eye closure and drowsiness (slow phase eye velocities <3deg/sec). In one monkey, both eyes were opened 3 weeks after the reverse suture. In this animal the nystagmus remained unchanged for the duration of the study (90 days).

The waveform of the nystagmus induced by reverse eye lid suture in infant monkeys resembles congenital nystagmus in human beings. Further studies are underway to determine the effects of reverse suture on visual acuity and eye tracking ability.

49.10 VESTIBULAR OCULAR ORGANIZATION IN VERTEBRATES IS A LARGELY CONSERVED PHYLOGENETIC PLAN. R. Baker, J.M. Delgado-Garcia*, W. Graf and R.F. Spencer. Dept. of Physiol. & Biophys., NYU Med. Ctr., New York, N.Y., Fac. Biol., Seville, Spain., Rockefeller Univ., New York, N.Y. and MCV, Richmond, Va.

Teleosts are uniquely different from other vertebrates in that the abducens nucleus exhibits two separate subdivisions and is located ventral in the medulla. In previous intracellular recording, HRP injection and light/electron microscopy of oculomotor motoneurons in the goldfish, Carassius auratus, we described the characteristic synaptic profiles producing excitation and inhibition. Herein we compare those findings with morphological, physiological and immunohistochemical studies of the goldfish abducens nucleus. Electron microscopy of HRP labeled abducens motoneurons showed numerous axo-somatic/dendritic synaptic endings containing spheroidal synaptic vesicles establishing both chemical (asymmetrical pre-/postsynaptic specialization) and extensive electrotonic (apposed membranes exhibiting gap junctions) synaptic contacts. Intracellular recording from antidromically identified abducens motoneurons revealed robust short latency electrotonic EPSPs (0.5 msec latency) followed by chemical depolarizations (1.3 msec) evoked by electrical stimulation of the contralateral vestibular nerve. Other axosomatic synaptic endings containing pleiomorphic vesicles established only chemical synaptic specializations. As expected, stimulation of the ipsilateral vestibular nerve revealed IPSPs (1.4 msec) that were reversed following injection of current and/or chloride ions. Like the mammalian abducens nucleus, efficacy of inhibition was demonstrated by blockade of the antidromic field potential following isolated electrical stimulation of the horizontal canal nerve. Immunohistochemical labeling in both the goldfish oculomotor and abducens nucleus was consistent with our findings in mammals demonstrating glycine and GABA to be the inhibitory neurotransmitter of second order vestibular neurons related to the horizontal and vertical vestibulo-ocular reflex, respectively. Collectively these data from the abducens and oculomotor nuclei demonstrate a common arrangement of vestibular inhibition and excitation across diverse vertebrate species. Although our detailed comparisons reveal quantitative differences in density of presumed inhibitory and excitatory contacts including gap junctions, it is the overall similarity of vertebrate vestibular ocular organization that is most striking. Viewed on an evolutionary scale these observations are noteworthy, because each of the many species studied to date are far removed, in divergent directions, from the 'truly primitive' vestibular ocular plan. Supported by NEI 02007, 04613 and 02191.

49.11 THE SOMA-DENDRITIC ORGANIZATION OF PHYSIOLOGICALLY CHARACTERIZED AVIAN EXTRAOCULAR MOTONEURONS. Juan Carlos Letelier*, Craig Evinger, and Josh Wallman. Dept. of Biology, City College, CUNY, New York, NY 10031 and Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

The physiological organization of the avian oculomotor system differs from that of mammals in that two separate types of motoneurons within a nucleus control tonic eye position ("tonic" neurons) and saccadic trajectory ("phasic" neurons). The tonic motoneurons are involved in positional control and, between saccades, fire at a steady rate correlated with eye position. All tonic motoneurons pause for every saccadic eye movement, regardless of direction. In contrast, the phasic motoneurons are completely silent except during saccadic eye movements, when they discharge with the 25 Hz oscillations typical of avian saccades. Trochlear motoneurons of this type exhibit a brief burst of spikes just prior to and during the beginning of each intorsional phase of the oscillatory eye movement.

To investigate the anatomical organization of these two classes of motoneurons, physiologically characterized neurons from the trochlear and oculomotor nuclei of alert chickens were intracellularly impaled and injected with horseradish peroxidase. Eye position was monitored with eye coils so that neurons could be classified as phasic or tonic.

Chicken extraocular motoneurons are similar in appearance to those of other species. Both trochlear and oculomotor motoneurons had five to seven primary dendrites. Although often beaded, the dendrites were rarely spiny. The dendrites of each motoneuron extended throughout the entire subdivision corresponding to the muscle that they innervate. Except for medial rectus motoneurons, the dendrites also extended outside of the nucleus into the medial longitudinal fasiculus (MLF) and adjacent reticular formation. In following motoneuron axons until they exited the brain, we never found any collaterals. While the overall appearance of "phasic" and "tonic" motoneurons was similar, phasic motoneurons tended to have thicker primary dendrites than tonic motoneurons. In addition to motoneurons, we stained axons within the MLF which exhibited phasic activity indistinguishable from that of phasic motoneurons. These axons terminated profusely within specific subdivisions of the oculomotor nucleus. They could be internuclear neurons involved in the synchonization of the different extrinsic oculorotary muscles during oscillations. In any event, this is indirect evidence that the bursts of activity of the phasic motoneurons are not the consequence of some biophysical property of their membrane, but rather that this oscillatory behavior is actively induced by their neuronal input.

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49.12 SOME ANTERIOR ECTOSYLVIAN CORTICOFUGAL PROJECTIONS IN CATS R.L. Segal and R.M. Beckstead. Dept. of Rehab. Med., Emory Univ. School of Med., Atlanta, GA 30322, and Dept. of Anatomy and Cell Biology, Med. Univ. of South Carolina, Charleston, SC 29425.

Several studies have focused on the physiological organization of the cortex surrounding the anterior ectosylvian sulcus (AES) of the cat. Neurons responding to visual, auditory and somatosensory stimuli have been identified. Thus, the AES must be considered a multimodal cortical region. Little information is available concerning the anatomical connections between AES and the multimodal deep layers of the superior colliculus.

In a previous report (Segal and Beckstead)[1] we observed retrogradely labeled neurons all along the banks of AES, but with the greatest density along the fundus and ventral bank. We recently examined the projection from the caudal two-thirds of the fundus and ventral bank of AES to the colliculus and thalamus using anterograde tracers [WGA-HRP or L-(2,3,4,5)-^{3}H-proline].

Anterogradely labeled axons are observed ipsilaterally in the following thalamic nuclei : interadjacent division of the lateral posterior complex, posterior complex, posterior nuclear group and in the magnocellular, dorsal and ventral divisions of the medial geniculate complex. In the colliculus, labeled axons are observed throughout the rostrocaudal extent, but are concentrated in the medial one-half. In the rostral colliculus labeled axons pass through the deep gray layer to apparently terminate in the dorsal intermediate gray layer. More caudally (reaching the mid-colliculus), dorsal and ventral tiers of axon-labeling are observed within the intermediate gray and stratum opticum. From the mid-colliculus and caudal, a single bank of labeled axons encompasses the intermediate gray layer ventrally and extends dorsally into the stratum opticum. Examination of the colliculus contralateral to the cortical injection reveals labeled axons primarily in the intermediate gray layer and stratum opticum of the medial portion of the rostral one-half.

The results indicate that neurons in the caudal two-thirds of the ventral bank and fundus of AES, the so called anterior ectosylvian visual area (Mucke et al., '82)[2], project to the superior colliculus. In addition, the pattern of axonal labeling is different than from injections in the rostal two-thirds of the dorsal bank of AES (Stein et al., '83)[3].

[1] Segal and Beckstead, J. Comp. Neurol. 225:259-275, 1984.
[2] Mucke et al., Exp. Brain Res. 46:1-11, 1982.
[3] Stein et al., J. Neurophysiol. 50:896-909, 1983.

49.13 THE NIGROTECTAL PROJECTION IN MAN. B.M. Johnson*, A.A. Sadun*, H. Chui#, and J.A. Mortimer@. *Dept. of Ophthalmology and #Alzheimer's Disease Center, USC School of Medicine, Los Angeles, CA 90033; and @Veterans Administration Medical Center, Minneapolis, MN 55417.

Several studies have documented the crucial role of the superior colliculus (SC) in the control of eye movements (Robinson, Vis. Res., 12:1795, 1972; Wurtz and Goldberg, Invest. Ophthalmol., 11:441, 1972). It has been shown in cat and monkey that one source of input to the superior colliculus is the substantia nigra (Graybiel, Brain Res., 143:139, 1978; Jayaraman et al., Brain Res., 135:147, 1977). There has been a dearth of investigations on a putative projection from the substantia nigra (SN) to the SC in humans, because of a previous absence of any techniques suitable for tracing this pathway. Recently, the paraphenylene-diamine staining technique (PPD) has been used to successfully trace degenerated human visual pathways, and has shown that degenerated axonal fibers persist in humans, even following lesions of long duration (Sadun et al., J. Neuropath. exp. Neurol., 42:200, 1983). Employing the PPD technique, examination of specimens from post-mortem brains has revealed eight retinofugal projections.

Parkinson's disease is a well-described syndrome in which the most characteristic pathological feature is degeneration in the SN. This would permit degeneration to be traced from the SN to any target nucleus. We therefore collected tissue from two Parkinson's disease patients at autopsy, both of whom had an otherwise normal ophthalmological history and lesion-free visual system. Blocks containing the entire mesencephalon in coronal section were processed for both paraffin and epoxy resin sectioning, and examined using both standard and special stains. Using the PPD method, degenerated axons were traced from the SN in a mid-lateral arc to the deep layers, but not to the superficial layers, of the superior colliculus. The degenerated fibers appear to arise from dorso-lateral areas in the SN pars compacta, and project in an arch laterally, bypassing the medial lemniscus, and terminate in the deep layers of the SC.

The presence of a nigrotectal projection in man establishes an anatomical substrate which allows us to speculate further on the role of the superior colliculus in eye movements. The termination of a nigral projection in the SC could provide a relatively direct route to elements of the oculomotor mechanism, including elements involved in visuo-motor control. The substantia nigra serves a site of projection for the basal ganglia (Wurtz and Hikosaka, Prog. Brain Res., 64:175, 1986), which in turn may serve as a 'primer' for eye movements (Denny-Brown and Yanasawa, Assoc. Res. Nervous and Mental Dis., 55:115, 1976). In view of such evidence, the nigrotectal pathway in man may contribute to the generation of signals in the initiation of eye movements. Injury to this projection might be the anatomical basis for abnormal eye movements (apraxia, cogwheel pursuits, etc.) commonly seen in Parkinson's disease patients.

49.14 ORIGIN OF CEREBELLAR PROJECTIONS TO THE REGION OF THE OCULOMOTOR COMPLEX, MEDIAL PONTINE RETICULAR FORMATION, AND SUPERIOR COLLICULUS IN CEBUS MONKEYS. G.R. Leichnetz and A. Gonzalo-Ruiz*, Dept. of Anatomy, Med. Coll. of Virginia, Virginia Commonwealth Univ., Richmond, VA 23298.

Horseradish peroxidase (HRP) microinjections, or transcannular HRP gel implants were made into the oculomotor complex (OMC), superior colliculus (SC), or medial pontine tegmentum of 16 adult capuchin monkeys (Cebus apella) to determine the origin of their afferents from the deep cerebellar nuclei. Some of the monkeys had been used in previous studies of cortical input to the same oculomotor-related structures (Leichnetz et al JCN 228:359-408, 1986).

Injections confined to the OMC only retrogradely-labeled a few cells in the dentate nucleus (DN). Larger injections that involved adjacent paraoculomotor nuclei, like the nucleus of Darkschewitsch (ND) and medial accessory nucleus of Bechterew (MAB), resulted in labeling within the anterior (AIN) and posterior (PIN) interpositus nuclei. When OMC injections involved the ventral periaqueductal gray (PAG), there was increased labeling of the fastigial nucleus (FN). A supratrochlear ventral PAG OMC-projecting cell group was found that received a significant projection from the FN. All of the OMC cases produced retrograde labeling in cell group Y, but not in the basal interstitial nucleus (BIN) as described by Langer ('85).

Injections into the caudal medial pontine reticular formation (mPRF) or pontine raphe resulted in large numbers of retrogradely-labeled cells in the FN. Only when the injection was in the more rostral mPRF, involving the nucleus reticularis tegmenti pontis (NRTP), did significant numbers of labeled cells occur in the DN. Such cases also contained labeling in the AIN, which was attributed to uptake of HRP by axons traversing the NRTP enroute to more caudal targets (eg. inferior olive).

The principal source of cerebellar input to the deep SC was from the PIN, although the ventrolateral FN also contained labeled cells.

The results are discussed in terms of the possible convergence of cerebellar projections with those of the frontal eye field to paraoculomotor and preoculomotor nuclei, and their interaction in the control of eye movement.

This study was partially supported by NSF Grant BNS 8113387 and VCU Grant-in-Aid to Faculty to G.R.L., and the U.S.-Spanish Joint Committee Grant IPB85-04059 to A.G.-R.

49.15 THE CELLS OF ORIGIN OF EFFERENT PATHWAYS FROM THE DEEP LAYERS OF THE SUPERIOR COLLICULUS IN THE RAT. M. E. Bickford* and W. C. Hall (SPON: B. R. Schofield) Dept. Anatomy, Duke Univ. Medical Center, Durham, N.C. 27710

These experiments address the question of whether the multiple efferent pathways which arise in the deep layers of the superior colliculus originate from different cell populations. The efferent pathways which have been studied include: (1) the tectothalamic pathway to the mediodorsal and intralaminar nuclei (2) the tectotectal pathway (3) the contralateral tectobulbar pathway and (4) the tectospinal pathway. The cells of origin of these pathways were compared by injecting fluorescent dyes into two structures in single brains. One structure was injected with Fluoro-Gold and the other with rhodamine labeled microspheres, and the neurons in the superior colliculus were examined to determine the distributions of cells with one or both labels.

Three of the pathways studied, the tectospinal, the contralateral tectobulbar and the tectothalamic, arise primarily from the intermediate grey layer (for nomenclature, see Weiner, S. I., J. Comp. Neurol. 244:137, 1986). The remaining pathway, the tectotectal, arises from cells distributed throughout the layers below the stratum griseum superficiale, with the majority located below the stratum griseum intermedium. Following injections of different labels in the contralateral brainstem and the ipsilateral thalamus, many cells containing both labels were found. In contrast, very few double-labeled cells were present when an injection in the contralateral superior colliculus was paired with an injection in either the contralateral brainstem or spinal cord.

The results obtained thus far suggest two main conclusions. First, the mediodorsal-intralaminar nuclear region of the thalamus may receive input from the same cells of the intermediate grey layer that project to premotor areas of the contralateral brainstem and spinal cord. Second, the tectotoectal pathway arises primarily from cells which are distinct from the cells of origin of the crossed descending pathways. (supported by NSF grant #BNS-86-07060).

49.16 THE AVIAN EDINGER-WESTPHAL NUCLEUS: SOURCES OF INPUT CONTROLLING ACCOMMODATION, PUPILLOCONSTRICTION, AND CHOROIDAL BLOOD FLOW. P.D.R. Gamlin and A. Reiner. Dept. of Physiological Optics, Univ. Alabama at Birmingham, Birmingham, AL 35294 and Dept. Anatomy and Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109

The Edinger-Westphal nucleus (EW) is the source of preganglionic fibers to the ciliary ganglion. In the bird, cells in the medial EW (EWm) project to cells in the ciliary ganglion that innervate choroidal blood vessels. Thus the EWm may modulate retinal blood flow. Cells in the lateral EW (EWl) project to neurons in the ciliary ganglion that innervate the sphincter pupillae muscle and the ciliary body. The EWl therefore appears to mediate pupilloconstriction and accommodation. Previously, we have shown in the pigeon that the suprachiasmatic nucleus has a predominantly contralateral projection to the EWm (Gamlin et al., PNAS 79:3891-3895, 1982). We have also shown that a projection from a single retinorecipient pretectal nucleus to the caudolateral EWl mediates the pupillary light reflex. We have therefore inferred that cells in the caudolateral EWl mediate pupilloconstriction and that cells in the rostral EWl mediate accommodation (Reiner et al., TINS 6:140-145, 1983).

In this report, we describe two further sources of input to the EW. In our previous studies, HRP injections that included EW labeled a number of regions that might be sources of afferents. We have examined some of these regions by injecting them with 0.04-0.1 µl of tritiated proline/leucine (S.A. 200 µCi/µl). We have found that cells in a portion of the caudal mesencephalon ventrolateral to the oculomotor nucleus project to all subdivisions of the contralateral EW. This input may contain 5-HT and enkephalin since our immunohistochemical studies show that many neurons in this region contain 5-HT or enkephalin and that many fibers and terminals throughout EW contain these substances. In addition to this input to the entire EW, we have found an input to EWl (and not EWm) that arises from cells in a localized area of the rostral lateral reticular formation (FRL) dorsolateral to nucleus subpretectalis. This projection is predominantly contralateral and includes the accommodative subdivision of the EWl.

These studies suggest that cells in the rostral FRL and dorsolateral raphe may be components of separate pathways to EW, with the former influencing accommodation and the latter influencing all three ocular functions controlled by EW.
(This research was supported by NIH grant #EY05298)

49.17 AN INEXPENSIVE ULTRASONIC CONTACT LENS TO MEASURE ACCOMMODATION
R.S. Remmel, J. Feeny*, M. Khodadoust*, C. Paulino*, and R. Sims*. Biomedical Engineering Dept., Boston Univ., Boston, MA 02215

Assessment of accommodation usually involves asking the patient to read at various distances--assuming he/she can read. Ultrasound probes usually obstruct vision. Refractometers costing >$10,000 involve measuring how light is focused upon the retina. An invalid output occurs if the patient blinks or moves.

We are trying to overcome these restrictions by placing upon a contact lens two piezoelectric crystals, a transmitter and a receiver, one on either side of the pupil. Vision is unaffected. The time delay between echoes from both eye lens surfaces yields lens thickness. For a 3.5 mm thick lens with a 1610 m/s speed of sound, the time difference is ≈4.3 μs (2*3.5 mm/1610 m/s).

The lead metaniobate crystals (EBL Co.) are 0.117 mm thick disks of 1 mm radius. A crystal is excited by a 50 V step, producing a wave. This is repeated at 2.8 KHz. When the echo exceeds a set threshold, a pulse is produced, which turns a clock on for the first echo and off for the second. A time-to-voltage converter outputs accommodation in diopters.

Echoes have been detected from objects immersed in water. The received signal was a 1 MHz sinusoid with a rise time of 2 μs and a fall time of 5 μs. The crystal rings. This 5 μs decay interferred with detection of a subsequent echo.

When the disk lies in the plane θ = 90 deg, the pressure $P(r,\theta,\phi,t)$ is calculated from the wave equation:

$$c^2 \nabla^2 P = \partial^2 P/\partial t^2$$

A solution for P involves the spherical harmonic $Y_{20}(\theta,\phi)$:

$$B[3\cos^2(\theta) - 1] [(3/k^3 - 1/k)\sin(k) - 3\cos(k)/k^2] \sin \omega t,$$

where $k=\omega r/c$, c=1497 m/s in water, $\omega=2\pi F$, and F=1 MHz, the measured resonance freq. B is a constant. This wave has a node at r=1.25 mm, which is approx. the crystal radius. This equation predicts that reducing the radius to 0.25 mm will increase the resonant freq. to 4 MHz--in accord with experiment. The damping time can thus be reduced if r is decreased.

A silicon-rubber annular lens with a hole over the pupil is being tested. The crystals can be glued on with silicon rubber glue, which is approved for use on the eye. A coil of wire can also be glued to the lens so that eye movements can be measured. Clinical evaluation of patient tolerance is planned.

Accommodation should be measurable with a device costing $300.

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49.18 **RETROGRADE TRANSNEURONAL LABELING OF SYMPATHETIC PREGANGLIONIC NEURONS FOLLOWING INJECTION OF TETANUS TOXIN C FRAGMENT INTO MUELLER'S EYELID MUSCLE.**
K.A. Manning, C. Evinger, and J.T. Erichsen. Dept. Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Mueller's muscle is a smooth muscle of the upper and lower eyelid that aids in lid opening. The muscle is innervated by cells in the superior cervical ganglion (SCG) and damage to the SCG produces lid ptosis. Although the preganglionic sympathetic neurons are situated in the spinal cord, it has not been possible to identify those cells which innervate specific targets via the SCG. We injected the atoxic C fragment of tetanus toxin (TTC) into the rat eyelid to localize: (1) the postganglionic SCG neurons that project to Mueller's muscle, and (2) the preganglionic spinal neurons that project specifically to these SCG neurons.

The eyelid of adult Sprague-Dawley rats was injected with 10ul of 15% TTC. After survival times of 5 to 55 hrs, the tissue was processed for immunohistochemistry and the TTC localized with a monoclonal antibody to TTC.

The data reveal a diffuse organization for the ganglionic and preganglionic innervation of Mueller's muscle. Retrogradely labeled cells are scattered throughout the SCG. At shorter survival times, the granular label appears to fill the cytoplasm. With longer survival times, the cell labeling diminishes except for the large grains that appear to outline the cell membranes. Although a majority of SCG cells remain unlabeled, light label is scattered throughout the ganglion, which may be the stained terminals of preganglionic fibers. Fibers of the SCG are poorly labeled. Lower cervical and upper thoracic spinal cord contain transneuronally labeled neurons. The majority of neurons are clustered in the intermediolateral nucleus. Others are scattered through the intercalated region and the lateral funiculus, although a few labeled cells lie in the central autonomic area. These regions have been previously reported to contain the neurons preganglionic to the SCG. Our data provide the first demonstration that preganglionic neurons for a specific motor target can be labeled transneuronally. The retrograde transneuronal labeling capabilities of TTC may help to eludicate the anatomical pathways of a wide variety of autonomic systems.

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49.19 CELLULAR INVESTIGATION OF THE ADAPTIVE GAIN CONTROL OF THE BLINK REFLEX: MODULATION OF ORBICULARIS OCULIS MOTONEURONS.
C. Evinger and K.A. Manning. Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794.

The blink reflex of mammals exhibits adaptive gain changes. The activity of the orbicularis oculis (OO) muscle (the lid closing muscle) increases when the eyelid experiences an opposition to lid closure. Likewise, OO activity decreases with facilitation of eyelid closure. As an initial step toward understanding the neural mechanisms underlying adaptive processes of the blink reflex, we have recorded the discharge of OO motoneurons before, during and after adaptation of the eyelid.

The blink reflex was evoked by electrical stimulation of the cornea in decerebrate rats. Electromyographic (EMG) electrodes in the eyelid provided a measure of OO muscle activity while microelectrodes in the facial nucleus recorded the activity of individual OO motoneurons.

Rats exhibited rapid and robust adaptation to restraint of the upper eyelid. Fewer than 20 consecutive blinks (mean interstimulus interval of 12 sec) during eyelid restraint resulted in an increase in the magnitude of the OO EMG. Within 30 trials the OO EMG reached its maximum increase of 50% over preadaptation levels. Upon freeing the eyelid, the OO EMG activity returned to preadaptation levels in 5 to 10 trials. The adapted OO EMG had a slightly shorter latency to corneal stimulation, but the primary change was the addition of longer latency bursts of activity. Individual OO motoneurons showed parallel changes during adaptation. The latency in response to corneal stimulation fell from 12-15 ms before lid restraint to a latency of 8-12 ms in the adapted state. The motoneurons also exhibited longer latency bursts of action potentials so that the overall level of discharge in response to corneal stimulation increased approximately 30-50% over preadaptation levels. Upon freeing the eyelid, motoneuron discharge returned to preadaptation levels in 5-10 trials. Thus, the OO EMG accurately reflects the discharge patterns of individual OO motoneurons. The rapid gain changes and the mechanical and neuronal simplicity of the blink reflex make this preparation ideal for investigating the cellular mechanisms of adaptation. Moreover, since the adaptive gain changes seen in this rat preparation match those occurring in alert rabbits and humans, similar processes may underly blink adaptation in all mammals.

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49.20 BILATERALITY OF THE SENSORY AND MOTOR PATHWAYS UNDERLYING BLINKING.
P.J. May and R. Baker. Dept. of Anatomy, Univ. of Mississippi Med. Ctr., Jackson, M.S. and Dept. of Physiology and Biophysics, New York Univ. Med. Ctr., New York, N.Y.

Blinking is most often a conjugate motor response requiring synchronous inhibitory control of both levator palpebrae muscles. The basis for this conjugacy was investigated in the cat. We previously described a population of cells lining the rostral and ventral aspects of the sensory trigeminal nucleus that were retrogradely labelled following injection of HRP into the caudal oculomotor nucleus. This pathway crossed the midline, traveled with the medial lemniscus to the level of the trochlear nucleus, turned dorsally and extended to terminate bilaterally, with a contralateral predominance, in and above the caudal pole of the oculomotor nucleus. Electrophysiological investigation revealed that antidromically activated levator palpebrae motoneurons were characterized by long latency (3.0-7.0 ms), small amplitude (<2.5 mV) hyperpolarizing synaptic potentials following electrical stimulation of either the ipsi- or contralateral orbital trigeminal nerves. EPSP-IPSP sequences (1.5 ms latency) were recorded following stimulation of either vestibular nerve. The latency of IPSPs evoked in levator motoneurons following bipolar central stimulation of the sensory trigeminal nucleus were shorter (1.8-3.0 ms). This IPSP was unaffected by acute transverse section (>3 mm deep) of the pons, including the MLF. Elimination of ipsilateral vestibular IPSPs in superior rectus motoneurons established the adequacy of the lesion, thereby indicating inhibition of the levator motoneurons was not the result of current spread to the ipsilateral vestibular nucleus, MLF or predorsal bundle fibers. Axons recorded within the levator subdivision displayed multiple action potentials, characteristic of trigeminal sensory division cells, and were activated from one set of orbital and central trigeminal electrodes with latencies of 3.0 and 1.5 ms, respectively. Intra-axonal HRP injection revealed that these axons terminated extensively within the levator subdivision and the adjacent supraoculomotor area. Intracellularly HRP injected levator motoneurons possessed ten or more primary dendrites that radiated to form a dendritic field (1.4 mm across) filling the levator subdivision and adjacent supraoculomotor area bilaterally. We conclude that the trigeminal sensory input from both sides reaches all levator motoneurons. More surprising, levator motoneurons were encountered that could be antidromically activated from both IIIrd nerves, demonstrating axon branches innervate both levator muscles. Thus the entire neuronal circuitry is organized to produce conjugate blinking.

Supported by EY05689 and NS13742.

51 WORKSHOP. COCAINE: MODULATION OF MONOAMINE FUNCTION. J.M. Lakoski, Univ. Texas Med. Br., (Chairperson); D.C.S. Roberts, Carleton Univ., J.E. Smith, Louisiana State Univ; F.J. White, Univ. Illinois; K.A. Cunningham, Univ. Texas Med. Br.; T.V. Dunwiddie, Univ. Colorado Health Sci. Center; M.P. Galloway, Wayne State Univ.; J.T. Williams, Oregon Health Sci. Univ.; and R.A. Wise, Concordia University.

A resurgence of interest in the neuropharmacology of cocaine has been prompted by the widespread abuse of this drug. Neuropharmacological effects of cocaine have recently been expanded with regard to interactions of cocaine with monoaminergic neurons. This workshop will provide a forum for discussion of current research to define the role of monoamine neurotransmitters in the potent pharmacologic effects of cocaine. Results obtained from behavioral, neurochemical, and electrophysiological approaches will be presented to provide new insights into the specific interactions of cocaine with monoaminergic neurons.

The workshop will first address the modulatory role of cocaine on monoaminergic function from a behavioral perspective. Studies identifying the neural substrates that underlie cocaine self-administration behavior, particularly with respect to dopamine, will be presented by David C.S. Roberts. Studies of the behavioral factors involved in the reinforcing properties of cocaine, with respect to monoaminergic function, will be presented by James E. Smith. These behavioral approaches will then be complemented by both in vivo and in vitro electrophysiological studies of cocaine's modulatory effects on monoamine function. Francis J. White will present electrophysiological data on the actions of cocaine on the mesoaccumbens dopamine system as related to cocaine's reward properties. Cocaine's specific interactions with serotonergic neuronal systems, as assessed by electrophysiological techniques, will be presented by Kathryn A. Cunningham. The modulatory effects of cocaine in the hippocampus and ventral tegmental area, utilizing both in vitro electrophysiological and neurochemical approaches, will be addressed by Thomas V. Dunwiddie. In addition, Matthew P. Galloway will discuss modulation of mesocortical and striatal dopamine synthesis following acute and chronic cocaine administration. These multidisciplinary approaches will provide state-of-the-art evaluations of the modulatory actions of cocaine on monoaminergic function.

An added feature of this workshop will be an open panel discussion of issues raised during the sessions which will be led by John T. Williams and Roy A. Wise. Major issues relevant towards understanding how cocaine produces its complex pharmacological profile will be addressed.

52 SYMPOSIUM. THE BASICS OF MOLECULAR BIOLOGY. J.L. Roberts, Stanford University; R. Goodman*, Tufts University; J. Brosius*, Columbia University.

This symposium will review many of the basic concepts and methodology involved in the understanding of and the use of molecular biological techniques to study questions of interest to neurobiologists. The first speaker, Dr. Scheller, will discuss how genes are organized in the chromosome and what technologies are used to isolate specific genes. There will be discussion of multiple gene families and some of the difficulties of deciphering their structures and functions. The next speaker, Dr. Richard Goodman will give a general description on how genes are transcribed by RNA polymerases, and how this transcription is regulated during development and after hormonal or environmental manipulation. Particular emphasis will be given to using molecular biological techniques to dissect the structure of the promotor regions of several important neuropeptide genes. Dr. Roberts, the third speaker, will discuss a later area of gene regulation, that of stability of cytoplasmic mRNA and efficiency of translation of those RNAs. This talk will cover how these post-transcriptional events become very important in determining exactly how much protein is synthesized from any specific gene product. Finally, Dr. Brosius, will speak on the use of eurkaryotic gene expression systems for the production of specific eukaryotic gene products. With the current ability to isolate intact genes and express them in heterologous cells, this approach has proved very advantageous to producing large quantities of a specific protein for subsequent biological studies to elucidate the function of these proteins. This series of talks should give the audience a brief overview of the events in the production of a specific gene product as well as an understanding of how the modern techniques of molecular biology are used in characterizing these gene products.

ACTION POTENTIALS AND ION CHANNELS V

53.1 A NEW TYPE OF K CHANNEL THAT CONTROLS THE RESTING POTENTIAL OF THE NERVE AXON. D.C. Chang. Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

Unlike the case of the action potential, the membrane pathways responsible for the generation of the resting potential of a nerve cell have not been well understood. It is unclear whether the resting current passes mainly through a small number of excitable K and Na channels that stay open in the resting state, or, as some investigators have suggested, the pathway for resting current may simply be a "leakage" conductance. Using the internally perfused squid axon as a biological model, we studied the effects of various cations on the membrane potential, membrane conductance, and isotopé-labelled efflux in the resting state. We found evidence that the semi-permeable property of the resting membrane is controlled by a new class of K channel. This resting K channel has a slight tendency of inward rectification and is activated at a voltage below the normal resting potential. It's ion-selectivity sequence, as determined from our V-clamp studies, $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+ \geq Li^+$, is similar to that of the resting membrane. The pharmacological properties of this resting channel seem to differ from those of the delayed rectifier K channel. For example, this resting channel is less sensitive to TEA^+ (tetraethylammonium) but is affected by internal Rb^+. Furthermore, the K efflux through this resting channel responds to a change of the external ionic environment in a manner different from that of the delayed rectifier. When the external concentration of K^+ increases, the K^+ efflux is actually elevated. These findings suggests that this new class of K channel may be structurally different from the delayed rectifier K channel. (Supported by ONF Contract N00014-85-K-0424 and NSF grant BNS-84-06932.)

53.2 CHARACTERISTICS OF 4-AP, TEA AND CA^{2+} INSENSITIVE VOLTAGE-DEPENDENT SLOW OUTWARD CURRENT IN STATOCYST HAIR CELLS OF HERMISSENDA CRASSICORNIS. L.M. Grover & J. Farley. Prog. Neurosci. & Behav., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Voltage-clamp analysis of ionic currents in statocyst hair cells of Hermissenda has revealed the presence of three distinct components of outward current. Two appear to be identical to currents observed in a number of other preparations. These are: (1) A fast, transient, 4-AP sensitive potassium current (I_A). Activation of this current is steeply voltage dependent at potentials positive to -40 mV. At a steady-state membrane potential of -45 mV I_A is half-inactivated. (2) A delayed, TEA sensitive outward current (I_K), that shows substantial activation only at potentials above 0 mV.

We have also observed a third outward current which resists block by 5-10 mM 4-AP and 100 mM TEA. Although the kinetics of this current are similar to a calcium-activated potassium current seen in other central neurons of Hermissenda, this current is not calcium dependent. Removal of calcium ions from the bath does not reduce the magnitude of this current, although an inward calcium current is markedly reduced by this treatment. Elevation of extracellular calcium (from normal 10 mM to 100 mM) does not enhance the outward current, although I_{Ca} is increased. Calcium channel blockers (5 mM cadmium or 10 mM cobalt) which suppress the calcium current do not reduce the 4-AP and TEA resistant current. Intracellular injections of EGTA also do not reduce this current. Finally, the current-voltage relationship of the 4-AP and TEA resistant current does not display the characteristic "N" shape expected of a calcium-dependent current. Measurements of the reversal potential of this delayed current (made in 5 mM 4-AP, 100 mM TEA and 10 mM Co^{2+}) indicate that this is a potassium current, since it reverses near -70 mV, and varies in the expected direction with changes in $[K^+]_o$. This current shows no sign of inactivation, even with maintained depolarizations (5-15 sec) to relatively positive potentials (0 to +20 mV).

In preliminary patch-clamp experiments (cell attatched) we have recorded a 35 pS channel that may underlie this delayed outward current. As one would expect from the macroscopic current, the channel is open a large fraction of the time (steady-state) at potentials more positive than +20 mV.

It is likely that this current contributes in important ways to the integrative properties of the hair cell, since the other outward currents present in the soma membrane are either largely inactivated (in the case of I_A) or insufficiently activated (in the case of I_K) at the normal resting potential of these cells (-35 to -50 mV). Supported by NASA grant NAG2-397.

53.3 POTASSIUM CURRENTS UNDERLYING AFTERHYPERPOLARIZATIONS (AHPs) AND SPIKE REPOLARIZATION IN RAT HIPPOCAMPAL PYRAMIDAL CELLS (CA1).
J.F.Storm, Dept.Neurobiology & Behavior, SUNY Stony Brook, NY11794.

Action potentials in CA1 cells are followed by three AHPs: a fast (fAHP), a medium (mAHP), and a slow one (sAHP). The sAHP is due to a slow Ca-activated K-current, I_{AHP} [Lancaster & Adams, J.Neurophys. 55:1268]. Spike repolarization (s.r.) and the fAHP involve a fast Ca-activated K-current which is sensitive to 1mM tetraethylammonium (TEA) [Storm,J.Physiol.385:733]. Here, two questions are addressed: (1) Which currents underlie the mAHP ? (2) Is the Ca-dependent TEA-sensitive current underlying s.r./fAHP, transient [Zbicz & Weight,J.Neurophys.54:1034] or persistent (I_C [L.&A.]) ? These problems were studied in slices (28-33°C) using current clamp and single electrode voltage clamp (v.c.).

The mAHP, following trains of 2-8 spikes elicited by current injection, was (a) apparently less voltage sensitive than sAHP; (b) resistant to 0.2mM Cd or 4mM Mn, but reduced by 0.5-2mM Ba; (c) reduced 40-80% by 40µM carbachol (reversed by atropine) when the membrane potential was close to -60mV, but not at -80mV; (d) abolished by 2mM Cs at -80mV but not at -60mV. The mAHP seems also to be TEA-sensitive. This suggests that three currents contribute to the mAHP: I_M (a slow K-current blocked by carbachol or Ba [Halliwell & Adams,Brain Res.250:71]), I_Q (a Cs-sensitive inward current activated at hyperpolarized potentials, and deactivated by depolarization [H.&A.]), and I_K ("delayed rectifier" [Segal & Barker,J.Neurophys.51:1409], blocked by TEA, but not by carbachol). The contribution of each may vary with the membrane potential: I_M seems to dominate around -60mV (c.f. carbachol effect), whereas I_Q dominates at -80mV (c.f. Cs effect). The recruitment of I_Q may explain why mAHP was not reversed by hyperpolarization (a).

Under v.c. (V_h=-60mV), the fast tail current (τ~50ms) following 0.1-0.5s commands to -10 to -40mV (which should activate I_C [L.&A.]), showed (a) surprisingly little Ca-dependence (0-15% reduced by Mn/low Ca); (b) was reduced 40-60% by 40uM carbachol (reversed by atropine); and (c) was reduced 20-70% by 1-10mM TEA. Thus, this fast tail seems to correspond to the Ca-independent mAHP (rather than s.r./fAHP), and may represent a mixture of a carbachol-sensitive (I_M ?) and a carbachol-resistant current (I_K?). On the other hand, a Ca-dependent, TEA-sensitive (1mM) transient (lasting 5-20ms) current [Z.&W.], here called I_{CT}, was observed during v.c. commands positive to -45mV. I_{CT} may be the current involved in s.r./fAHP. Finally, a Ca-independent, carbachol-resistant, TEA-sensitive (10mM) outward current (I_K?) activated (τ~130ms) and subsequently decayed (τ~1.7s at -35mV) during commands positive to -40mV. The M-current also appeared to be TEA-sensitive.

Conclusions: (1) I_M, I_Q and I_K may underlie the medium AHP; (2) a transient Ca-dependent TEA-sensitive K current may underlie spike repolarization and the fast AHP. (Supported by NIH grant NS18579.)

53.4 SMALL CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNELS IN HIPPOCAMPAL NEURONS.
B. Lancaster, D.J. Perkel* and R.A. Nicoll, Depts. of Pharmacol. and Physiol., Univ. of Calif., San Francisco, CA 94143.

Single-channel recordings were made from inside out patches of membrane obtained from hippocampal neurons in primary culture. Patches were positioned in a continuous flow system which allowed exchange of solution at the cytoplasmic face of the patch. Except where noted, solutions bathing the patch were symmetrical, containing (mM) 140 KCl, 10 NaCl, 1 $MgCl_2$, 10 HEPES, 80 µM EGTA.

When 1 µM free Ca^{2+} was perfused across the cytoplasmic surface, two channel types were activated. 1. Large conductance channels (~200 pS) which opened for longer periods at depolarized potentials (Brett and Lancaster, Soc. Neurosci. Abstr., 11:954, '86). 2. Channels with slope conductance~19 pS. This second channel could be observed at hyperpolarized potentials, where the larger channel is closed due to its voltage sensitivity. At membrane potentials between -40 and -110 mV, 1 µM $[Ca^{2+}]_i$ caused the small conductance channel to remain open most of the time. Both channel types were observed if KCl was replaced by $KMeSO_4$ and are thus likely to be Ca^{2+}-activated K^+ channels (Blatz and Magleby, Nature, 323:718, '86; Lang and Ritchie, Soc. Neurosci. Abstr., 12:560, '86). Neither channel type was sensitive to apamin (25 nM) in the pipette.

Under normal conditions, the small conductance channel displayed inward rectification, i.e. no clear outward currents were observed positive to 0 mV. This rectification was not affected if Na^+ was removed from either side of the membrane. When Mg^{2+} was removed from the cytoplasmic face of the patch, Ca^{2+}-activated outward currents could be observed at the depolarized potentials. Under these conditions addition of 1 mM Mg^{2+} induced channel flickering. The observations are consistent with a small conductance Ca^{2+}-activated K^+ channel, lacking the voltage dependence of the large channel and which displays inward rectification due to Mg^{2+} block from the intracellular face (Vandenberg, Biophys. J., 51:366a, '87).

Cultured hippocampal neurons can display a long-lasting slow afterhyperpolarization (AHP) following a burst of spikes. As in the slice preparation, this can be abolished by 8-Br cAMP (2 mM final bath concentration). Cell-attached patches have revealed long-lasting activation of a K^+ channel following a burst of spikes. Activation of this channel parallels the time course of the AHP and the conductance is estimated to be no greater than 15 pS (pipette contained 140 NaCl, 10 HEPES, 4 $MgCl_2$, 1.3 KCl). The relation between channel activation and the AHP is being investigated.

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53.5 CALMODULIN METHYLATION AND CALCIUM-DEPENDENT CURRENTS IN PARAMECIUM,
M.A. Wallen-Friedman, Y. Saimi*, D.-Z. Lu*, J.E. Colquhoun*, D.L. Nelson*, C. Kung*, Neuroscience Training Program, Department of Molecular Biology and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, 53706.

We used the eukaryotic unicell Paramecium tetraurelia as a model for excitable cells to study ion-channel function and regulation. It has been shown that a mutant, pntA, with a defect in calmodulin (CaM), has a greatly reduced current through the Ca^{++}-dependent K^+ channel. Normal current and behavior is restored when wild-type CaM is microinjected into pntA (Hinrichsen et al, 1986, Science 232, 503-506). pntA CaM has a single substitution of phenylalanine for serine at residue 101 of the wild-type CaM (Schaefer et al, 1987, PNAS, in press).

We have isolated a mutant, pntD, which also has a greatly reduced Ca^{++}-dependent K^+ current, but unlike pntA, has an increased Ca^{++}-dependent Na^+ current as well. The CaM from pntD differs from wild-type CaM and from pntA CaM in its migration pattern on native, on acid-urea and on isoelectric-focusing gel electrophoresis. Cytoplasmic fractions containing either pntD or wild-type CaM, when microinjected into pntA, restore equally wild-type behavior in pntA. Thus, it appears that the pntD lesion differs from and complements the pntA defect. Microinjection of wild-type CaM into pntD does not restore normal behavior to the pntD mutant.

CaM from wild-type Paramecium has 2 sites of post-translational lysine-N-methylation. Both of these sites are almost fully methylated in vivo; thus wild-type CaM is a poor substrate for N-methylation in vitro. In contrast, pntD CaM is heavily N-methylated in vitro, suggesting that the mutant CaM is undermethylated in vivo. Methylating activities in pntD and wild-type supernatant fractions appear to be similar. Experiments are underway to compare demethylating activity in wild type and pntD.

Ion channel regulation by cytoplasmic proteins is important in many neurophysiological processes, e.g. short-term memory. How CaM mediates or modulates the Ca^{++}-dependent K^+ current and the Ca^{++}-dependent Na^+ current may be addressed using this unique combination of mutations, functional restoration, biochemical analysis and electrophysiological measurements. Supported by NIH GM22714, GM36386, GM34906 and GM32514.

53.6 QUANTITATIVE CALCIUM-DEPENDENCE OF WHOLE-CELL NONSPECIFIC CATION AND POTASSIUM CURRENTS ACTIVATED BY INTRACELLULAR CALCIUM RELEASED FROM CAGED CALCIUM (NITR) CHELATORS.
R. S. Zucker and L. Landò. Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

The relationship between whole-cell membrane current and intracellular calcium concentration was measured for two calcium-activated currents in large neurons in the abdominal ganglion of Aplysia californica. We used new tetracarboxylate 2-nitrobenzhydrol chelators, which are converted in 2 msec from a high affinity form (K_D = 630 nM for NITR-5, 215 nM for NITR-7, at 300 mM ionic strength) to a low affinity nitrosobenzophenone (K_D = 18 µM and 7.7 µM respectively) on exposure to a 0.5 ms flash of about 0.2 J/cm^2 of ultraviolet light between 340 and 380 nm. Computer simulations were developed to predict the average changes in free calcium concentration, depending on the average light intensity after absorption in a spherical cell, the concentrations of NITR and total calcium, and the rate of extrusion of calcium by active pumps. The simulated effects of successive flashes of light were confirmed by in vitro and in vivo spectrophotometric calibrations of 5-30 mM NITR, 60-90% bound to calcium, mixed with 0.25 mM arsenazo. The arsenazo absorbance changes at several visual wavelengths were used to detect calcium concentration change. We also measured the change in calcium concentration as a function of incident light-flash intensity.

Calcium-activated potassium currents were recorded from neurons voltage-clamped to the reversal potential for the nonspecific cation current (about -25 mV), or in which the nonspecific current was blocked in sodium- and calcium-free sea water. Calcium-activated nonspecific cation currents were isolated with 50 mM tetraethylammonium to block the calcium-dependent potassium current. The magnitudes of both currents were linearly related to calcium concentration changes over the range 0.1-20 µM, using different degrees of calcium loading of NITR-5 and NITR-7. This first-order stoichiometry was independent of membrane potential between -10 and -40 mV.

A computer model of the redistribution of calcium and free and bound nitrobenzhydrol, nitrosobenzophenone, and native buffer following a flash replicated the time course of decay of calcium-dependent currents from their peak after a flash to a steady-state level about one minute later. This decay is sensitive to the stoichiometry of calcium activation, and again indicated first-order calcium-dependence for both currents at all potentials between -10 and -40 mV. Sodium/calcium exchange hastened the decay of the currents slightly. Our diffusion model also predicted the slower decay of currents following a weak continuous light, due to the diffusional equilibration taking place during the light exposure.

The results suggest that different decay rates of these currents following a burst or depolarizing pulse in bursting pacemaker neurons are not due to differences in calcium stoichiometry, but perhaps to differences in voltage-dependent relaxations. Supported by NIH Grant NS 15114.

53.7 CO-LOCALIZATION OF Ca CHANNELS WITH Ca-ACTIVATED K CHANNELS IN HAIR CELLS OF THE BULLFROG'S SACCULUS. W. M. Roberts and A. J. Hudspeth. Department of Physiology, University of California School of Medicine, San Francisco, CA 94143-0444.

Hair cells from the bullfrog's inner ear exhibit electrical resonance that contributes to their frequency selectivity. The resonance arises from an interaction among calcium (Ca) channels, calcium-activated potassium (K_{Ca}) channels, and membrane capacitance; it occurs at a frequency that is largely determined by the time course of the K_{Ca} current. The K_{Ca} current is activated very rapidly in these cells by step depolarizations, rising to half its peak value in 1-4 ms. During this time, the Ca^{2+} concentration at a K_{Ca} channel is probably influenced by the number of Ca channels within a radius of less than 1 μm. The spatial distributions of these two channel types may therefore play an important role in determining the activation rate of the K_{Ca} current.

Using loose-seal pipettes to record ionic currents through small patches (10-50 μm^2) of membrane, we mapped the distributions of Ca channels and K_{Ca} channels on the basolateral surfaces of enzymatically isolated hair cells. Voltage-clamp steps were applied intracellularly through a separate tight-seal pipette, which was also used to measure the whole-cell current. A collagen plug at the tip of each loose-seal pipette prevented damage to the membrane when suction was applied to maintain an adequate seal resistance.

Ca-current density varied more than ten-fold among patches, with as much as 20% of a cell's current sometimes confined to a single patch. All patches that displayed Ca current also showed K_{Ca} current in a ratio similar to that observed in the whole-cell current; the two types of channels are thus highly co-localized.

To test the possibility that K_{Ca} channels are present in patches devoid of Ca channels, but cannot be activated due to a lack of local Ca^{2+} influx, we perfused cells internally with solutions in which the free Ca^{2+} concentrations were buffered at 10-100 μM with 60-100 mM HEDTA. Under these conditions, depolarizing voltage steps from a holding level of -70 mV elicited a K_{Ca} current with an exponential, rather than the usual sigmoidal, time course. This result confirms that the Ca^{2+} concentration at K_{Ca} channels was effectively elevated. Even with high intracellular concentrations of free Ca^{2+}, which should have activated all of the K_{Ca} channels in a cell, depolarizing voltage steps nevertheless elicited a K_{Ca} current of an amplitude similar to that seen with normal intracellular solution. Most K_{Ca} channels are therefore located close enough to Ca channels to be activated by the influx of Ca^{2+}. The clustering of Ca and K_{Ca} channels is expected to result in a more rapid rise in intracellular Ca concentration at the K_{Ca} channels, and hence a more rapid activation of the K_{Ca} current, than would occur if the channels were uniformly distributed across the cellular surface.

Supported by NIH grants NS22389 and NS07904.

53.8 **MESSENGER RNA SYNTHESIS DEPENDENCE OF POTASSIUM CURRENTS AND A CRITICAL PERIOD FOR MATURATION OF THE ACTION POTENTIAL.** Angeles B. Ribera and Nicholas C. Spitzer. Department of Biology, University of California, San Diego, La Jolla, CA 92093.

The action potential of embryonic *Xenopus* spinal neurons matures from a long duration, largely calcium dependent impulse to a brief sodium dependent spike during the first day of development in culture (Spitzer and Lamborghini, 1976). Whole-cell voltage clamp analysis shows that changes in outward potassium current are more substantial than those in inward sodium and calcium currents (O'Dowd, 1983a; Ribera et al., 1986) and suggests that potassium currents play a major role in the maturation of the action potential. The development of the Na dependent spikes is blocked by chronic inhibition of RNA synthesis begun before the end of a sensitive period (3-18 hr in culture), but not at later times (O'Dowd, 1983b); the development of inward currents is largely unaffected (O'Dowd, 1985). We have determined whether mRNA synthesis is required for development of outward currents. In addition, we have examined whether there is a critical period for the mRNA synthesis required for development of the action potential.

The reversible inhibitor of mRNA synthesis, 5,6-dichloro-1-β-D-ribobenzamidazole (DRB; 50 μM), was added to cultures 3 hr after plating. Continued presence of the drug from the time of plating prevented extension of neurites; when the drug was removed within a few hr, however, neurite extension was delayed by a comparable interval, suggesting that the action of the drug is reversible in these neurons.

Whole-cell voltage clamp methods were used to record voltage dependent potassium current. Chronic inhibition of mRNA synthesis blocked the developmental 3.5X increase in current density and doubling of activation rate previously reported for potassium current (Ribera et al., 1986). Intracellular recording under current clamp was used to elicit and record action potentials. The duration of the impulse, providing an index of its ionic dependence and maturity, was 1.6±0.2 ms ($n \geq 10$, mean±SEM) after 1 day in culture. In young neurons (6-9 hr), the action potential lasted 34±9 ms. Neurons that were subjected to chronic mRNA synthesis inhibition for 1 day had impulse durations of 72±16 ms, probably since transcription was arrested 3-6 hr before the time at which young neurons were examined. This value declined to 33±11 during the second day but is unlikely to reflect renewal of mRNA synthesis, since controls show that the drug is stable over this period. When DRB was applied acutely for 15 hr and the cells examined 1 day after washing out the inhibitor, the action potential lasted 23±8 ms.

These results indicate that chronic inhibition of mRNA synthesis blocks maturation of potassium currents, consistent with a major role for these currents in determining the mature form of the action potential. Furthermore, acute inhibition is sufficient to prevent the shortening of the impulse during neuronal differentiation, suggesting that a critical period of mRNA synthesis is associated with the development of the action potential.

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53.9 EXPRESSION OF mRNA FOR THE CHICK BRAIN EARLY K CURRENT IN FROG OOCYTES; A BIOASSAY FOR cDNA CLONING OF THIS mRNA. I. Lotan*, A. Volterra, P. Dash*, S.A. Siegelbaum and P. Goelet*. HHMI, Ctr. for Neurobiol. & Behav., Columbia Univ., and NYS Psych. Inst., NY, NY 10032.

To clone the early potassium channel gene (A channel), for which there is no sequence information, we are developing a genetic approach based on hybrid arrest of translation of mRNAs microinjected into Xenopus oocytes.

Poly-A^+ RNA from chick embryo brain was injected into Xenopus oocytes (50-100 ng mRNA per oocyte). Four days later, the oocytes were assayed for ionic currents using a two microelectrode voltage clamp. The oocytes consistently expressed, in a concentration-dependent manner, both transmitter and voltage activated ionic currents, including GABA and kainate induced currents as well as fast TTX sensitive sodium current and early (A) K^+ current.

The early K^+ current was blocked by 4 mM 4-AP but was insensitive to 30 mM TEA, 4 mM Co^{2+}, and the calcium chelator BAPTA. The current activated following depolarizations positive to -50 mV, reached a peak outward value within 10-20 msec, depending on the donor, and then rapidly inactivated. Steady-state inactivation showed an S-shaped dependence on membrane voltage, with half-inactivation occurring at a holding potential of -77 ± 1.28 mV, and a slope factor for inactivation of -4.9 ± 0.5 mV (mean ± S.D., n = 4). These properties are very similar to the properties of the A current measured in both invertebrate and vertebrate neurons. The early K currents were never observed in control oocytes or in oocytes injected with liver mRNA (at up to 100-fold higher concentrations).

We have used chick ovalbumin and rabbit globin mRNAs to determine appropriate conditions for hybrid arrest in the Xenopus oocyte. Although both complementary RNA and DNA sequences to these mRNAs, generated from pKSM13 cDNA recombinants, selectively and quantitatively block in vitro translation of ovalbumin and globin mRNAs in reticulocyte lysates, in vivo translation of these mRNAs in the Xenopus oocytes was quantitatively arrested only by complementary DNA sequences.

To generate antisense DNA from chick brain mRNA that could be screened for the arrest of the expression of the early K^+ current, we have constructed an orientation-specific cDNA library from chick brain mRNA in pKSM13. This plasmid vector permits the preparation of strand-selected, single-stranded cloned DNAs. We are currently testing the ability of single-stranded DNA prepared from this cDNA library to arrest expression of the mRNA in Xenopus oocytes. By testing successively smaller subpools of recombinant cDNA from this library, we hope to isolate a cloned sequence, which by hybrid arrest blocks the translation of potassium A current mRNA in the oocyte.

53.10 DIVERSITY OF VOLTAGE DEPENDENT POTASSIUM CHANNELS INDUCED IN XENOPUS OOCYTES BY TOTAL AND FRACTIONATED RAT BRAIN mRNA. J.H.Hoger*, I.Ahmed*, N. Davidson*, H.Lester, & B. Rudy. Divs. of Biology & Chemistry, Caltech, Pasadena, CA 91125. and Dept. of Physiology & Biophysics N.Y.U. Med. Ctr., N.Y., N.Y.. 10016.

K channels show a high degree of diversity, varying in voltage dependence, kinetics, pharmacology, and single-channel properties. The types of K channels present in the brain are incompletely characterized due to the difficulties of applying present electrophysiological techniques to many cell types and regions of the cell. In order to characterize brain voltage-dependent K channels we have prepared poly(A) RNA from rat brains by a LiCl-urea procedure. This poly(A) RNA was injected into prepared Xenopus oocytes and expression of voltage dependent K channels was measured by two micro-electrode voltage clamping. This technique may allow the study of the source of diversity of K channels, because all the different channels are expressed in the same system. The K currents observed include a fast transient component with very similar characteristics to the "A" current and a "delayed rectifying" current. The delayed rectifying current may be composed of more than one component as determined by inactivation properties, activation and deactivation kinetics, and sensitivity to externally applied 4-aminopyridine(4AP), tetraethylammonium(TEA), and toxin I from Black Mamba. We have fractionated the mRNA by centrifugation on linear sucrose gradients (6-20%). A delayed rectifier type current was expressed by mRNA comigrating with the sodium channel alpha subunit mRNA(7.5-9.5Kb). This delayed rectifier type current may still contain more than one component; it is not completely blocked by TEA and it still contains different deactivating components. However, the "A" current is not expressed or greatly reduced in this fraction. A fraction of smaller size (6-8Kb), in contrast, expresses an "A" current and a "delayed rectifier" component. These results indicate that different voltage-dependent K channels are encoded by different mRNAs and are therefore composed of different polypeptides which may in part account for the diversity of voltage dependent potassium channels. Supported by NIH GM-26976, GM-10991, GM-29836, and Fellowships from American Cancer Society and NIH.

53.11 EXPRESSION OF DIFFERENT "A" CURRENTS IN XENOPUS OOCYTES INJECTED WITH TOTAL OR FRACTIONATED RAT BRAIN mRNA. B. Rudy, J.H. Hoger*, N.Davidson* & H. Lester.(SPON:J.Pollock) Dept. of Physiology & Biophysics New York Univ. Med. Ctr., N.Y.,N.Y. 10016. and Divs. of Biology & Chemistry, Caltech, Pasadena, CA. Xenopus oocytes injected with rat brain mRNA express a fast transient K current with very similar properties to the "A" currents observed in molluscan and mammalian neurons. The current has a relatively low threshold, activating at around -60 mV before there is significant activation of delayed rectifying type current. The rate of activation of this current is voltage dependent and often it can only be resolved properly for small depolarizations, where the time constants are of the order of 5 msec at room temperature. The activation time constant decreases as the depolarization is increased. The current inactivated with a relatively voltage-independent time constant of about 30-40 msec at room temperature. Steady-state inactivation by long prepulses is complete at -50 mV and half inactivation occurs at around -65 mV. The current is completely blocked by 5 mM 4-AP and half blocked at 2 mM. The degree of block at a given 4-AP concentration decreases with increasing depolarization, and decays during sequential pulses at frequencies higher than 0.5 sec $^{-1}$. The "A" current is insensitive to external TEA at concentrations of up to 40 mM.We have observed the expression of this current to be of variable magnitude with the same RNA, suggesting that other factors probably related to oocyte variability are important for its expression. Oocytes injected with sucrose gradient fractionated RNA express a fast transient K current when injected with a fraction of 6-8 kB. This current differs from the current observed when total mRNA is injected in that; (a)its activation and inactivation kinetics are faster and; (b)it is less sensitive to 4-AP. 5 mM 4-AP blocks the current for small depolarizations only. Even 10 mM 4-AP does not completely block the current. However, 4-AP block of this current shows the same type of voltage and frequency dependence as that of the current observed in oocytes injected with total mRNA. Furthermore, steady-state inactivation of this current by prepulses remains unchanged. We propose that the altered pharmacology is the result of the change in kinetics, as can be expected for a state-dependent block. Several explanations for these results will be considered. For example, this current may represent a distinct, fast transient K current or its altered kinetics may result from the fact that the channel contains more than one polypeptide. We will report on experiments attempting to reconstitute the original kinetics and pharmacology. Supported by NIH GM-26976, GM-10991, GM-29836, and a Fellowship from American Cancer Society.

53.12 COMPUTER SIMULATION OF THE EFFECT OF NON-INACTIVATING SODIUM CHANNELS ON EXCITABILITY. I.Z. Steinberg* (SPON: U.Z. Littauer). Chemical Physics Dept., Weizmann Institute of Science, Rehovot 76100 Israel.

Non-inactivating sodium channels (NI-NaC) have been discovered in various cell types, like Purkinje cells (Llinas & Sugimori, J. Physiol., 305:171,1980), the Squid axon (Shoukimas & French, Biophys. J., 32:857,1980; Rakowski, De Weer & Gadsby, Biophys.J., 47:31a, 1985), and frog oocytes (L.C.Schlichter, Dev.Biol., 98:47,1983; Baud, Kado & Marcher, Proc.Natl.Acad.Sci.,USA, 79:3188,1982). Additionally, normal voltage-gated sodium channels lose their inactivation capabilities following treatment with pronase (Armstrong, Bezanilla & Rojas, J.Gen.Physiol.,62:375, 1973) or certain chemicals and toxins. The presence of NI-NaC in a cell membrane profoundly modifies the electrical properties of the cell, since opening of these channels and cell depolarization reinforce each other without intrinsic control. Computer simulations were performed to systematically explore the pattern of behavior of excitable cells which have NI-NaC in their membranes. The Hodgkin and Huxley (H&H) equations for space-clamped cells were used to describe the changes in electric potential, except that the sodium currents were represented by the sum of two terms:
$FI \cdot g_{Na} \cdot m^3 \cdot h \cdot (V-E_{Na}) + (1-FI) \cdot g_{Na} \cdot m^3 (V-E_{Na})$, where $FI (0 \leq FI \leq 1)$ is the fraction of sodium channels which inactivate normally, and the other symbols have their usual definition. The simulations thus quantitatively apply to pronase-treated squid axons, but may serve to qualitatively illustrate patterns of electrical activity introduced by NI-NaC generally.

A rich repertoire of possible types of electrical behavior is obtained: Normal behavior, including capability of firing action potentials, requires values of FI which are not far from unity, the permissible range depending on g_K. Bistability, at which the cell may exist in one of two stable states of different resting potential, occurs when the value of FI is lowered. Transitions from the polarized to the depolarized resting states, and vice versa, may be brought about by depolarizing and hyperpolarizing triggers, respectively. Such behavior is like that of memory storage devices. Monostability at depolarized potentials is favored by low FI values and can occur if g_K is less than the H&H value. Repetitive firing is obtained for values of g_K which are appreciably lower than the H&H value and for values of FI which are close to unity. Note, however, that it may occur also with the H&H values of the ionic conductivities if the deactivation kinetics of the NI-NaC is slowed down. Inverse action potentials, following a hyperpolarizing trigger when the cell is in the depolarized resting state, are favored by FI values which are in the vicinity of the transition between monostability at depolarized potentials and bistability.

EXCITATORY AMINO ACIDS: PHARMACOLOGY I

54.1 PERTUSSIS TOXIN INHIBITS THE STIMULATION OF INOSITOL PHOSPHOLIPID HYDROLYSIS BY GLUTAMATE IN PRIMARY CULTURES OF CEREBELLAR NEURONS J.T. Wroblewski*, F. Nicoletti*, E. Fadda*, J. Mazzetta* and E. Costa (SPON: W.J. Wojcik). Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Med. Sch., Washington, DC 20007.

In cerebellar granule cells the hydrolysis of membrane phosphatidylinositol (PI) is coupled to two subtypes of metabolotropic glutamate receptors. The G_{P1} receptor is activated by glutamate, aspartate and N-methyl-D-aspartate (NMDA), is inhibited by 2-amino-5-phosphonovaleric acid (APV), Mg^{2+} and negatively modulated by phencyclidine. The G_{P2} receptor is insensitive to the above inhibitory actions and is activated selectively by glutamate and quisqualate.

The incubation of cultured granule cells with pertussis toxin (PTX, 2 ug/ml, 16 h) results in a selective depression of the signal transduction at the G_{P2} receptor. After this treatment the stimulation of PI hydrolysis by quisqualate is reduced by 65%, while the stimulatory action of NMDA remains unaffected. PI hydrolysis stimulated by glutamate is reduced by only 30%, due to the action of glutamate at both PI-coupled receptors. When the action of glutamate at the G_{P1} receptor is eliminated by the addition of 1 mM APV, the remaining PI hydrolysis is inhibited by 70% by PTX treatment.

In cultured granule cells, PI hydrolysis is also stimulated by the cholinergic muscarinic agonist carbachol. However, treatment with PTX fails to affect this stimulation. Two ionotropic excitatory amino acid receptors are present in cultured granule cells, G_{C1} and G_{C2}, and are coupled to increased Ca^{2+} influx and cyclic GMP formation. PTX treatment does not reduce the signal transduction at these glutamatergic receptors.

The possible involvement of a GTP-binding protein in the signal transduction mechanism of the G_{P2} receptor was studied additionally in preparations of membranes obtained from cultured granule cells. Although membrane preparations did not preserve the stimulation of PI hydrolysis by transmitter receptor agonists, they responded with increased PI hydrolysis to the application of GTP-γ-S (stable GTP analogue). The same membrane preparations were used to study the effect of GTP on the specific binding of [^{3}H]glutamate. Additions of 50 uM GTP-γ-S resulted in a decrease of specific glutamate binding, reducing by 30% the number of binding sites, while not affecting the affinity.

These data indicate the presence in cultured granule cells of a PTX-sensitive GTP-binding protein which participates in signal transduction at G_{P2} receptors. This protein is not involved in the mechanisms of PI hydrolysis stimulated by other receptors present in the granule cells, nor in ionotropic signal transducing systems activated by excitatory amino acids.

54.2 SELECTIVE POTENTIATION OF EXCITATORY AMINO ACID RECEPTOR-STIMULATED PHOSPHATIDYLINOSITOL HYDROLYSIS BY LOW CONCENTRATIONS OF COBALT AND NICKEL. E. Fadda*, F. Nicoletti*, J.T. Wroblewski*, J. Mazzetta* and E. Costa (SPON: J.L. Meek). Fidia-Georgetown Institute for the Neurosciences, Georgetown University Med. Sch., Washington, D.C. 20007.

In primary cultures of cerebellar granule cells, excitatory amino acid recognition sites are coupled to ionotropic and metabolotropic signal transducing mechanisms. These include the hydrolysis of membrane phosphoinositides (PI) (G_P receptors) and the activation of receptor-operated Ca^{2+} influx with a consequent increase in cyclic GMP formation (G_C receptors). The two receptors coupled to PI hydrolysis differ by the pharmacology of their recognition sites and the molecular characteristics of their coupling mechanism. Thus, G_{P1} is activated by N-methyl-D-aspartate (NMDA), glutamate and aspartate, is antagonized by 2-amino-5-phosphonovaleric acid (APV), is sensitive to Mg^{2+} and allosterically modulated by phencyclidine (PCP). In contrast, G_{P2} is not sensitive to the above agents and is selectively stimulated by quisqualate and glutamate. The G_{C1} has a pharmacology similar to the G_{P1} receptor; however, is coupled to a Ca^{2+} influx

In the present work, we have demonstrated that micromolar concentrations of Co^{2+} and Ni^{2+} selectively potentiate signal transduction at the G_{P1} receptor. The dose-response curve for this potentiation was biphasic, reaching the maximal effect at 50 uM, while above 100 uM an inhibition could be seen, similar to that produced by Mg^{2+}. Among other divalent cations tested Mn^{2+}, Cu^{2+}, Fe^{2+}, La^{2+}, Zn^{2+} and Cd^{2+} shared the properties of Mg^{2+} but not those of Co^{2+} and Ni^{2+}.

The potentiation by Co^{2+} and Ni^{2+} was selective for PI hydrolysis stimulated by glutamate, NMDA and aspartate (G_{P1}), but not for that stimulated by quisqualate (G_{P2}) or the muscarinic cholinergic agonist carbachol. Moreover, it was inhibited by APV, Mg^{2+} and PCP, which act at the G_{P1} receptor. Despite large similarities in the pharmacological profiles of the G_{P1} and G_{C1} recognition sites, Co^{2+} and Ni^{2+} failed to affect Ca^{2+} influx and cyclic GMP formation mediated by the G_{C1} receptor.

These results indicate that in primary cultures of cerebellar granule cells Co^{2+} and Ni^{2+} show a selectivity of action which is restricted not only to a specific signal transducing mechanism (PI hydrolysis), but also to a specific subtype of excitatory amino acid receptors (G_{P1}). The results will be discussed in relation to the possibility that the supramolecular structure of the G_{P1} receptor may include two cation-dependent modulatory sites, one negative - sensitive to Mg^{2+}, the other positive - sensitive to Co^{2+} and Ni^{2+}.

54.3 PROTEIN KINASE C TRANSLOCATION AND ACTIVATION IN PRIMARY CULTURES OF NEURONS: REGULATIONS BY GLUTAMATE AND BY SPHINGOGLYCOLIPIDS. F. Vaccarino, A. Guidotti and E. Costa. FIDIA-Georgetown Institute for the Neurosciences, Washington D.C. 20007.

The amino acid glutamate and NMDA are ligands for receptor-operated cation channels that allow a substained increase in the influx of Ca^{2+} in neurons. One possible consequence of such an intracellular Ca^{2+} increase might be the stimulation of Ca^{2+}-dependent kinases such as protein kinase C (PKC). PKC stimulation involves two separate steps: translocation or priming, by which the enzyme becomes membrane-bound, and activation by membrane diacylglycerols (DAGs). In rat cerebellar granule cells in primary culture, glutamate activates PI turnover and increases Ca^{2+} influx (Nicoletti et al., 1986; Wroblewski et al., 1985). Using this model, we investigated whether such glutamate-activated signal transduction results in PKC translocation from the cytosol to the neuronal membrane with subsequent PKC activation. Treatment of intact granule cells with excitatory amino acids such as glutamate and NMDA increases the number of binding sites (Bmax) for ^{3}H-beta-phorbol-12,13-dibutyrate (PDBu) (a specific ligand for PKC). This increase was blocked by phencyclidine and by aminophosphonovaleric acid (APV), was not mimicked by cGMP or by carbachol, and was dependent upon the extracellular Ca^{2+}. These data suggest that glutamate elicits a Ca^{2+} influx thereby triggering PKC translocation from the cytoplasm to the membrane compartment. Moreover, if granule cell homogenates are preincubated with Ca^{2+} and centrifuged, a redistribution of ^{3}H-PDBu binding sites is observed from the cytoplasmic (supernatant) to the membrane (pellet) fractions. In parallel, the PKC-mediated phosphorylation of endogenous substrates disappears from the cytoplasmic fraction, while PKC-mediated phosphorylation of membrane proteins of approximately 55, 66, 76 and 84-kDa is stimulated in the membrane fraction after Ca^{2+} preincubation. Similar proteins were found to be phosphorylated after glutamate stimulation of intact granule cells and this increase was antagonized by APV. The action of glutamate was mimicked by the phorbol ester tetradecanoylphorbolacetate (TPA) and by DAG. The ganglioside G_{T1b} does not affect basal ^{3}H-PDBu binding to intact granule cells but it antagonizes the increase in Bmax induced by glutamate. This trisialoganglioside also inhibits the phosphorylation of the membrane substrates induced by glutamate. Gangliosides are normal components of neuronal membranes and in particular G_{T1b} is enriched in cerebellar granule cells but is absent in cerebellar astrocytes. The inhibitory action on PKC translocation and activation exerted by G_{T1b} in intact cells may imply an important role of these glycolipids in the physiological down regulation of PKC.

54.4 ACTIVATION OF GLUTAMATE RECEPTORS INDUCES THE EXPRESSION OF C-FOS PROTO-ONCOGENE IN PRIMARY CULTURE OF RAT CEREBELLAR GRANULE CELLS. A.M. Szekely, M.L. Barbaccia and E. Costa (SPON: E. Adler-Graschinsky). FIDIA-Georgetown Institute for the Neurosciences, Georgetown Univ. Med. Sch., Washington DC 20007.

Proto-oncogenes, whose altered retroviral counterparts cause tumorigenic transformation, are thought to regulate normal processes of growth and differentiation in many cell types, including neurons. C-fos belongs to a large class of oncogenes, whose protein products are present in the cell nucleus and presumably participate in the regulation of expression of other genes. This effect might induce modifications that have a time dimension outlasting the duration of the initializing stimulus. Since hippocampal signal transduction of excitatory amino acids has been implicated in memory processes, it became of interest to us to investigate the possible linkage between activation of excitatory amino acid receptor(s) and induction of proto-oncogenes using primary culture of cerebellar granule cells. These cells contain ionotropic (GC_1) and metabotropic (GP_1) receptors transducing the transmitter signal via Ca^{2+} influx or activation of phosphatidylinositol (PI) metabolism, respectively, in a Mg^{2+} sensitive manner (Wroblewski, J., Proc. Natl. Acad. Sci., in press). N-methyl-D-aspartate (NMDA) is the synthetic agonist of GC_1 and GP_1, kainate and quisqualate are the synthetic agonists for GC_2 and GP_2 receptors, respectively. We analyzed the change of c-fos specific mRNA level following selective stimulation of granule cells via GP_1, GP_2, GC_1 and GC_2 receptors. Total cellular RNA was isolated, poly (A^+) RNA was purified and analysed by Northern blotting using ^{32}P nick translated mouse c-fos cDNA probe. The c-fos mRNA level was expressed using as a reference β-Actin cDNA probe. The GP_1 and GC_1 receptor agonists glutamate (10^{-5} M) and NMDA ($5x10^{-5}$ M) increased c-fos mRNA level. Kainate (GC_2) and quisqualate (GP_2) ($5x10^{-5}$M) failed to modify the basal expression. While activation of protein kinase C by tetradecanoylphorbolacetate (TPA) (10^{-7}M) resulted in a dramatic increase of c-fos mRNA level, the increase of cytosolic Ca^{2+} concentration by the ionophore A23187 ($5x10^{-7}$M) failed to elicit a change of the basal signal. Our results indicate that the increase in PI turnover (GP_1) and/or activation of Ca^{2+} influx (GC_1) can increase c-fos mRNA level, while depolarization due to kainate receptor (GC_2) stimulation fails to change this mRNA signal. These inferences are consistent with a c-fos mRNA increase elicited by the phorbol ester-induced activation of protein kinase C.

54.5 EFFECTS OF APV, KETAMINE, PHENCYCLIDINE AND KYNURENIC ACID ON HIPPOCAMPAL SLICES BATHED WITH PENICILLIN OR Mg^{++}-FREE MEDIUM. J.H. Schneiderman and J.F. MacDonald. University of Toronto, Toronto, Ontario, Canada.

In order to examine the role of excitatory amino acid (EAA) receptors in generating epileptiform discharge, we compared the effects of specific NMDA blockers (APV, ketamine and phencyclidine) and a non-specific EAA blocker (kynurenic acid) on penicillin and Mg^{++}-free bursts in the same hippocampal slices.

APV (50 uM), ketamine (10 uM), PCP (1 uM) and kynurenic acid (1 mM) reduced the amplitude and duration of spontaneous bursts in Mg^{++}-free medium and usually blocked them. The burst rate tended to decrease when the bursts became small. These effects were identical to increasing magnesium above 500 uM. The actions of all the drugs were reversible; however, recovery from PCP required several hours of drug-free rinse. Kynurenic acid prolonged the interval between penicillin bursts without significantly reducing their amplitude, whereas the other drugs had little effect. Ketamine 200 uM reduced the amplitude and frequency of penicillin bursts but did not block them.

Therefore, it appears that the NMDA mechanism is activated in Mg^{++}-free medium but not in the presence of the GABA-blocker, penicillin. The ability of kynurenic acid to selectively decrease penicillin burst frequency without reducing burst amplitude suggests that a non-NMDA EAA receptor may be involved in generating the spontaneous rhythmic synaptic activity which triggers the bursts but not in the PDS itself.

54.6 EXTRACELLULAR STRIATAL CONCENTRATIONS OF GLUTAMATE AND ASPARTATE DURING STIMULATION OF FRONTAL CORTEX, IN VIVO. H. Perschak* and M. Cuénod (SPON: M.-C. Hepp-Reymond). Brain Research Institute, Zürich University, CH-8029 Zürich, Switzerland.

Corticostriate neurons are thought to use excitatory amino acids (eAA) such as glutamate (Glu) or aspartate (Asp) as transmitters. In vitro, eAA are being released from depolarized striatal tissue in dependence of intact corticostriate terminals. In preliminary in vivo experiments, however, striatal extracellular concentration of Asp was unchanged and Glu showed a significant decrease by 16% during a four min activation of frontal cortex. Under physiological conditions eAA are being inactivated by reuptake; thus, release and uptake are principal determinants of extracellular concentrations of eAA in vivo. In the present experiments activity dependent changes in extracellular levels of eAA were further investigated by shortening sampling time and/or stimulation duration.

Striata of anesthetized rats were perfused with a Ringer solution by means of a push-pull cannula. One min effluent fractions were collected before, during and after four min or one min electrical stimulation periods of frontal cortex. Endogenous Glu and Asp were measured using glass-capillary/mass-fragmentography.

The fraction collected during the first min of a four min stimulation period contained elevated levels of Glu and especially Asp. During the following three min concentrations of Glu and Asp decreased and dropped, in the case of Glu significantly, to a level below pre-stimulation conditions. The increase of Glu and Asp during one min stimulation compared to the resting situation was 58% and 56% respectively and was significant for the latter.

These observations are consistent with the following hypothesis: Stimulation dependent release of Glu and Asp, detectable during the first min, is later overcompensated by activity induced enhancement of eAA uptake. Such stimulation dependent uptake has been proposed by Nieoullon et al. (Neurosci Lett, 43:191, 1983).

54.7 EVIDENCE THAT GLUTAMATE IS A NEUROTRANSMITTER IN AVIAN RETINAL GANGLION CELLS. S.E. Raiguel* and R.E.Marc Dept. of Sensory Sciences, U.T. Health Sci. Ctr. at Houston, Houston, TX. 77030

Glutamate has been previously proposed as a neurotransmitter of retinal ganglion cells (GC's). In the present study, three approaches have been used to demonstrate an association between avian GC's and the receptors and high-affinity transport mechanism for glutamate.

(1) High-affinity uptake/autoradiography (HAU/ARG) of ^{3}[H]-L-Glutamate or ^{3}[H]-L-Aspartate in the optic tecta (OT) of normal birds showed a concentration of activity in the superficial layers of the OT in which the axons of the GC's terminate. Enucleation reduced both the thickness of these laminae and the density of silver grains over them. Conversely, in a developmental study using chick embryos, we found that Glutamate HAU first appeared in these layers at the time of GC axon arrival.

(2) Receptor binding/contact ARG of ^{3}[H]-L-Glutamate in the OT showed that Na^+-independent binding of this ligand was highest in the layers of the tectal SGFS which receive visual afferents, and in the nucleus of the basal optic root, which has been shown to the target of the axons of displaced ganglion cells in the pigeon and chick. Glutamate binding was displacable either with unlabeled Glutamate or with quisqualate. ^{3}[H]-Kainate showed identical patterns of radioactivity in the OT.

(3) Anti-glutamate peroxidase-antiperoxidase immunocytochemistry showed a pattern of immunoreactivity in the OT of normal birds similar to the silver grain distribution in glutamate HAU/ARG experiments, with activity concentrated in the visual layers of the fibrous gray layer (SGFS). Enucleation resulted in a decrease in the thickness of those layers with a loss of immunoreactivity throughout the SGFS. Moreover most of the cells of the ganglion cell layer of the retina, and a population of large cells in the amacrine cell layer, presumably displaced ganglion cells, were strongly immunoreactive.

Our data support the hypothesis that ganglion cells, both conventional and displaced, use glutamate as their neurotransmitter.

54.8 DIFFERENTIAL BLOCKADE OF SYNAPTIC INPUT TO NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONS BY SELECTIVE EXCITATORY AMINO ACID ANTAGONISTS. B.D. Miller*, T.S. Donta*, and R.B. Felder. Department of Medicine and CV Center, University of Iowa Hospitals, Iowa City, Iowa 52242.

We recently demonstrated that selective antagonists for l-glutamate (l-glu) receptor subtypes blocked synaptic transmission in NTS in an in vitro rat brain slice preparation. We pursued this observation further in the present study by determining the effective blocking bath concentrations of these agents and by testing the effects of blocking concentrations of each antagonist on synaptic input to single NTS neurons activated by two different afferent pathways.

Single units evoked by stimulating the tractus solitarius (TS) (1 Hz, 0.3 ms) with ultrafine concentric bipolar electrodes were recorded from medial NTS using glass microelectrodes filled with 2 M NaCl (impedance 4-15 mohm). The ability of selective excitatory amino acid antagonists to block synaptic input to these units was then tested. Dose response relationships for 2-amino-5-phosphonovaleric acid (APV), kynurenic acid (KY) and glutamate diethylester (GDEE) were obtained by infusing increasing concentrations of each antagonist into the recording chamber. Maximum inhibition of the single unit response to APV (NMDA antagonist) was 94±4% at a calculated bath concentration of 40 μM, (n=6); KY (NMDA and kainate antagonist) caused a 90±6% inhibition at 290 μM (n=5) bath concentration, and GDEE (quisqualate antagonist) caused an 80±10% inhibition at a 240 μM (n=6) bath concentration.

Numerous cells in medial NTS responded to bilateral TS stimulation. Four units have been studied to determine the effects of more than one antagonist on synaptic input evoked by stimulation of each TS. Complete data for all three antagonists at blocking concentrations are available for two such units. The response of one cell to ipsilateral TS stimulation was inhibited by APV but not KY or KA while the response to contralateral TS stimulation was inhibited by KY and GDEE but not APV. The response of the second cell to ipsilateral TS stimulation was inhibited by KY but not APV or GDEE; contralateral input was not significantly inhibited by any of the antagonists studied.

These data suggest that more than one l-glutamate receptor subtype may be intrinsic to synaptic mechanisms in the NTS and that the presence of multiple l-glutamate receptor subtypes on single NTS neurons may provide a mechanism for selective activation by different neural pathways.

54.9 MEDIATION OF DIFFERENTIAL GLUTAMATE RESPONSES BY NMDA AND KAINATE RECEPTORS ASSOCIATED WITH DEGLUTITIVE NEURAL SUBSTRATES WITHIN THE RAT SOLITARIUS COMPLEX. M. A. Hashim* and D. Bieger. Faculty of Medicine, Division of Basic Medical Sciences, Memorial University of Newfoundland, St. John's, Nfld. A1B 3V6 Canada.

The complex motor act of swallowing comprises three subsynergies, viz., the linguopalatal, the pharyngeal and the oesophageal stages, governed by at least two closely linked central pattern generators associated with the nucleus tractus solitarii (NTS). As shown in previous studies (Bieger, D., Neuropharmacol., 23(12A): 1451-1464, 1984; Hashim, M. A. & D. Bieger, Brain Res.Bull., 18: 355-363, 1987), S-glutamate (Glu), applied pneumophoretically to the NTS, will elicit either complete deglutitive sequences (CDS) or the component subsynergies, depending upon the site of stimulation, muscarine, on the other hand, is selective in its action, producing primarily rhythmic oesophageal contractions at Glu-responsive NTS subnucleus centralis loci. In the present study, the involvement of different Glu receptor subtypes was investigated in urethane-anaesthetised rats with respect to their possible association with the individual subsynergies. For the microstimulation of deglutitive neurons, multibarrelled glass micropipettes (tip diameter 2-5 μm) were stereotaxically positioned in the NTS via an occipital craniotomy. Drugs contained in the micropipettes were pressure-ejected (400-500 kPa; 50-300 ms) by means of a nitrogen-pressured pneumophoresis pump. The three Glu-receptor agonists, N-methyl-D,L-aspartate (NMA), quisqualate (QA) and kainate (KA) were applied 0.1-10 pmol in pulses of 20-100 pL at NTS loci yielding pharyngeal and/or oesophageal responses. At pharyngeal sites, the rank order of agonist potency was KA>>NMA>QA; and the rank order of efficacy, KA>NMA>>QA, as indicated by repetitive swallowing frequency. However, at oesophageal sites, agonist potency followed the order NMA>QA=KA The specific NMDA-receptor blocker, 2-amino-5-phosphonovaleric acid (APV) selectively and reversibly antagonised the Glu-evoked oesophageal responses with no corresponding effect on rhythmic oesophageal responses elicited by muscarine; at CDS sites, APV spared the pharyngeal component while selectively blocking the oesophageal components. Unlike APV, the nonselective Glu-receptor antagonist, gamma-D-glutamylglycine failed to discriminate between Glu-evoked pharyngeal and oesophageal responses. In contrast to both the muscarine-evoked rhythmic oesophageal contractions and Glu-evoked pharyngeal swallows, Glu-evoked oesophageal responses displayed a typical self-sensitization. Application of equal volumes of the vehicle was ineffective. It is concluded that within the NTS, KA receptors are associated with the pharyngeal premotor elements whereas NMDA-receptors are linked to the oesophageal pattern generator. Both the KA- and the NMDA-mediated deglutitive mechanisms can operate under physiological conditions.

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54.10 NMDA INDUCED FREQUENCY-DEPENDENT POTENTIATION OF CORTICALLY EVOKED EPSPs IN CAT CAUDATE. P. L. Herrling. Sandoz Research Institute Bern Ltd., P.O. Box 2173, CH-3001 Bern, Switzerland.

Previous work had shown that iontophoretically applied NMDA agonists can strongly potentiate EPSPs evoked by electrical stimulation of the prefrontal cortex in caudate neurons of halothane anaesthetised cats. (Do et. al.,J. Neurosci. 6:2226, 1986). This potentiation was defined as occurrence of EPSP amplitudes, -durations and number of synaptically evoked action potentials that were significantly larger during application of NMDA agonists than during equal depolarizations elicited by applications of non-NMDA excitatory amino acid receptor agonists such as quisqualate.

In subsequent experiments it became apparent that the probability for the occurrence of potentiated EPSPs varied with different stimulation frequencies. At 0.5Hz about 57% of cells (N=14) displayed potentiation. At 2Hz only 22% (N=9) of cells showed potentiation, however, at this stimulation frequency the cortical stimulus occurred in 89% of cells during a NMDA elicited depolarization plateau (for description of plateaus, see: Herrling et al., J. Physiol. 339:207, 1983). In these cases the depolarizations were abruptly terminated at a constant latency from the stimulation, independent of the previous duration of the plateaus. This phenomenon was called plateau cut-off. At 5 and 10 Hz potentiation probability increased to 100% (N=6 and 5, respectively) while plateau cutoff probability sank to 30 and 20%, respectively. At 20Hz, NMDA induced plateaus and EPSP potentiation disappeared and only regular firing occurred.

Measures on the time sequence of synaptic events triggered by cortical stimulation indicate that the frequency dependency of EPSP potentiation by NMDA might be a consequence of circuit properties of the caudate nucleus.

54.11 EXCITATORY AMINO ACID ANTAGONISTS DEPRESS ACOUSTIC STARTLE AFTER INFUSION INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS OR PARALEMNISCAL ZONE. R.F. Spiera* and M. Davis (Spon. C.A. Sorenson), Dept. of Psychiaty, Yale Univ. Sch. Med , 34 Park Street, New Haven, CT 06508.

The startle reflex is a simple behavior which has proven useful for examining the neural mechanisms underlying behavioral plasticity. The neural pathway that mediates this short latency response consists of the ventral cochlear nucleus, the paralemniscal zone (an area just medial to the ventral nucleus of the lateral lemniscus - VLL), nucleus reticularis pontis caudalis (RPC) and spinal motor neurons. The startle reflex can be modulated by neurotransmitters such as serotonin, dopamine, norepinephrine, glycine, GABA, acetylcholine, and various peptides. However, none of these substances appear to actually mediate startle (i.e., released by one neuron within the startle pathway onto the next neuron within the pathway) since depletion or antagonists of these transmitters do not eliminate startle. The present study begins to define possible neurotransmitters that might actually mediate the acoustic startle reflex.

The role of excitatory amino acid transmitters in acoustic startle was chosen for study. The startle reflex has an extremely short latency so that rapidly acting transmitters such as amino acid transmitters might mediate this very fast reflex. Moreover, excitatory amino acid transmitters appear to to be released by the auditory nerve, indicating that they mediate auditory transmission at the cochlear nucleus. The present study sought to evaluate the role of excitatory amino acid transmitters at the VLL since this auditory nucleus may be somewhat specific for startle.

Male albino rats were implanted with bilateral cannulas into the VLL under chloral hydrate anesthesia. After one week recovery, animals were tested for acoustic startle response by presenting noise bursts (100-115 dB) every 20 sec before and after bilateral infusion of either 0, 2.5, 5.0 or 10.0 μg/side of either λ-glutamylglycine (DGG), λ-D-glutamyl-aminomethyl sulfonate (GAMS) or 2-5-phosphonovalerate (APV). Infusions of DGG, APV, and GAMS produced a rapid, marked, dose-dependent depression of the acoustic startle response. All three drugs had similar potency and efficacy over this dose range. These effects only occurred in animals in which the cannulas were found histologically to be located in the VLL or paralemniscal zone. Moreover, these depressant effects were very specific to blocking the startle reflex, since drug infusions into the VLL did not produce any other obvious behavioral changes and an auditory lick-suppression technique demonstrated that infusions into the VLL did not eliminate hearing.

These results indicate that excitatory amino acid transmitters may play an important role in either modulating or mediating startle at the level of the VLL and paralemniscal zone.

54.12 DUAL-COMPONENT SYNAPTIC POTENTIALS IN XENOPUS EMBRYO INTERNEURONES: SPONTANEOUS AND EVOKED RELEASE OF EXCITATORY AMINO ACID TRANSMITTER FROM CUTANEOUS AFFERENT FIBRES. K.T.Sillar* and A.Roberts* (SPON: W.J.Heitler). Dept. of Zoology, Univ. of Bristol, Bristol BS8 1UG, U.K.

The trunk and tail skin of Xenopus laevis embryos near the time of hatching is innervated by the unmyelinated and free nerve endings of Rohon-Beard neurones. These have somata in the dorsal spinal cord and longitudinal axons in spinal marginal zones, where they make monosynaptic contacts with dorsolateral sensory interneurones. Transient indentation, or electrical stimulation of the skin with short current pulses evokes impulses in Rohon-Beard neurones and short latency EPSPs in dorsolateral interneurones (Clarke et al, J.Physiol. 348:511-525 (1984); Clarke & Roberts, J.Physiol. 354:345-362 (1984)). We now show that the EPSPs are dual-component, consisting of separate fast and slow potentials (cf. Dale & Roberts, J.Physiol. 363:35-59 (1985)), which are both blocked by 1-2 mM kynurenic acid. The dual-component EPSPs are therefore mediated by an excitatory amino acid neurotransmitter. The slow components, but not the fast are specifically blocked by the NMDA receptor antagonists APV and magnesium indicating that the dual-component EPSPs result from the simultaneous activation of NMDA and kainate/quisqualate receptors.

Dorsolateral interneurones also receive spontaneous depolarizing and hyperpolarizing potentials. The hyperpolarizing potentials are abolished by 1 uM strychnine, while the depolarizing ones are resistant to both 1 uM strychnine and 1 uM TTX. The depolarizing spontaneous potentials are abolished by 1-2 mM kynurenic acid and are therefore excitatory amino acid-mediated. Like the evoked EPSPs, they have fast and slow components. Unlike evoked EPSPs in which fast and slow potentials are usually evoked simultaneously, the spontaneous events are predominantly fast. However, a significant proportion (ca. 30%) are either slow or dual-component. The spontaneous EPSPs show the same pharmacological properties as the evoked EPSPs and they therefore result from a transmitter action at both NMDA and kainate/quisqualate receptors.

The major source of excitatory input to the dorsolateral interneurones is from the Rohon-Beard neurones. Given the similarities in timecourse and pharmacological sensitivity we suggest that the spontaneous EPSPs, like those evoked following skin stimulation derive from the presynaptic release of excitatory amino acid transmitter from Rohon-Beard neurones.

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CELL LINEAGE AND DIFFERENTIATION I

55.1 DIFFERENTIATION OF CHOLINE ACETYLTRANSFERASE-IMMUNOREACTIVE CELLS IN QUAIL NEURAL CREST CULTURES. G. G. Leblanc* and M. E. Bronner-Fraser. (SPON: L. Smith-Thomas) Developmental Biology Center, University of California, Irvine, CA 92717.

When quail neural crest cells are grown in vitro, they differentiate into several of their normal in vivo derivatives, including melanocytes, adrenergic cells, and cells which resemble sensory neurons. Choline acetyltransferase (CAT) activity and acetylcholine synthesis have also been detected in these cultures, suggesting the presence of cholinergic cells. However, until recently there have been no histochemical markers for cholinergic cells. Hence, nothing is known about the distribution and morphology of cholinergic cells in neural crest cultures.

We have examined the differentiation of cholinergic cells in cultures of quail cranial and trunk neural crest using two different antibodies to CAT: a polyclonal antibody raised against CAT purified from chicken brain by Johnson and Epstein (J. Neurochem. 46, 968), and the monoclonal antibody 4D7, which was raised against CAT purified from rat brain by Crawford et al. (Proc. Natl. Acad. Sci. 79, 7031). Similar staining patterns were observed with both antibodies, consistent with the conclusion that the immunoreactive material present in cultured neural crest cells is genuine CAT.

The time course of appearance of CAT-immunoreactivity was similar to that previously reported for CAT catalytic activity in neural crest cells cultured under conditions identical to those used in the present study (Howard and Bronner-Fraser, Dev. Biol. 117, 45). Low levels of CAT-immunoreactivity were observed in some neural crest cells by 5 days in vitro. Seven day old cultures of both cranial and trunk neural crest contained numerous CAT-immunoreactive cells. These cells tended to occur in large clusters. Most of the CAT-immunoreactive cells seen in 7 day cultures appeared morphologically undifferentiated: they were ovoid or polygonal in shape and lacked processes. However, some of the cells had neuronal morphology: they had round cell bodies, formed ganglion-like aggregates, and extended long processes which contacted other aggregates of CAT-immunoreactive cells. The number of CAT-immunoreactive cells decreased substantially by 14 days, but extensive plexuses of CAT-immunoreactive fibers were seen at this time. Experiments are now in progress to determine whether the CAT-immunoreactive cells exhibit neuronal markers such as neurofilament immunoreactivity. We are also interested in examining what other neurotransmitters may be coexpressed with acetylcholine in cultured neural crest cells.

Supported by NIH grant RO1 HD15527, NSF grant DCB-8502604, and a basic research grant from the March of Dimes.

55.2 A MONOCLONAL ANTIBODY AGAINST A LAMININ-HEPARAN SULFATE PROTEOGLYCAN COMPLEX PERTURBS CRANIAL NEURAL CREST MIGRATION IN VIVO. Marianne Bronner-Fraser and Thomas Lallier, Developmental Biology Center, University of California, Irvine, Ca. 92717.

INO is a monoclonal antibody that blocks neurite outgrowth, probably by functionally blocking a laminin-heparan sulfate proteoglycan complex (Chiu, A.Y., Matthew, W.D. and Patterson, P.H., J. Cell Biol. 103: 1383, 1986). In the present study, we examined the effect of this antibody on avian neural crest migration by microinjecting INO into the pathways of cranial neural crest migration. After injection lateral to the mesencephalic neural tube, the injected antibody had a primarily unilateral distribution. INO was observed in the basal laminae surrounding the neural tube, ectoderm, and endoderm, as well as within the cranial mesenchyme on the injected side of the embryo. The INO distribution pattern was indistinguishable from staining patterns observed after injection of antibodies against laminin or heparan sulfate proteoglycan. The injected antibody remained detectible for eighteen hours after injection, with the intensity of immunoreactivity decreasing with time.

Embryos ranging from the neural fold stage to the 9-somite stage were injected with INO and subsequently allowed to survive for up to twenty-four hours after injection. Of the 64 embryos injected, 74% demonstrated severe abnormalities in cranial neural crest migration. The predominant defects were: 1. ectopic neural crest cells external to the neural tube (observed in 39% of the injected embryos); 2. neural crest cells within the lumen of the neural tube (observed in 20% of the injected embryos); and 3. neural tube deformities (observed in 36% of the injected embryos). In contrast, embryos injected with antibodies against laminin or heparan sulfate proteoglycan were unaffected. No effects were noted when embryos with greater than ten somites were injected with INO, suggesting that embryos are sensitive for only a limited time during their development. These results indicate that functional blockage of a laminin-heparan sulfate proteoglycan perturbs cranial neural crest migration. This is the first evidence that laminin is involved in aspects of neural crest migration in vivo.

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55.3 CHANGES IN THE HOMING BEHAVIOR OF NEURAL CREST CELLS OF AVIAN EMBRYOS AS THE RESULT OF TREATMENT WITH A PHORBOL ESTER DRUG. Gary Ciment, Rosalie Sears* and Lisa Hess* (SPON: M. Forte). Dept. of Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97201

The neural crest is a transient structure seen during early vertebrate embryogenesis. Neural crest cells originate within the neural tube, but leave the tube and migrate along characteristic pathways in the embryo, eventually giving rise to a wide variety of different cell types in the adult, including neurons and glial cells of the peripheral nervous system, as well as pigment cells of the integument.

In earlier studies, we found that neural crest-derived cells of early quail embryos change some of their developmental properties in response to the phorbol ester drug 12-O-tetradecanoylphorbol-13-acetate (TPA) [Ciment et al., Develop.Biol.118:392, 1987]. That is, organ cultures of either sympathetic ganglia, sensory ganglia, or peripheral nerves were found to contain pigmented cells when the medium contained 1.0 uM TPA, but never in the absence of this drug. These pigmented cells were judged to be true melanocytes by their ability to invade the integument and produce normal-appearing pigmented feather primordia in vivo. We interpret these and other results to mean that TPA reverses the developmental restriction of melanogenesis that normally occurs in neural crest-derived cells that migrate to sites in avian embryos where melanogenesis does not occur.

In order to determine whether TPA affected other properties of neural crest cells, we performed a series of heterospecific grafting experiments. In this studies, either dorsal root ganglia or peripheral nerves were dissected from 5-10 day quail embryos, cultured in the presence or absence of 1.0 uM TPA, and then grafted in ovo into the neural crest migratory space of 2.0 day embryonic chicken hosts. These chimeric hosts were allowed to develop for an additional 5 days, and then were fixed, embedded in parafin, sectioned, and stained according to the Feulgen-Rosenbeck procedure in order to differentiate between graft (i.e., quail) and host (i.e., chicken) cells.

We report here that TPA has dramatic effects on the homing behavior of quail neural crest-derived cells. Whereas cells cultured in the ABSENCE of TPA localized to sites characteristic of non-cultured crest cells, cells cultured in the PRESENCE of TPA localized to specific sites in which neural crest cells are normally never seen. These ectopic sites, for example, include regions of the central nervous system. These changes in the homing behavior are NOT simply the result of cell crowding or overflow, etc., since TPA-treated crest cells were not seen at sites to which untreated or non-cultured neural crest-derived cells normally homed.

55.4 RECONSTITUTED BASEMENT MEMBRANE COMPONENTS STIMULATE ADRENERGIC DIFFERENTIATION IN NEURAL CREST CULTURES. G.D. Maxwell and M.E. Forbes*. Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

Considerable evidence indicates that neural crest cell development is affected by environmental cues during embryogenesis. In the course of their development some neural crest cells encounter basement membranes which line several epithelial structures in the embryo. We have examined the effect of a reconstituted basement membrane (RBM) preparation derived from the EHS tumor on the differentiation of quail trunk neural crest cells in tissue culture.

Secondary cultures were prepared from primary neural crest outgrowths after 2 days in vitro and grown in the presence or absence of an overlay of RBM gel. After one week the cultures were analyzed for the number of catecholamine-positive (CA+) cells that were present. Cultures grown in the presence of the RBM gel showed a 25-50 fold greater number of CA+ cells than sister control cultures without RBM gel. The morphology of the CA+ cells was similar in both conditions. Total cell number was not dramatically altered by the presence of the RBM gel, indicating that the effect was selective for the CA+ phenotype and not the result of the production of increased numbers of all cell types. Melanocytes were present with and without the RBM gel, although the amount of pigmentation in the cultures was somewhat reduced in the presence of the RBM gel.

The effect of the RBM gel was specific as shown by experiments in which type I collagen gels, type I collagen gels plus laminin, and laminin alone failed to mimic the effect of the RBM gel. Also, the timecourse of CA+ cell development was similar in the presence and the absence of the RBM gel, indicating that the effect was not one of simply increasing the rate at which CA+ cells develop.

When secondary cultures were prepared using a range of initial plating densities, all in the presence of RBM gel overlays, it was found that the development of CA+ cells was dependent on initial plating density. At initial densities below 20 cells/mm^2 no CA+ cells were observed (although melanocytes and unpigmented cells developed) while at initial densities of 80-320 cells/mm^2 the number of CA+ cells which developed was proportional to the initial plating density.

These results indicate that the presence of reconstituted basement membrane components results in a dramatic and selective stimulation of the differentiation of the CA+ phenotype by neural crest cells in tissue culture. This work was supported by NIH grants NS 16115 and RCDA NS 00696 to GDM.

55.5 A ROLE FOR THE EXTRACELLULAR MATRIX IN CNS NEUROGENESIS IN VITRO. T.A. Reh and K. Radke*. Dept. Med. Physiol., Univ. Calgary, Alta. Calgary, Alta. T2N 1N4.

Virtually nothing is known concerning regulation of proliferation of the germinal neuroepithelial cells (GNCs) that give rise to the vertebrate CNS. Among the reasons for the current lack of information about GNCs is that the cells do not remain mitotically active in vitro for any length of time. It is not clear why GNCs do not continue to proliferate in vitro. These cells may be programmed for terminal differentiation after a certain number of cell divisions since in the chick and rodent embryonic CNS, neuroepithelial proliferation only occurs in vivo for short periods. Alternatively, dissociation may promote terminal differentiation, by disrupting cellular associations or contacts with the extracellular matrix normally present in GNCs. In frogs, development of the CNS proceeds much more slowly than in homeothermic vertebrates. Frog retina retains a specialized zone of GNCs at the margin throughout the animal's life; therefore, this system might enable us to determine why GNCs don't normally prolierate in vitro and provide a source for a GNC line.

Midlarval staged Rana pipiens tadpoles were anaesthetized, the eyes removed, and the retinas, with RPE still attached, were embedded in low melting point agarose, quickly chilled at 4°C and sectioned at 100 to 200 µm. Retinal slices were cultured in media consisting of L-15, 1-10% fetal bovine serum, glucose, and antibiotics. To identify dividing cells in the cultures, [H^3]-thymidine was added to the media at concentrations of 1 µCi/ml. We found that GNCs in the proliferative zone of these retina slice cultures continued to incorporate [H^3]-thy and undergo mitosis in vitro for up to three weeks at the levels observed in vivo. Throughout this period the germinal cells retain their pseudostratified columnar epithelial morphology. Using neuron-specific and glial-specific monoclonal antibodies, we were able to determine that most of the new cells generated by the GNCs in culture were neurons.

Since these slice cultures preserve the normal cellular and matrix associations present in vivo, we were able to determine if proliferation of GNCs requires contact with the external basement membrane that surrounds the developing CNS by removing this matrix with collagenase. In the cultures labelled with H^3-thy one day after the removal of the external basement membrane, germinal cells still incorporate the label at near normal rates; however, by three days, the collagenase treated cultures have a significantly reduced level of H^3-thy uptake. In addition, this zone is beginning to differentiate to give a laminated pattern of cell distribution. By five days after removal of the basement membrane, virtually all GNC proliferation has stopped; no signs of degeneration were observed in this region following the enzyme treatment, but rather the germinal cells have apparently terminally differentiated. This suggests that the contacts of the germinal cells with their normal basement membrane may be important in keeping them in a proliferative state.
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55.6 IMMORTAL NEUROPEITHELIAL PRECURSOR CELL LINES. K. Frederiksen, P-J. Jat, N. Valtz, D. Levy, R. McKay. Depts. Brain and Cognitive Science, Biology, MIT, Cambridge, MA 02139.

It is now clear that the functional ability of the adult brain is achieved by mechanisms which require many molecularly distinct neuronal types. As a consequence the methods of molecular biology are increasing applied to the outstanding problems in neuroscience. In vertebrates traditional genetic methods are tedious but as recent advances in cancer biology proove the tools of recombinant DNA based genetic manipulation can be applied powerfully to vertebrate cells in tissue culture. The precursor cells of the embryonic brain become specialized before neurons differentiate setting in motion a cascade of events which generate specific numbers and types of neurons. If precursor cell lines were available it would be possible to apply recombinant genetic methods to study the molecular basis of neuronal specificity.

We have used retroviruses carrying oncogenes to establish immortal cell lines with the properties of neuroepithelial precursor cells. Using hybridoma technology we have generated an antibody, Rat 401, which recognizes neuroepithelial precursor cells in vivo (Frederiksen and McKay, J. Neurosci. in press, 1987). The large SV 40 T antigen, v-myc and neu oncogenes establish stable cell lines expressing the Rat 401 antigen. These cell lines are clonal by Southern blot analysis of the integrated oncogene. The SV40 T antigen used was a temperature sensitive variant which can be inactivated by growing the cells at 39°C. When these cell lines are induced to differentiate by growth at the elevated temperature or by providing other differentiating signals some of these cell lines loose the precursor marker and express either neuronal or glial properies. The same clonal cell line can give rise to differentiated products which express antigens characteristic of neurons, astrocytes or oligodendrocytes. This data suggests that Rat 401 marks a multipotential precursor cell type in the developing cerebellum.

Our results suggest that functionally competent precursor cell lines can be obtained with conditional oncogenes. The differentiation potential of these cell lines can be rigorously assessed by transplantation into the developing rat brain. Our preliminary data shows that we can detect the transplanted cells upto six months after injection. There is no indication of tumors and the injected cells are found in large numbers in the gray matter. These data raise the exciting prospect of using genetically manipulated cell lines to identify the molecular bases of neuronal specificity both in tissue culture and in vivo.

55.7 CELL LINEAGE IN MOUSE CEREBRAL CORTEX STUDIED WITH A RETROVIRAL MARKER. M.B. Luskin, A.L. Pearlman, and J.R. Sanes. Departments of Anatomy & Neurobiology, Cell Biology, and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

We recently began using a recombinant retrovirus to study postimplantation cell lineage in mouse embryos. The virus, LZ1, integrates into a chromosome but is defective in replication; it is therefore confined to the progeny of the initially infected cell. LZ1 contains the E. coli β-galactosidase (lacZ) gene, expression of which is detectable histochemically or immunohistochemically. By analyzing the clonal progeny of single infected cells, lineage relationships in yolk sac and skin were determined (Sanes et al., EMBO J. 5: 3133, 1986). Here, we report extension of this approach to the cerebral cortex.

To ask which cortical cell types can be infected by LZ1, we used primary cultures. Cells were dissociated from cerebral hemispheres of E12-13 mice (during the peak of neurogenesis) and virus was added ~6h after cells were plated; 3-8d later, cultures were doubly stained with antibodies to lacZ and to one of a group of cell type-specific proteins: neurofilaments (NF), neural cell adhesion molecule (NCAM), the cell adhesion molecule L1, glial fibrillary acidic protein (GFAP), or fibronectin (FN). Four types of homogeneous clones, each with 2-20 lacZ-positive cells, were identified: neurons (small, round, process-bearing, $NCAM^+$, $L1^+$, NF^+, $GFAP^-$, FN^-), flat astrocytes (medium sized, process-poor, NCAM-poor, $L1^-$, NF^-, $GFAP^+$, FN^-), star-shaped astrocytes (small, process-bearing, NCAM-poor, $L1^-$, NF^-, $GFAP^+$, FN^-), and fibroblasts (large, flat lamellipodia-bearing, $NCAM^-$, $L1^-$, NF^-, $GFAP^-$, FN^+). In addition, some clones were heterogeneous; these are being characterized to seek evidence for bipotential precursor cells. We conclude: 1) that an exogenous gene introduced by a retrovirus can be expressed in several brain cell types, and 2) that E12-13 cortex contains separate populations of neuroblasts and glioblasts.

In parallel experiments, we injected viral concentrate directly into the telencephalon of E12 or 13 embryos in utero, then fixed and stained intact brains for lacZ pre- or postnatally. Pieces of cerebral cortex (as well as of olfactory bulb, diencephalon, striatum, and cerebellum) containing clones of lacZ-positive cells were subsequently embedded and serially sectioned. Clones in cortex contained 2-10 cells, often arranged in interrupted columns that spanned several laminae, and comprising cells of several distinctive sizes and morphologies. We are analyzing these clones with cell type-specific markers, and comparing results obtained in vivo and in vitro, to document lineage relationships in the cerebral cortex. (Supported by NIH.)

55.8 PATTERNS OF NEUROGENESIS IN CHICK OPTIC TECTUM STUDIED WITH A RETROVIRAL MARKER. J.C. Glover, G.E. Gray* and J.R. Sanes (Spon: M.B. Luskin). Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

We are using a recombinant retrovirus as a lineage marker to analyze the fates of clonally-related cells in the chick optic tectum. The virus inserts a β-galactosidase (lacZ) gene into the genome of an infected cell; that cell's lacZ-positive progeny are detected histochemically in whole mounts and characterized in sections (Sanes et al., EMBO J 5: 3133, 1986, and preceding abstract). For use in chickens, the host range of the virus was extended by passage through an amphitrophic helper cell line (Miller and Buttimore, Mol. Cell Biol. 6: 2895, 1986). The optic tectum was chosen because of its prominent laminar and columnar organization, well-characterized inputs and outputs, and accessibility in ovo. By infecting cells and analyzing clones at appropriate times, we hope to learn the spatial and phenotypic fates of lineally related cells.

As a first step, we injected viral concentrate into the tectal ventricle during the period of active cell proliferation (Stages 10-24 or E2-4), and then analyzed clones when proliferation is nearly over but before laminae form (Stages 30-34 or E7-8; LaVail and Cowan, Brain Res. 28: 421, 1971). The 60 clones examined so far are comprised of isolated clusters of 2-100 morphologically similar, closely spaced, lacZ-positive cells. The number and arrangement of cells within clones varies systematically with the time at which LZ1 was injected: Clones resulting from injections at Stages 10-13 contain multiple closely-spaced vertical arrays of cells extending the full thickness of the tectum. Clones resulting from injections at Stages 15-20 generally consist of 1 or 2 vertical arrays of cells extending the full thickness of the tectum. Clones resulting from injections at Stages 22-25 consist of vertical arrays that do not extend the full thickness of the tectum. Thus, clones marked progressively later are spatial subsets of clones resulting from earlier injections.

From these results, we draw 2 conclusions: 1) During the period of neural proliferation, clonally related cells mingle little. 2) An orderly temporal progression exists, such that early-born progeny are displaced laterally (giving rise to multiple vertical arrays) while later-born progeny are displaced vertically (giving rise to single or partial vertical arrays). We are now analyzing older embryos, to learn the extent to which migration and differentiation lead cells in a near-coherent clone to diversify during later stages of tectal ontogenesis. (Supported by NIH.)

55.9 THE CELLULAR SOURCE OF PERINEURIUM DETERMINED WITH A RETROVIRAL MARKER. M.B. Bunge, J.R. Sanes, P.M. Wood*, L.B. Tynan*, and M.L. Bates*. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

In peripheral nerves, fascicles of axons and Schwann cells are ensheathed by a layer of cells and extracellular matrix called the perineurium. The cellular source of perineurium in vivo remains in question, although fibroblasts (Fbs) are leading candidates (Scaravilli, J. Anat. 139: 411, 1984). In culture, perineurium forms when pure populations of Fbs and Schwann cells (SCs) are added to cultured nerve cells (NCs) (Williams, Bunge and Wood, J. Cell Biol. 95: 2a, 1982). Since perineurial cells exhibit characteristics of both SCs and Fbs, but are distinct from either, perineurial cells could arise from either Fbs or SCs. To distinguish these alternatives, we have used a technique in which a heritable marker (the lacZ gene) is introduced into the genome by means of a defective recombinant retroviral vector; the product of this gene, β-galactosidase, is then detected histochemically (Sanes et al., EMBO J. 5: 3133, 1986; and see preceding abstracts by Luskin et al. and Glover et al.).

Our strategy was to prepare NC + SC + Fb cultures containing either virus-infected Fbs or virus-infected SCs, and then to ask which combination would give rise to lacZ-positive perineurium. Because the retrovirus infects only dividing cells, SCs were exposed to virus as they proliferated following addition to NCs; proliferating Fbs were infected in isolation and then added to NC + SC cultures. Following 6 weeks in differentiation-supporting medium, cultures were fixed, reacted for lacZ, and processed for electron microscopy (EM). Fascicles with labeled cells were located by light microscopy, then sectioned for EM. In NC + infected SC + Fb cultures, marked SCs were found along axons and throughout fascicles. EM showed that the lacZ histochemical reaction product is electron dense and readily detectable in SC cytoplasm around axons; however, reaction product was absent from perineurium. In NC + SC + infected Fb cultures, some marked Fbs appeared at fascicle perimeters; EM demonstrated the presence of reaction product in typical perineurial cells. We conclude that perineurial cells differentiate from fibroblasts in culture and, by implication, in vivo as well. (Supported by NIH Javits Award NS 09923.)

55.10 **RE-EXPRESSION OF TYROSINE HYDROXYLASE DURING SENESCENCE AND ONCOGENIC TRANSFORMATION OF MURINE PANCREATIC BETA CELLS.** G. Teitelman, S. Alpert* and D. Hanahan*, Division of Neurobiology, Cornell Univ. Med. Coll., New York, NY and Cold Spring Harbor Lab, Cold Spring Harbor, NY.

The catecholamine enzyme tyrosine hydroxylase (TH) is transiently expressed by pancreatic insulin (β) cells during development and reappears in some β cells of postnatal and adult mice. In this study we used immunocytochemical and autoradiographic techniques to characterize the adult TH-insulin cells during islet growth in pregnant mice, mutant obese (ob/ob) mice, and transgenic mice that develop beta cell tumors. The latter group contains a hybrid gene comprised of the 5' flanking region of the rat insulin II gene linked to the protein coding information for the Simian virus 40 large T antigen (Tag). The number of TH-insulin cells increased during normal pregnancy (from 1.5±0.2 per islet at gestation day 10 (G-10) to 3.3±0.3 per islet at G-18; $n \geq 20$) and as hyperplasia developed in ob/ob mice (from 1.2±0.2 per islet at 3 months to 3.9±0.5 per islet at 7 months; $n \geq 20$; controls 3 mo: 0.7±0.1 and 7 mo: 0.1±0.02). However, the TH-insulin cells of postnatal mice did not incorporate radiolabeled thymidine (^{3}H-tdR) into their nuclei indicating that they did not divide. In contrast, the insulin "only" cells proliferated and, thus, were responsible for the increase in beta cell numbers. This indicates that (a) islet growth was produced by proliferation of insulin only cells; (b) insulin cells underwent a limited number of cell divisions, then withdrew from the cell cycle, and re-expressed an embryonic marker (TH). To determine the fate of postnatal TH-insulin cells, ^{3}H-thymidine was injected into pregnant mice at G-17 and the pancreas of the progeny examined 7 days after birth. At this time, the nuclei of some TH cells were heavily labeled with the isotope. However, at 14 days these heavily labeled cells had vanished. Since postnatal TH-insulin cells do not divide, the disappearance of these cells cannot be due to dilution of the label. Thus, it seems likely that insulin cells that re-express TH are senescent cells destined to die.

In transgenic mice, the TH-insulin cells proliferated and their number increased dramatically during the pre-neoplastic period (from 9.3±1.2 per islet at 4 weeks postnatal to 50.7±5.2 per islet at 9 weeks, $n \geq 20$). In the neoplastic period, the TH-insulin cells did not die but increased their rate of proliferation. Thus, in the presence of an oncogene, the TH-insulin cells are apparently unable to complete terminal differentiation to a postmitotic state and instead escape from the senescence pathway to produce immortal tumor cells.

We conclude that TH is expressed in three different conditions of pancreatic beta cells: during maturation in embryos, in a terminal differentiation pathway to cellular senescence and elimination and during malignant transformation. The mechanism that mediates the number of times the beta cell divides before re-expressing TH and then determines their fate remains to be elucidated.

56.1 NGF SYNTHESIS IN VIVO AND IN VITRO. R.H. Edwards[1], M.J. Selby[1]*, S. Weinrich[2]*, D. Hruby[2]* and W.J. Rutter[1]*. [1]Hormone Research Institute, University of California, San Francisco, CA 94143 and [2]Dept of Microbiology, Oregon State University, Corvallis, Oregon 97331

We have used a recombinant vaccinia virus system to express NGF in a variety of cell lines. Two recombinant viruses were constructed, corresponding to the two major NGF transcripts: one (A) predicts a 34 kd precursor with mature NGF at the C-terminus and the sole hydrophobic domain internal; the other (B) predicts a 27 kd precursor lacking the N-terminal 7 kd of A, and consequently having the hydrophobic region at the N-terminus. Infection of a permissive cell line (BSC-40) with the recombinants showed substantial accumulation of NGF mRNA. Infection of this and other cell lines, including L929, G8, PC12, HIT, AtT20, MDCK and primary Schwann and fibroblast cultures, shows that all secrete mature NGF into the medium as indicated by immunoprecipitation of metabolically labeled protein. Furthermore, the medium induces sprouting from PC12 cells.

Using the recombinant viruses, we have studied the processing of NGF in infected cells. Both recombinants produce an NGF precursor of ~35 kd, in all the cell lines tested. Pulse-chase experiments show processing of the prohormone within the cell to mature NGF, followed by secretion ($T_{\frac{1}{2}}$ ~2 hr); and both recombinants show roughly the same kinetics. A 22 kd intermediate can occasionally but inconsistently be detected for A and B. The precursors appear to have substantial glycosylation, with N-glycanase digestion reducing both in size to ~30 kd on Laemmli gels. Tunicamycin at doses not affecting protein synthesis reduces the amount of NGF secreted and the precursor becomes undetectable within cells. Immunofluorescence shows NGF localized to the region of the Golgi apparatus.

We have used precursor from infected cells as a substrate for putative NGF-processing enzymes. γ-NGF is found as part of a complex with mature β-NGF in the mouse salivary gland, and has proteolytic activity on artificial substrates. NGF precursor synthesized in vitro using SP6-generated NGF RNA and a cell free translation system has previously been shown to be improperly cleaved by γ-NGF, resulting in the destruction of the mature hormone. A coupled translation-translocation system also failed to yield an adequate substrate. Precursor obtained from infected cells can, however, be cleaved to mature β-NGF by γ-NGF. Presumably, the precursor generated inside the cell but not in vitro has the conformation required for correct processing. Sequencing of precursor metabolically labeled with ^{3}H-valine from infected cells is consistent with prior cleavage of the signal peptide.

56.2 **REGULATION OF NGF GENE EXPRESSION IN A MOUSE CELL LINE.** **R. Houlgatte, D.Wion, N.Barbot, E.Dicou and P.Brachet. INSERM U.298, CHR, 49033 ANGERS Cx, FRANCE.**

Mouse L-929 cells are known to synthetize and secrete nerve growth factor (NGF), and these cells provide a simple model system suitable for the search and characterization of extracellular effector molecules able to influence the level of expression of the NGF gene.

L-cell proliferation can be obtained in serum-containing medium as well as in serum-free medium. Hybridization experiments performed with a cDNA probe have previously shown that serum itself contains some factor(s) which increase specifically the cellular levels of NGF mRNA (1). Correspondingly, serum induces an enhancement of the amounts of NGF secreted by the cells, as was evidenced by a double site ELISA assay (2). Recent experimental data indicated that the positive effect of serum was dose-dependant. It was elicited by sera from various vertebrate species, such as foetal calf, horse, rat or human. Although the modulatory activity was distroyed after a heat treatment of 5 min at 100°C at neutral pH, about 80% of the activity was recovered when heating was performed on serum samples previously adjusted to pH4. This heat and acid-resistant effector displayed an apparent MW of 100-130 k Da, as observed by gel filtration chromatography.

L cells were also cultured in a serum-free medium supplemented with human α,β or γ globulins, or serum albumin. α globulins exerted a marked effect on the biosynthesis of NGF. The compound responsible for this stimulation appeared heat-stable at pH4 and heat-sensitive at neutrality. β globulins exerted some stimulation too but the effector was heat-sensitive at either pH value. γ globulins and albumin were depleted of effect.

Serum appears to act in a very different way than another positive effector studied in this laboratory, retinoic acid. This analogue of vitamin A also enhances the cellular levels of NGF mRNA and NGF protein in L-cells cultured in serum-free medium. Relative levels NGF mRNA are increased by a factor of 3 in cells exposed to micromolar amounts of retinoic acid. However, the time course of this increase is long, since maximal stimulation is obtained after 3 days of treatment, while the effect elicited by serum is already detected 4h after its addition to the medium, and reaches maximal values after 24h or less. Both effectors, therefore, seem to differ in their mode of action.

(1) Wion,D. et al (1985) FEBS Letters, 189, 37.
(2) Houlgatte,R. et al (1986), in : Molecular Aspects of Neurobiology, R.Levi-Montalcini ed., Springer V., page 40.

56.3 SYNTHESIS AND RELEASE OF NERVE GROWTH FACTOR BY RAT MACROPHAGES U. Otten, G. Weskamp*, M. Hardung* and D. K. Meyer*
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Nerve growth factor (NGF) is a polypeptide known to regulate the development and function of peripheral sympathetic and sensory nerve cells and of cholinergic neurons of the basal forebrain. Recent studies indicate that NGF in addition to its neurotrophic effects may also be involved in the physiological modulation of inflammatory responses. The present study aimed to further investigate the latter NGF effects. We used a sensitive enzyme-linked immunosorbent assay for determination of NGF protein and an optimized Northern blot technique for quantification of NGF mRNA to characterize the regulatory mechanisms involved in NGF production and to identify the cell types synthesizing NGF.

1.) Nanomolar concentrations of immunoreactive NGF (irNGF) were found in inflamed tissues and in inflammatory pleural and peritoneal exudates induced by carrageenin or sterile paraffin-oil in adult rats.

2.) IrNGF released in inflammatory exudates is biologically active as monitored by the neurite outgrowth response in PC 12 cells and sensory neurons.

3.) The increase in irNGF-content in the peritoneal exudate after paraffin-oil injection reached peak values (16fold as compared to controls) after 4 days correlating with the accumulation of mononuclear cells.

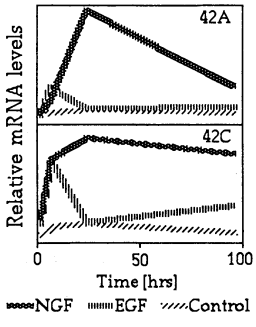
4.) Approximately 90 % of irNGF was found in the macrophages after separating them from the remaining inflammatory cells by their ability to bind to plastic surfaces.

5.) NGF mRNA content in peritoneal macrophages revealed a 4 - 5 fold increase over basal values as early as 24 h after onset of inflammation and decreased to basal levels within 7 days.

Thus, upon inflammatory stimuli peripheral macrophages enhance the synthesis and release of NGF. These results suggest that NGF may play an important role in various functions of macrophages including those in inflammatory and neuronal regeneration.

56.4 NERVE GROWTH FACTOR INDUCES IN PC12 CELLS TWO GENES CODING FOR S100-RELATED PROTEINS. P. Masiakowski* and E. M. Shooter. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305

The molecular mechanisms involved in NGF-induced neuronal differentiation can be conveniently studied in rat pheochromocytoma-derived PC12 cells. To identify genes which may be involved in this process, we have screened PC12 cDNA library with cDNA probes from PC12 cells grown for 7 days in presence or absence of NGF. Two of the clones, 42A and 42C, hybridize on Northern blots to short (700-800 nucleotides) RNA bands whose intensities increase within few hours of NGF addition and remain high for several days. EGF causes only brief increase in the levels of 42A and 42C mRNAs (see figure).



DNA sequence analysis allowed us to deduce the amino acid sequences of the putative proteins coded for by these mRNAs. Computer search of NBRF Protein Database revealed a strong homology of both peptides to the subunits of S100, a calcium-binding, nervous tissue specific protein. In addition, our peptides are highly homologous to porcine p11 (regulatory subunit associated with p36, a major target of tyrosine protein kinases) and human 2A9 (peptide deduced from a message inducible by factors stimulating fibroblast proliferation). All these proteins constitute a closely related family, as they are all about 100 amino acid long, with identical residues in 16 positions and conservative substitutions in 17 positions. Furthermore, in the predicted secondary structure, they all share a characteristic strongly hydrophilic region with turns in the part of sequence homologous to the calcium-binding site of well characterized calcium-binding proteins. This region is immediately followed by a strongly hydrophobic, possibly membrane-interacting, domain. To test the hypothesis that these conserved features reflect an important cellular role played by proteins corresponding to our clones, we are now transfecting PC12 cells with the copies of 42A and 42C recloned from a library constructed in an eukaryotic expression vector.

56.5 HIGH LEVELS OF NERVE GROWTH FACTOR mRNA IN THE CHICKEN EMBRYO COINCIDE WITH MAXIMAL RESPONSIVENESS TO RECOMBINANT NGF PROTEIN.

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Nerve growth factor (NGF) is a well characterized trophic protein necessary for the maintenance of sensory and sympathetic neurons in the peripheral nervous system, as well as a subset of cholinergic neurons in the central nervous system. We have used the eucaryotic expression vector p91023(B) to produce both a rat and a chicken recombinant NGF. The constructs were transfected into a monkey cell line (COS-cells) and NGF expression was analyzed by: Northern-blot analysis; immuno-precipitation with affinity purified anti-mouse NGF antibodies; and the ability of COS-conditioned medium to stimulate neurite outgrowth in a bio-assay using cultured sympathetic ganglia from 9 day old chick embryos. The transfected COS-cells produced NGF transcripts of predicted size and secreted a protein with the mol wt of 14k which was precipitated with anti-NGF antibodies. Transfection with both the rat and the chicken NGF constructs stimulated neurite outgrowth in the NGF assay.

Using a DNA probe from the cloned chicken NGF gene (Ebendal et al., 1986, EMBO J. 5:1483-1487) NGF mRNA was detected in the chicken as early as embryonic day 3.5 . The level of NGF mRNA increased fourfold from day E3.5 to day E8, remained high until day E12 and decreased subsequently. The transient rise in expression of NGF mRNA paralleled the fiber outgrowth response in sympathetic and neural crest-derived sensory neurons to the recombinant chicken NGF. The high NGF mRNA levels of embryonic day 8 were mainly derived from NGF mRNA expression in the skin and eye, possibly serving a trophic function for sensory neurons.

56.6 CHANGES IN BETA-NERVE GROWTH FACTOR mRNA AND PROTEIN LEVELS IN HIPPOCAMPUS FOLLOWING LESIONS OF THE SEPTOHIPPOCAMPAL PATHWAY IN RATS.

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According to recent evidence nerve growth factor (NGF) acts as a neurotrophic agent for cholinergic neurons of the basal forebrain: it is synthesized in target fields of cholinergic neurons such as hippocampus and retrogradely transported to the respective perikarya in the septum.
We investigated whether NGF gene expression in adult rat hippocampus is regulated by innervating cholinergic axons, the major part of the septohippocampal pathway.
4 and 15 days after bilateral surgical transection of the septo-hippocampal pathway, immunoreactive NGF protein (irNGF) and NGF mRNA levels were determined.
As compared to controls, the lesion decreased choline acetyl-transferase activity to 8 % and 30 % respectively in dorsal and ventral hippocampus after 4 days. At this time, irNGF content was increased by 50 % in dorsal and by 30 % in ventral hippocampus as compared to sham operated animals. In contrast, NGF mRNA levels declined to 50 and 75 %.
15 days after lesioning significant changes in irNGF and NGF mRNA in both dorsal and ventral halves of the hippocampus were no longer observed.
In summary, we found a good correlation between the lesion-induced decrease in cholinergic innervation and changes in NGF mRNA and irNGF in adult rat hippocampus. Thus, we conclude that NGF gene expression in adult rat hippocampus is modulated by cholinergic innervation. Whether increase in NGF protein concentration is due to a regulation at the posttranscriptional level or to accumulation of NGF following lesion-induced blockade of retrograde transport is being currently investigated.

56.7 **Transplants of NGF-rich tissue facilitate survival and regeneration of axotomized cholinergic neurons in the medial septum and diagonal band.**

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Transections of the fimbria-fornix (F/FX) result in a loss or shrinkage of cholinergic neurons in the medial septum/and vertical limb of the diagonal band (MS/VDB), possibly due to the deprivation of a retrogradely transported, target-derived trophic factor(s) in the hippocampal formation. From recent evidence reported in this and other labs, it is suggested that one of these trophic factors may be nerve growth factor (NGF). To provide axotomized MS/VDB neurons with a constant source of NGF we have utilized a transplantation model using intraventricular grafts of male mouse submaxillary glands (a rich source of NGF). Animals received unilateral transections of the F/FX, followed by intraventricular grafts of male mouse submaxillary gland. At 1-6 weeks following transection, a time at which axotomized MS/VDB neurons would normally have degenerated, animals with grafted submaxillary gland tissue exhibit marked survival of MS/VDB neurons which stain positive for acetylcholinesterase (AChE) and are immunoreactive for the NGF receptor (antibody provided by E. Johnson). In addition, fibers which are positive for both markers appear to course from the medial septum into the lateral septum. In some instances, AChE- and NGF receptor-positive fibers were found to penetrate the graft adjacent to the lateral septum. To determine the presence of NGF-like trophic activity in the graft at 3-4 weeks following transplantation, the graft was removed from the host brain and placed in a culture of PC12 cells, which are known to be responsive to NGF. Within 36 hours following attachment of the graft onto the cell layer, PC12 cells were observed to differentiate and extend neurites towards the direction of the submaxillary gland. NGF treatment is also known to increase choline acetyltransferase (ChAT) activity in the basal forebrain of neonate rats and in adult rats that have sustained F/FX damage. The MS/VDB was dissected from the host brain and the effect of the submaxillary gland on ChAT activity in the MS/VDB was determined 3-4 weeks following surgery. As reported by others, we found a recovery of ChAT activity in the MS/VDB of those animals that received only F/FX transections. However, in those animals that also received submaxillary gland grafts, ChAT activity in the MS/VDB was increased 40% above the recovery levels observed in animals receiving transections alone. From these results it is suggested that submaxillary gland transplants are able to i) survive for extended periods of time (3-4 weeks) in a cross-species grafting procedure ii) influence the survival and regeneration of axotomized MS/VDB cholinergic neurons, and iii) increase the biochemical activity of these basal forebrain neurons which are thought to be sensitive to NGF.

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56.8 NGF STIMULATES GROWTH OF CHOLINERGIC FIBERS INTO THE HIPPOCAMPUS OF RATS WITH PARTIAL FIMBRIAL TRANSECTIONS.

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NGF acts as a neurotrophic factor for cholinergic neurons of the basal forebrain. We earlier reported that NGF prevents the lesion-induced degeneration of these neurons after transection of the septo-hippocampal pathway (Hefti, J. Neurosci. 6:2155,1986; Montero, Proc. Soc. Neurosci. 12:1099,1986). We also studied the requirements for the NGF treatment to be effective and found that one month of treatment does not result in permanent surival of the cholinergic neurons. In addition, delayed onset of NGF treatment failed to rescue these cells. The effect of NGF was specific for cholinergic neurons, inasmuch as GABAergic neurons, which represent the majority of non-cholinergic septo-hippocampal neurons, did not respond.

The fimbrial transection performed in our studies destroys part of the septo-hippocampal pathway, leaving the most medial fibers intact. We earlier reported that NGF treatment during 10 weeks did not stimulate the growth of cholinergic fibers into the partially lesioned hippocampus (Hefti et al., Brain Res. 293:305, 1984). We now tested whether NGF treatment for up to 22 weeks promotes cholinergic fiber growth under these conditions.

Female adult rats with a unilateral partial transection of the the septo-hippocampal pathway were implanted with a cannula ipsilateral to the lesion. The animals were injected intraventricularly twice a week with 2.5S NGF (2.5ug in 5ul) or an equal ammount of cytochrome c. They were treated for up to 22 weeks and were then taken for histochemical visualization of AChE-positive fibers in the hippocampus. (AChE is a reliable marker for cholinergic fibers in the hippocampus). Control animals showed a marked loss of AChE-positive fibers in the CA3 area of the hippocampus ipsilateral to the lesion. In NGF-treated animals the density of hippocampal AChE-positive fibers on lesioned sides was higher than that of control animals in the same area which were treated for equal intervals of time. Furthermore, the fiber density on the lesioned side increased with the duration of NGF treatment.

These results suggest that intraventricular injections of NGF stimulate fiber growth of septo-hippocampal cholinergic neurons into the partially denervated hippocampus.

56.9 ACQUIRED DEPENDENCE OF BASAL FOREBRAIN NEURONS ON NGF. J.N. Barrett, D. Nonner* J. Hartikka*and F. Hefti (SPON: D.C. Mash). Depts. of Physiology and Biophysics and of Neurology, Univ. of Miami School of Medicine, Miami, FL 33101.

Neurons from fetal rat basal forebrain and/or septal area were cultured in a medium which supports long-term neuronal survival without glial proliferation (Kaufman and Barrett, Science, 220: 1394, 1983). Addition of 100 biological units/ml of Nerve Growth Factor (NGF) to this medium enhanced the cholineacetyl-transferase (ChAT) activity of the cultures by 2 to 10 times after growth periods of 1 to 6 weeks. A substantial increase (2 to 5 times) in ChAT activity was observed even when the cultures were first maintained in the presence of anti-NGF for 2-4 weeks prior to exposure to NGF. However, if the neurons were grown in medium containing NGF for 4 weeks or more and after washing out the NGF, were then exposed to anti-NGF, then ChAT levels fell to less than 50% of the levels found in control cultures never exposed to NGF. Later treatment of these cultures with NGF (after removal of anti-NGF) did not enhance ChAT activity. Treatment of cultures which had previously been exposed to NGF for 4 weeks with anti-NGF greatly reduced the number of neurons stained with ChAT immunocytochemistry. Damaged ChAT-positive neurons were observed in these cultures after exposure to anti-NGF for 2 days. Withdrawal of NGF, without addition of anti-NGF, did not usually reduce the number of ChAT-positive neurons or their ability to respond to later NGF treatment.

It is unlikely that the damage to cholinergic neurons observed in cultures treated with anti-NGF was complement-mediated, since complement was inactivated or removed from the antibody preparation. The findings therefore suggest that basal forebrain and septal cholinergic neurons exposed to NGF in culture for 4 weeks or more become dependent upon the presence of NGF and degenerate when this factor is removed. The finding that cholinergic neurons degenerated only after the addition of anti-NGF, but not after simple removal of NGF, suggests that other cells in the cultures produced sufficient amounts of NGF to keep these neurons alive without inducing maximal levels of ChAT activity. It seems likely that NGF was produced by astrocytes (Assouline et al., Proc. Soc. Neurosci. 11:933, 1985). Supported by NIH grant NS 12207 and the National Parkinson Foundation.

56.10 EXPRESSION OF NERVE GROWTH FACTOR RECEPTORS BY BASAL FOREBRAIN CHOLINERGIC NEURONS. J. Hartikka, W. Strauss and F. Hefti. Depts. of Neurology and Pharmacology, University of Miami School of Medicine, Miami FL 33101.

Nerve growth factor (NGF) acts as a neurotrophic factor for the cholinergic neurons of the mammalian basal forebrain. The effects of NGF are mediated through specific receptors located on the cell membrane. We have studied the expression of NGF receptors (NGFR) using immunocytochemical and molecular biological techniques.

Primary cultures of dissociated cells were prepared from fetal (E 17) rat septum. Cells were plated at high density (300,000 cells per sq cm) on poly-lysine coated dishes and grown in a modified L-15 medium. We earlier showed that NGF increases the activity of choline acetyltransferase (ChAT) in these cultures without affecting the survival of cholinergic neurons. After ten days cultures were fixed and taken for immunocytochemical visualization of NGF receptors using monoclonal antibody 192-IgG. In untreated septal cultures, only weakly stained neurons were visible. In NGF treated cultures, however, a large number of strongly stained neurons were found.

To further characterize the NGFR positive cells, cultures taken for immunocytochemical visualization of NGFR were co-stained for acetylcholinesterase (AChE). AChE has been shown to co-localize with ChAT and, therefore, to be a reliable marker for cholinergic neurons in our cultures. Double staining for NGFR and AChE was assessed by comparing photographs from the same visual fields. All of the 146 NGFR positive cells were also positively stained for AChE. Thirty-eight of the 184 AChE-positive neurons (i.e. 21% of the total) were not labeled by the NGF receptor antibody.

Co-staining for NGF receptors and AChE indicates that NGF receptors are exclusively located in cholinergic neurons in cultures prepared from rat basal forebrain. The finding that NGF treatment increases the staining intensity of neurons by NGFR immunocytochemistry suggests that NGF either increases the synthesis or decreases the degradation of its own receptor. Currently, we are studying the expression of the NGF receptor gene by measuring the level of NGFR mRNA in septal cultures using a cDNA clone for the rat NGF receptor (Radeke et al., Nature 325:593, 1987).

56.11 LOCALIZATION OF NGF RECEPTORS ON SPECIFIC CELL TYPES IN RAT BASAL FOREBRAIN CULTURES. C.F. Dreyfus, H.J. Martínez,* S.J. Rubin,* P. Bernd and I.B. Black. Div. Devel. Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021 and Dept. Anat., Mt. Sinai Sch. of Med. of C.U.N.Y., New York, N.Y. 10029.

Previous work has indicated that nerve growth factor (NGF) specifically and selectively increases choline acetyltransferase (CAT) and acetylcholinesterase in cultures of rat basal forebrain-medial septal area (BF-MS). To determine whether these actions are potentially receptor-mediated, and to identify cells expressing receptors, dissociated BF-MS cultures were examined. Two independent methods, radioautography following ^{125}I-NGF binding and immunocytochemistry with a monoclonal NGF receptor antibody (192-IgG, generously provided by Dr. E. Johnson, Jr.) detected specific receptors. The NGF receptors were localized to two different cellular populations, flat, large (70 µm), non-neuronal-like cells, and small, round, process-bearing neuron-like cells.

To define and localize receptor subtypes present in the cultures, dissociation studies were performed. Taking advantage of the dissimilar K_ds exhibited by low- and high-affinity receptor subtypes, cultures were incubated with ^{125}I-NGF (5ng/ml) followed by non-labelled NGF (5 µg/ml, 4°C, 10 min). Only the neuron-like cells exhibited silver grains following this procedure, suggesting that high-affinity receptors were localized to the neuron-like population, while only low-affinity receptors were localized to the non-neuron-like cells.

The BF-MS area is composed of a heterogeneous population of neuron-like cells. To define the individual cell types that express receptor, we examined cultures for co-localization of receptor and transmitter traits. Initially, cholinergic cells were identified immunocytochemically using a specific monoclonal antibody to CAT (kindly provided by Crawford and Salvaterra). Simultaneous radioautography detected ^{125}I-NGF binding sites on the CAT-positive cells. However, only a subset of CAT-positive cells exhibited NGF binding sites in these cultures, suggesting that CAT positive cells may comprise several populations. We tentatively conclude that NGF may elicit cholinergic effects by directly binding to high-affinity receptors on neurons, quite possibly cholinergic. However, future studies will be required to further identify populations expressing NGF receptors. (Supported by NIH Grants NS 20788, NS 10259, AG 00086-07, NSF Grant BNS 86-00256,and grants from the Alzheimer's Disease and Related Disorders Assoc. Inc., and March of Dimes Defects Fdn. HJM is a Fellow from the Instituto de Investigaciones Clínicas, Universidad de Zulia, Maracaibo, Venezuela. IBB is a recipient of a McKnight Research Project Award).

56.12 NERVE GROWTH FACTOR STIMULATES PRION PROTEIN GENE EXPRESSION IN DEVELOPING BRAIN. W. C. Mobley, S. J. DeArmond*, S. B. Prusiner, M. V. Johnston and M. P. McKinley*. Depts. of Neurology and Pathology, University of California, San Francisco, CA 94143; Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Rodents manifesting clinical signs of scrapie, a neurodegenerative disorder, demonstrate deficits in forebrain cholinergic presynaptic markers (McDermott et al., *Lancet* II:318, 1978). Nerve growth factor (NGF) exerts prominent, dose-dependent effects on developing cholinergic neurons of the rodent forebrain and may serve as a trophic factor for these cells (Mobley et al., *Mol. Brain Res.* 1:53, 1986). Because trophic factors have been suggested to participate in the pathogenesis of some neurodegenerative disorders, we undertook a study of NGF in scrapie. Considerable evidence indicates that a protein encoded by a cellular gene, designated scrapie prion protein (PrP^{SC}), plays a central role in the pathogenesis of scrapie. *In situ* hybridization studies indicate that most CNS neurons contain PrP mRNA (Kretzschmar et al., *Am. J. Pathol.* 122:1, 1986). Recent studies show that PrP mRNA levels are regulated in a regionally specific fashion in neonatal hamster brain. Interestingly, we found that developmental increases in PrP mRNA levels in hamster brain septum were almost coincident with those in ChAT activity, raising the possibility of coordinate control of these markers in basal forebrain cholinergic neurons. Since ChAT activity in neonatal rat brain had been shown to be stimulated by NGF (Mobley et al., op. cit.), we examined the possibility that PrP gene transcription might also be regulated by NGF. Neonatal hamsters were injected intraventricularly with NGF (30 ug) or vehicle on postnatal days (PD) 3, 5 and 7 and septum was assayed on PD9. ChAT activity was increased by 160% (NGF = 52+3 nmol ACh/hr/mg protein; vehicle = 20+2). In three separate experiments the level of PrP mRNA was increased approximately 14-fold, rising 40% above adult values. NGF effects on ChAT activity and PrP mRNA were both apparent within 48 hr of injection. Septal PrP mRNA was increased 7.5-fold following a single NGF injection at PD7. A lesser (70%) but significant increase in PrP mRNA was also found in caudate-putamen. No effect of NGF was registered in the thalamus, brainstem or cortex. ChAT activity was increased by 100% in septum and 45% in caudate-putamen in the same experiments. Although the magnitude of ChAT activity and PrP mRNA increases induced by NGF are different, the regional specificity and kinetics of these changes in developing forebrain are similar and raise the possibility that both changes are occurring in cholinergic neurons. *In situ* hybridization studies in progress may help to define the locus of NGF actions. It will be interesting to determine if NGF levels change during scrapie infection and whether exogenously administered NGF alters the course of disease.

57.1 AN ELECTRON MICROSCOPIC ANALYSIS OF THE MORPHOLOGY AND CONNECTIVITY OF INDIVIDUAL RAT VIBRISSA PRIMARY AFFERENT CENTRAL TERMINAL ARBORS LABELLED WITH INTRA-AXONAL HRP. W.E. Renehan, R.D. McCall*, R.W. Rhoades and M.F. Jacquin. Dept. of Anatomy, Univ. of Louisville Sch. of Medicine, Louisville, KY 40292; Dept. of Neuroscience, NY Inst. of Tech., Old Westbury, NY 11568; Dept. of Anatomy, Univ. of Med. and Dent. of NJ, Piscataway, NJ 08854.

In a previous light microscopic investigation of the morphology of the brainstem projections of individual physiologically identified trigeminal primary afferent neurons, we found that vibrissa, guard hair, nociceptive, hairy skin and mucosal afferents could be distinguished from each other on the basis of their central terminal arbor morphology (Jacquin, et al., 1986, J. Neurophys. 55:1153-1186). It was not possible, however, to differentiate the various types (e.g. rapidly adapting, slowly adapting, nociceptive-biased) of vibrissa-associated afferents from each other using that technique. The present study extends this analysis to the electron microscopic level in an attempt to increase our understanding of information processing and vibrissa connectivity in the rat trigeminal brainstem nuclear complex.

Individual physiologically characterized vibrissa primary afferent neurons were impaled with beveled glass microelectrodes (Microstar, R= 70-120 MΩ), filled with 6% HRP in 0.05 M Tris and 0.3 M KCl (pH 8.3) and processed for electron microscopy according to the method of Semba et al. (JCN, 1983, 221:466-481). To date, we have examined serially sectioned terminals from a slowly adapting type IIb afferent which supplied the E1 vibrissa and a D7 slowly adapting type I neuron. In both cases, all the HRP labelled boutons contained predominantly round, clear vesicles approximately 20-60 nm in diameter. We have observed terminals synapsing with dendritic spines (characterized by smooth endoplasmic reticulum but no microtubules) and dendritic shafts (containing parallel arrays of microtubules and neurofibrils, mitochondria, ribosomes and occasionally rough ER). The two slowly adapting afferents examined thus far were rarely postsynaptic to unlabelled axons or dendrites, a feature consistent with slowly adapting axons in the spinal cord (Ralston, et al., 1984, J. Neurophys., 51:777-792). It is also interesting that virtually every spinal cord or trigeminal intra-axonally labelled primary afferent investigated thus far, including those examined in the present study, has contained only clear round vesicles (see Semba et al., 1983, JCN:466-481 for review). Whether or not these axons represent some subpopulation of primary afferents awaits further analysis and an increase in our sample size. (Supported in part by DE-07734, DE-06528, NRSA NS-07444 and DE-07662).

57.2 TRIGEMINAL PRIMARY AFFERENT PROJECTION TO CONTRALATERAL DORSAL HORN: CENTRAL EXTENT, PERIPHERAL ORIGIN, AND PLASTICITY. M. Barcia*, N.L. Chiaia, R.W. Rhoades & M.F. Jacquin (SPON: L.C. Massopust). Dept. of Neuroscience, N.Y. Coll. of Osteo. Med., Old Westbury, NY 11568 & Dept. of Anatomy, N.J. School of Osteo. Med., Piscataway, NJ 08854.

Prior studies have documented a trigeminal (V) mandibular primary afferent (PA) projection to contralateral caudal medullary and cervical dorsal horns in cat, hamster and rat. We now report the existence of a much more substantial V ophthalmic PA projection to the extreme ventrolateral portion of contralateral caudal medullary and cervical dorsal horns in rat. Unilateral HRP injections, into the V ganglion or spinal tract (TrV), anterogradely labeled a large number of PA's exiting the caudal ventrolateral TrV to form a rostrocaudally continuous, transversely oriented, V PA decussation. These fibers terminated in each of laminae I-V along the ventrolateral border of the dorsal horn in contralateral caudal medulla, C1, and C2, though terminal label was heaviest in laminae III-V. Unilateral HRP injections, into medullary and cervical dorsal horns, also retrogradely labeled V PA collaterals contralateral to the injection site. Decussating V PA parent fibers (mean diameter ± SD: 1.49±0.85um, range: 0.43-3.91um) gave off collaterals in corresponding regions of dorsal horn, and in ventromedial subnuclei interpolaris, oralis, and principalis, rostral to their decussation. Thus, a given axon terminated both ipsi- and contralateral to its cell of origin. V ganglion cells were also labeled in dorsomedial (ophthalmic) portions of the contralateral V ganglion. 40.4±13.0 HRP-labeled cells were counted (N=5, corrected for split soma). Their average diameter was 29.9±3.3um, vs 26.3±5.4um for a spatially equivalent sample of ipsilaterally labeled ganglion cells. Double labeling studies were carried out to determine the ophthalmic receptor surface innervated by centrally crossing PA's. Diamidino yellow (DY) was injected into right medullary and cervical dorsal horns, and HRP was applied to the left cornea, ethmoid nerve, or the dura overlying cerebral cortex. The latter 3 structures are known to be represented in portions of ipsilateral dorsal horn receiving the crossed PA projection. Though DY labeled from 75-125 left ganglion cells per animal, no cells were double labeled. In conjunction with the relatively large ganglion cells and axons giving rise to this projection, and their central terminations, these data suggest that nociceptive-biased ophthalmic nerves are not the source of the crossed ophthalmic PA projection.

We have previously shown that ipsilaterally projecting mandibular and ophthalmic PA's maintain normal topography subsequent to neonatal infraorbital nerve damage. In adult rats subjected to left infraorbital nerve section at birth, HRP injections into right V ganglion or TrV labeled decussating ophthalmic PA's which were of normal density and terminal distribution in all left V brainstem subnuclei, C1 and 2. These data provide further evidence of an absence of central sprouting in spared V PA's following neonatal V deafferentation.

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57.3 THE CAPACITY OF HUMAN SUBJECTS AND PRIMATE S-I CORTICAL NEURONS TO PROCESS DIRECTIONAL INFORMATION PROVIDED AT TWO SKIN SITES. G.K. Essick* and B.L. Whitsel (SPON: A. Stuart). Dept. of Physiol. and Dent. Res. Cntr., UNC, Chapel Hill, NC 27514.

The ability of 6 human subjects to discriminate direction of tactile stimulus motion on the dorsum of the hand was determined (i) in the absence and (ii) in the presence of a temporally overlapping moving stimulus delivered to a second skin site on the ipsi- or contra-lateral forelimb. Similarly, the activity evoked from 16 single primate (Mac. fas.) directionally selective S-I neurons by two moving stimuli applied to different parts of their receptive fields was examined. The effects on directional sensitivity of stimuli moving in both the same and in opposite directions over the two skin sites was assessed.

It was found that when the two brushing stimuli move in the same direction, the directional sensitivity of the human observer is typically below that predicted for a hypothetical subject who can independently process information provided at each of the two skin sites. Moreover, the directional information provided at each skin site is often completely lost when the temporal order of site stimulation (ie, the long-range motion cues) provides conflicting directional information. In contrast, the directional sensitivity of invariant neurons was found to approximate that predicted for independent processing even when conflicting directional information was provided by delivering the two stimuli non-simultaneously.

In both the psychophysical and neurophysiological experiments, linear summation of the directional information provided by the two stimuli was observed only when the two stimuli were timed and positioned so that they approximated one continuous movement across the skin. Moreover, directional information was lost when the two stimuli moved in opposite directions.

The differences and similarities between the human and single neuron data lead us to suggest that while the S-I neurons cannot be solely responsible for the global motion processing capacity of human subjects, the behavior of the directionally selective cells of the primate S-I cortex is consistent with the idea that they may subserve an early, local, short-range process in tactile motion perception.

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57.4 FOUR CHANNELS MEDIATE THE SENSE OF TOUCH. S.J. Bolanowski, Jr., G.A. Gescheider, R.T. Verrillo and C.M. Checkosky*, Institute for Sensory Research, Syracuse University, Syracuse, NY 13210, Center for Brain Research, University of Rochester Medical School, Rochester, NY 14642 and Department of Psychology, Hamilton College, Clinton, NY 13323.

Previous physiological experiments have identified four groups of afferent fibers in glabrous skin of the human somatosensory periphery that are capable of signaling tactile stimuli: Pacinian (P), Rapidly adapting (RA), Slowly adapting type I (SA I) and Slowly adapting type II (SA II). Psychophysical studies, however, have shown that only two (P and non-Pacinian I or NP I), or perhaps three (P, NP I and NP II) channels participate in the perceptual process. This fact has been corroborated in microneurographic studies in which one of the fiber types (SA II) fails to evoke a sensation, even when electrically excited at high rates. By using stimuli to selectively mask the previously determined psychophysical channels, we now demonstrate that a fourth channel does, indeed, exist. The fourth channel responds to vibrations over a frequency range between 0.4 and 100 Hz and is unaffected by changes in skin-surface temperature (15 to 40° C), stimulus size (0.008 to 2.9 cm^2) and stimulus duration (700 to 2500 ms). Adhering to previous psychophysical nomenclature, we call the fourth channel NP III. The four channels combine, at threshold, to create an operating range for the perception of vibration which extends from 0.4 to greater than 500 Hz. Each channel mediates a specific portion of the overall threshold-frequency characteristic, although the sensitivities of the channels partially overlap. Thus, suprathreshold stimuli may activate several channels simultaneously, suggesting that the perceptual qualities of touch may be determined by the combined inputs from all four channels. Since the exact neural code used by each afferent fiber group is not presently known, it is possible that the failure to evoke a sensation from SA II fibers may be explained by inappropriate electrical stimulation.

Work supported by NIH and NSF.

57.5 ANTI- AND ORTHODROMIC SPONTANEOUS ACTIVITY IN RAT SAPHENOUS NERVE
Lisa C. Russell, Ph.D. & Kim J. Burchiel, M.D. Dept. Neurological Surgery, University of Washington & Veterans Administration Medical Center, Seattle, Washington

Spontaneous centrifugal activity was recorded in both acutely cut and chronically axotomized rat saphenous nerve. In 34 male Sprague-Dawley rats, a modification of the microfilament recording method was used to conduct surveys of spontaneous activity in saphenous nerve. Under deep pentobarbital anesthesia, six rats underwent L3 cordotomy, then unilateral saphenous axotomy. The proximal stump was then dissected into 30-50 small microfilaments and surveyed for spontaneous activity. Afterward, the nerve was cut back; gallamine (20 mg) was administered; and the nerve was once again surveyed in the same fashion. Twenty-eight rats underwent unilateral saphenous axotomy 1-8 weeks prior to similar recordings, and the neuroma was excised just before microfilament dissection. Spontaneous discharges originated from three foci: (1) the in-continuity dorsal root ganglia (DRG); (2) sympathetic vasoconstrictor neurons; and (3) dichotomizing afferent axons in peripheral nerve. There was significantly more antidromic activity from DRG in rats with prior axotomies than in control animals ($t=2.38$; $p < .025$), and gallamine produced a significant increase in DRG activity in chronically lesioned rats ($t=2.43$; $p < .025$) but not in acutely lesioned controls. However, 90% of the spontaneous activity in these recordings appears to be from sympathetic efferents. This activity was not increased by chronic neurotomy ($t=1.9$; $p > .05$), nor was it affected by gallamine administration ($t=.957$; $p > .1$). Usually 4-6 C-fibers exhibited synchronous firing at approximately 2 Hz each, and was coupled with the respiratory rhythm. Body cooling or hyperventilation induced a delayed increase in discharge rate. This activity was temporarily abolished by a sympathetic ganglion blocker (trimethaphan, 1 mg I.V.). These data correspond to sympathetic efferent activity recorded in human and cat cutaneous (sensory) nerve fascicles.

In only 2 microfilaments were spontaneous antidromic action potentials observed with receptive fields on blood vessels in the nearby fascia. Both branched axons were in acutely cut nerve thus were not the result of retrograde sprouting from a neuroma.

Conclusions: (1) Chronic axotomy of sensory afferents produced ectopic activity in their in-continuity DRG. (2) Potassium channel-blocking agents increased spontaneous activity from DRG in chronically axotomized rats. (3) On-going sympathetic efferent activity in rat saphenous nerve was not affected by distal axotomy for up to 8 weeks. (4) Rare branched sensory afferents in peripheral nerve occasionally exhibit spontaneous activity.

57.6 HYPOALGESIA AND DECREASES IN SPINAL CORD SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE ASSOCIATED WITH CAPSAICIN DESENSITIZATION IN ADULT RATS.
M.J. Iadarola, C.M. Flores* and R. Dubner, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Recently, calcitonin gene-related peptide (CGRP) has been colocalized with substance P (SP) in primary afferent neurons. Treatment of adult rats with capsaicin, unlike neonatal treatment, does not destroy the primary afferent neuron but does deplete spinal cord SP content. The present experiments examine the effect of adult capsaicin treatment on the content of spinal cord CGRP and SP and the relationship to behavioral hypoalgesia.

CGRP was measured with a c-terminally directed antibody that does not crossreact with cholecystokinin or FMRFamide. HPLC analysis of acidic extracts of rat spinal cord indicated that the major immunoreactive species coeluted with authentic rat CGRP. SP was measured in parallel with a second RIA. Capsaicin was administered by two routes: (1) s.c. in ascending daily doses of 30, 60, 60, 100 and 150 mg/kg or (2) direct application of a 1.5% solution to the sciatic nerve for 15 min. Nociceptive responses, paw withdrawal latencies (PWL), were assessed by application of a radiant heat source to the plantar surface of the foot.

Systemic administration of capsaicin produced a pronounced hypoalgesia (PWL: 14.0 vs 9.3 sec) as early as 24 hr after the first injection of capsaicin and lasting throughout the injection series. PWL returned to control levels over the ensuing 16 days. Depletion of CGRP (55%) occurred within 3 days of the first injection and was maintained at approximately this level for at least 3 weeks. SP was depleted in a precisely parallel fashion, but the decrease (36%) was less than that of CGRP. Thus, peptide depletion is concommitant to but outlasts the behavioral hypoalgesia. Application of capsaicin directly to the sciatic nerve produced a profound hypoalgesia (PWL reached the 25 sec cut-off) which lasted at least one week; peptide depletion did not exceed 35% for either CGRP or SP in spinal cord or lumbar dorsal root ganglia.

Our experiments show that treatment with capsaicin produces parallel effects on both CGRP and SP levels in dorsal spinal cord. Depletion with single nerve application was less robust than with systemic treatment. With systemic treatment, depletion is prolonged and exceeds the duration of hypoalgesia. This recovery of thermal sensitivity suggests that adaptive changes may occur to restore noxious sensitivity and that under certain conditions, there exists a dissociation between peptide depletion and the behavioral hypoalgesia seen after capsaicin treatment.

57.7 MULTIPLE RESPONSE CLASSES OF PERIPHERAL AFFERENT UNITS TO INTRA-ARTERIAL INJECTION OF BRADYKININ AND T-KININ.
R.H.Cohen, T.E. Paulson* and E.R. Perl. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514.

Kinins are potent pain-producing peptides formed as a consequence of the tissue injury. T-kinin (Ile-Ser-bradykinin) is the primary kinin formed in rats 24 hrs. after injection of carrageenin into an air pouch[1]. We examined and compared the afferent response elicited by bradykinin and T-kinin by recording from small fiber bundles teased from the great auricular nerve of an isolated rabbit ear preparation maintained *in vitro* by perfusion with an oxygenated Krebs-Henseleit solution. Typically, the threshold neural response to kinin, a gradual increase in spontaneous activity beginning more than 180 sec. after injection of 1 to 3 ηgms of kinin, occurred only once in multifiber recordings. At subsequent higher doses a shorter latency (<30 sec.) response appeared. Threshold sensitivity to bradykinin varied in multifiber recordings from 3 ηgms to 1000 ηgms. T-kinin was approximately 1/3 as potent. All C-polymodal nociceptors responded to the kinins in a similar fashion with a characteristic 20 to 30 sec. latency in onset and a low frequency discharge which reached a peak rate of less then 3 spikes per second at 1 - 2 minutes post injection. We could not demonstrate consistent changes in sensitivity to mechanical and thermal stimuli following each series of kinin presentations. Some unidentified units had a short latency response that began within seconds following injection and terminated within 1 minute post injection. In several multifiber recordings, units responded to the kinins with irregular bursting that lasted for many minutes post injection. Others responded with a maintained high frequency (>20/sec.) discharge that lasted up to 10 seconds or with trains of bursts that lasted many seconds. This multiplicity of response types and wide range of sensitivity to kinins indicates the need to reexamine categories of nociceptive afferent units with regard to chemoreceptive properties.
Supported by NINCDS grant NS10321 with computer facilities supplied by NS14899; RHC was the recipient of a NINCDS fellowship NS07788.

[1] Barlas, Sugio & Greenbaum. *Fed. Eur. Bioch. Soc.*, *190*: 268-270.

57.8 CORNEAL MECHANORECEPTOR RESPONSES TO REPETITIVE STIMULI.
H.W. Thompson,* B.M. Dupuy,* and R.W. Beuerman. LSU Eye Center, New Orleans, LA 70112.

The cornea is covered by an epithelial sheet containing free nerve endings originating from the ophthalmic branch of the trigeminal nerve. Many of these free nerve endings are mechanically sensitive, but have not been examined with stimuli of reproducible micron range displacements.

In anesthetized New Zealand rabbits, ciliary nerve branches were dissected free at the back of the globe of the eye, and single unit activity was obtained via conventional extracellular recording techniques. The eye was held firmly by tying the conjunctiva to a stainless steel ring. Mechanical stimuli of known force, displacement, rate of onset, and frequency were applied to the cornea by a piezoelectric stimulator driven by a function generator. Displacements were between 10 and 60 microns. In some experiments, the cornea was preloaded by probes of different areas and shapes (cylinders, spheres, and monofilament fibers) applied to different locations within a receptive field.

Two classes of mechanoreceptors were distinguished. The first class responded with bursts, or a single action potential, to the onset of only a few cycles of repeated stimulation before fatiguing. The second class, which did not fatigue rapidly, responded with bursts of potentials to the onset or offset of preloading displacements of 100 microns or more, and with single action potentials to each stimulus cycle of repeated stimuli. Single action potential responses were seen at frequencies up to 50 Hz, in response to displacements as small as 10 microns, and followed reliably for up to 10 minutes. Conduction velocities (1.5-4.0 m/s) for both groups were in the A-delta range. The two unit classes were often observed to have overlapping receptive fields, since a series of repeated small displacement pulses to the cornea frequently evoked two units (distinguishable by waveform and latency), one of which fatigued rapidly, and the other of which was nonfatiguing. Units of both types were highly sensitive to the rate of stimulus onset. Ramps or sinusoidal waveforms of fibers (0.13 mm dia.) were more effective stimuli than the larger spheres (1.7, 1.2, 0.7 mm dia.) or cylinders (1, 2, or 3 mm dia.). Sensitivity to rapid stimulus onset and the more effective nature of punctate stimuli for the two classes of corneal mechanoreceptors suggest that both may be involved in the perception of foreign bodies introduced into the eye. Comparison of the brainstem response to the output of rapidly fatiguing and nonfatiguing mechanoreceptors may be useful in understanding the processing of corneal pain information in the trigeminal nervous system.

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57.9 EVIDENCE THAT THE DISTRIBUTION OF POLYMODAL NOCICEPTORS DOES NOT DETERMINE BOTH THERMAL AND MECHANICAL PAIN THRESHOLDS OF HUMAN GLABROUS AND HAIRY SKIN. Casey, K.L., Butler, J.*, Lewis, K.G.* and Morrow, T.J., V.A. Medical Center, Ann Arbor, MI 48105

Polymodal nociceptors (PMNs) innervating mammalian skin respond to both mechanical and thermal noxious stimuli. If the distribution of PMNs is the major determinant of both mechanical and thermal pain threshold in humans, then both types of pain threshold should show the same relative difference among different sites on the cutaneous surface.

We measured mechanical and thermal pain thresholds in normal volunteers of both sexes aged 18 to 75. Mechanical skin pinch stimuli were delivered with a modified hemostat to 10 points within each of 6 body sites of 38 subjects. Pinch force was increased over a measured area at a rate of 3.7 $N \cdot sec^{-1}$ until the subject signaled the onset of pain. Average mechanical pain thresholds (N/mm^2) were computed for each body site. Thermal stimuli were delivered wtih an infrared CO_2 laser (45 msec, 5mm beam diameter) to 10 points within each of 6 body sites, 5 of which were identical to those tested with mechanical stimuli. The thermal pain threshold (watts) for each body site of 39 subjects was estimated by successive incremental adjustment of stimulus intensity from point to point.

For mechanically induced pain, the rank order of sensitivity (N/mm^2 ± SE) was: upper lip (0.19 ± .004), dorsal forearm (0.31 ± .007), ventral forearm (0.33 ± .004), finger pads (0.37 ± .009), dorsal foot (0.39 ± 0.14), and palm (0.50 ± .009). For laser-induced thermal pain, the rank order of sensitivity (watts ± SE) was: lower back (3.36 ± .21), ventral forearm (4.18 ± .31), dorsal foot (4.28 ± .21), dorsal forearm (4.66 ± .37), palm (9.28 ± .49), and finger pads (10.57 ± .50). This rank order of sensitivity and inter-site threshold differences for thermal pain agrees with previous results obtained using radiant heat (Hardy et al, Proc. Soc. Exp. Biol. Med. 80:425-427, 1952) except that we find glabrous skin thresholds higher relative to the minimum (lower back).

Mechanical and thermal pain thresholds are similar in showing no differences among the hairy skin sites of the forearms and dorsal foot, and in being significantly higher on the glabrous skin of the palm than on hairy skin. The thermal pain threshold of the finger pads, however, was even higher than that of the palm, while the mechanical pain threshold was the same as that of hairy skin. We conclude that the distribution of PMNs is not the major determinant of both mechanical and thermal pain threshold in hairy and glabrous skin and that other central and/or peripheral mechanisms determine the thresholds for each of these different types of pain.

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57.10 NEURAL MECHANISMS OF CUTANEOUS HYPERALGESIA IN HUMANS: PERIPHERAL OR CENTRAL? R.H. LaMotte, E. Torebjörk*, and L. Lundberg*. Dept. of Anesthesiology, Yale University Sch. of Med., New Haven, CT 06510 and *Dept. of Clin. Neurophysiol., University Hosp., Uppsala, Sweden.

Does intensely painful activation of cutaneous nociceptive peripheral nerve fibers sensitize (lower the thresholds and enhance the responses of) peripheral nociceptors or the central neurons to which they project? Either mechanism might account for hyperalgesia after cutaneous injury. To test for central sensitization without the complications of changes in response sensitivities of receptors, we delivered electrical test stimuli (TS) through a recording microelectrode inserted into the common peroneal nerve in awake humans and elicited pain sensations referred to a localized region (the projection field, PF) on the dorsum of the foot. Subjects judged the magnitude of pain sensation produced by each of 5-10 moderately painful TS (3 sec pulse trains of 10 Hz every 5 minutes). The TS were delivered before and after either of two intensely painful conditioning stimuli (CS). One CS was capsaicin (CAP) (100 ug in 10 ul tween-saline) injected intradermally within or adjacent to the PF; it produced intense pain and a wide area of hyperalgesia characterized by tenderness or pain to lightly stroking the skin. The alternative CS was an intensely painful, barely tolerable, intraneural electrical pulse train (1 Hz) delivered continuously for durations from 20 sec to 10 minutes. The magnitude estimates of evoked pain were not significantly different for TS given before and after either CS. Thus, an intensely painful CS, which presumably produced vigorous nociceptive input, failed to sensitize higher order neurons.

To test for peripheral mechanisms of hyperalgesia, evoked responses in 11 single C-fiber polymodal nociceptive afferent fibers (CPNs) were recorded from the common peroneal nerve in humans before and after intradermal injection of CAP. Injection of CAP 9-20 mm away from the CPN receptive field (RF) produced tenderness to skin stroking in an area encompassing the RF but did not sensitize the CPN. CPNs injected within or close to ($\leq$ 8 mm) their RFs were not sensitized although they typically did respond weakly to CAP, but ceased responding well before the termination of CAP pain. Injections within the RF desensitized responses to all cutaneous stimuli. Thus, activity of these CPNs could not account for CAP pain or hyperalgesia. Despite evidence that intense pain of peripheral origin can produce cutaneous hyperalgesia without "central sensitization," the peripheral nociceptors responsive for CAP pain and hyperalgesia have yet to be identified. (Supported by grants from the US PHS (14624) and the Swedish Medical Research Council)

57.11 OPIATES INCREASE REACTION TIME LATENCY TO FIRST AND SECOND PAIN SENSATIONS. R.H. Gracely*, R. Dubner, D. Walther*, P.J. Wolskee* and L. Lota*. Clinical Pain Section, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Two experiments assessed the influence of IV opiates on thermally-evoked first and second pain sensations. A contact thermode delivered trains of six 1.4 sec 51°C pulses (baseline 39°C) at 3 sec intervals. Subjects pressed a button to the first pain felt for each pulse in the train. Previous studies showed that for pulses 1 and 2, the first pain felt is a pricking, A-delta mediated, first pain sensation. This activity is suppressed after 4 pulses, and pulses 5 and 6 evoke only a diffuse, C-fiber mediated second pain sensation. Reaction time latencies to pulses 1 & 2 and 5 & 6 provided a relative measure of A-delta and C-fiber functioning. Reaction time latencies to auditory stimuli presented at the same intervals served as a motoric control.

In exp I, 24 pain-free subjects received 16 thermal and 10 auditory trains before and after double-blind IV infusion of either 1.1 ug/kg fentanyl (n = 9) or saline placebo (n = 15). In comparison to placebo, fentanyl increased reaction time to both first (132 msec, $t(22) = 2.42$, $p < 0.05$) and second (154 msec, $t(22) = 2.27$, $p < 0.05$) pain sensations while corresponding auditory latencies (pulses 1 and 2, 26 msec; pulses 5 and 6, 22 msec) were nonsignificantly increased.

In exp II, 21 chronic low back pain patients received 18 thermal (and 14 received 10 auditory) trains before, after and between sequential double-blind infusions of either morphine then 0.4 mg naloxone, or saline then morphine, presented on counterbalanced alternate days. Individually-determined morphine doses averaged 12 mg. In comparison to saline and naloxone, morphine increased latency to both first (291 msec, $t(21) = 3.69$, $p < 0.01$) and second (180 msec, $t(21) = 3.10$, $p < 0.01$) pain sensations, and decreased the detection frequencies of second pain sensations (2.57, $t(21) = 4.34$, $p < 0.001$). Auditory latencies (pulses 1 and 2, 38 msec increase; pulses 5 and 6, 70 msec increase) and detection frequency (pulses 5 and 6, 0.07 increase) were unaltered by morphine.

The present results suggest that opioid modification of both A-delta and C-fiber mediated pain sensation can be demonstrated in controlled studies of normal and chronic pain populations, and implicate these mechanisms in clinical analgesia.

57.12 PAINFUL SYNDROME NEWLY RECOGNIZED: POLYMODAL HYPERALGESIA WITH CROSS MODALITY THRESHOLD MODULATION AND RUBOR. ITS BASIS: 1 SENSITIZED NOCICEPTORS, 2 PLUS ANTIDROMIC VASODILITATION. [1]J.Ochoa, [1]M.Cline, [1]W.Comstock,* [2]W.J.Culp,* [1]R.Dotson, [3]P.Marchettini,* [4]H.E.Torebjork.* [1]Portland, OR, USA, [2]Dartmouth, NH, USA, [3]Milano, Italy, [4]Uppsala, Sweden.

Combined application to patients and volunteers of microneurography, intraneural microstimulation, thermography, quantitative sensory tests, sympathetic monitoring, differential nerve blocks, and capsaicin experiments, have allowed to define a specific neuropathic pain entity (Ochoa, 1986).

Key symptom is Stimulus-induced pain from weak stimulation of symptomatic skin. Spontaneous pain often occurs. Both mechanical and thermal stimulus modalities induce pain: Polymodal Hyperalgesia (PH). Cooling abolishes spontaneous and induced pains. Warming aggravates both.

Influence of one stimulus energy (thermal) upon perception threshold for pain elicited by another energy (mechanical) (Cross-modality Threshold Modulation: XTM) is explained at primary receptor level rather than through central gating. The possibility that specific thermal (cold) afferents may shut CNS gates is negated by persistence of XTM during selective afferent blockade (A-block resistent XTM). It is assumed that different energies alternatively excite or depress specific transducers within the same polysensitive ending in polymodal nociceptors (PNs). Sensitization of such units is suggested by PH and is endorsed by results of A-fiber blocks: when C fibers alone are conducting, PH persists. C nociceptor sensitization is proven by microrecording of single C-fiber PNs (Cline and Ochoa, 1986).

Key sign is skin warming from vasodilatation: it can occur in absence of sympathetic vasoconstrictor failure and probably reflects antidromic release by PNs of vasoactive transmitters. The same chemicals can lead to sensitization of nociceptors.

Symptoms and signs are similar to capsaicin-induced vasodilatation and sensitization of PNs via axon-reflex neurosecretion.

Symptoms up-modulated by warming may aggravate during sympathetic blocks due to the thermal increase.

M.Cline & J.Ochoa, CHRONICALLY SENSITIZED C NOCICEPTORS IN SKIN. PATIENT WITH HYPERALGESIA, HYPERPATHIA AND SPONTANEOUS PAIN. Society for Neuroscience Abstracts, Vol.12, Nov., 1986.
J.Ochoa, THE NEWLY RECOGNIZED PAINFUL ABC SYNDROME: THERMOGRAPHIC ASPECTS. Thermology, Vol. 2: 65-107, 1986.

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58.1 THE DISTRIBUTIONS OF DOPAMINE D1 AND D2 RECEPTORS ARE PARTIALLY COMPLEMENTARY IN THE CAT BASAL GANGLIA. R.M. Beckstead. Department of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425.

The patterns of dopamine D1 and D2 receptors were examined in the basal ganglia of the cat brain by quantitative autoradiography after in vitro radioligand binding with [^{3}H]SCH23390 and [^{3}H]spiperone, respectively. Highly specific binding for both radioligands occurs in striatal, pallidal and nigral components. However, the density of binding varies from one structure to another, and the density distribution in some striatal regions is heterogeneous. Both D1 and D2 radioligand binding occurs in irregular high and low density zones in the caudate nucleus and putamen. Such patches of high density D2 radioligand binding appear mainly in the dorsolateral half of the caudate head. For the D1 radioligand, the high density patches are more widespread throughout the caudate nucleus, nucleus accumbens and putamen. Low density zones of D2 radioligand binding occur at more caudal levels of the caudate nucleus. These low density D2 zones are often in register with, and matched in size and shape by the high density zones of D1 radioligand binding. These observations are consonant with the concept of neurochemical and connectional compartmentation in the striatum.

Pallidal and nigral structures show marked disparities in binding of the two different radioligands. The D2 radioligand binding in the globus pallidus is about twice that in the entopeduncular nucleus and pars reticulata of the substantia nigra, the latter two having approximately equal levels. No measurable binding of the D2 radioligand occurs in the ventral pallidum. In contrast, D1 radioligand binding is highest in the entopeduncular nucleus and in the pars reticulata of the substantia nigra, and moderate in the ventral pallidum, but no binding of D1 radioligand occurs in the globus pallidus. The binding density of D1 radioligand is comparable in both subdivisions of the substantia nigra, but a pronounced difference is apparent between the two subdivisions regarding the D2 radioligand binding: that in the pars compacta greatly exceeds that in the pars reticulata. Thus, the pattern of receptor binding conforms to the notion of duality in the striatifugal system.

Lesions of the nigral dopamine neurons with 6-OHDA or lesions of the striatum or entopeduncular nucleus with ibotenic acid indicate that the pars compacta D2 receptors are autoreceptors, whereas the nigral and entopeduncular D1 receptors are mainly in the membranes of striatonigral axons.

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58.2 **RECEPTOR BINDING PROPERTIES OF [I-125] LABELED IODOBENZAMIDE (IBZM): A SPECIFIC D2 DOPAMINE RECEPTOR LIGAND FOR SPECT.** T. Bruecke*, Y.F. Tsai*, W. Singhaniyom*, C. McLellan*, H.F. Kung*, R.M. Cohen* and C.C. Chiueh, (SPON: J.G. Kenimer), NIMH, Bethesda, Md, 20892 and Univ. of Pennsylvania, Pa, 19104.

Currently, several positron emitting ligands are in use to label and visualize dopamine (DA) receptors in the living human brain by using positron emission tomographic (PET) procedures. Because PET research is expensive and limited to a relatively small number of research centers, the search for ligands suitable for use in single photon emission computerized tomography (SPECT), a more readily available scan modality, seems advantageous. Benzamide derivatives appear to be specific ligands for D2 DA receptors while their reversibility permits receptor kinetic determinations within a convenient scanning time frame. In the present study we evaluated an I-125 labeled benzamide by investigating its binding properties in vitro.

I-125 labeled S(-)-N-(1-ethyl-2-pyrrolidinyl)methyl-2-hydroxy-3-iodo-6-methoxybenzamide (IBZM; sp.act. 600 Ci/mmol) was synthesized and purified by Kung et al (1986). In vitro binding experiments were performed using bovine and mouse caudate nucleus membrane preparations. I-125-IBZM binding is shown to be highly specific for D2 DA sites, since IC50 values for the D2 receptor antagonists, YM-09151-2 and spiperone, were in the nM range, which was 4 orders of magnitude lower than the IC50 value for the D1 antagonist SCH-23390. The S(-)-IBZM isomer was 10 times more potent than the R(+)-isomer in competing for the I-125-IBZM binding. The dissociation constant Kd of I-125-IBZM was 2.1 nM as measured by saturation analyses and calculated by Scatchard plots. DA agonists also competed with the I-125-IBZM binding. The IC50 values for DA, 6-F-DA and LY-171555 (DA agonist) were in the μM range and for Terguride (partial DA agonist) 10^{-8} M. 10^{-5} M clonidine and yohimbine (α-2 receptor ligands) displaced 15% and 40% of I-125-IBZM binding, respectively. A low affinity of S(-)-IBZM for the serotonin S2 receptors in frontal cortex was observed which was 2 orders of magnitude lower than the affinity of S(-)-IBZM for D2 sites in the striatum.

These results indicate that I-125-IBZM binding is highly specific for D2 DA receptors with a low affinity for D1, S2 and α2 binding sites. Furthermore, this ligand has been used successfully for in vivo imaging of denervation-induced increase in Bmax of D2 DA receptors in rat striatum (Kung et al., 1986; 1987). S(-)-IBZM, when labeled with a short half-life isotope, such as I-123, may prove to be a useful tracer for in vivo imaging of D2 DA receptors by SPECT procedures.

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58.3 NONSTEADY-STATE MEASUREMENT OF RADIOLIGAND-RECEPTOR BINDING IN VIVO USING PET AND 18F-SPIPERONE: IMPLEMENTATION IN HUMANS. JS Perlmutter*, J Markham*, M Kilbourn*, M Welch*, ME Raichle (SPON: T Videen). Washington Univ Sch of Med, St. Louis, MO 63110

We have developed a method using positron emission tomography (PET) and ^{18}F-spiperone (SP) for the measurement of radioligand-receptor binding in vivo. We now describe the implementation of this method in normal human subjects.

We studied 10 normal volunteers (8 male and 2 female) with a mean age of 26 years (range, 20 to 38). In each, we measured regional blood flow and blood volume with PET and rapidly administered ^{15}O-labeled radiotracers. The fraction of ^{18}F-SP bound in arterial blood, f_1, was determined with ultracentrifugal microfiltration. Following these measurements, up to 5 mCi of no-carrier-added ^{18}F-SP (containing < 2 to 4 ug of SP) was injected IV. Thirty-nine sequential PET scans and measurements of arterial-blood radioactivity due to ^{18}F-SP and its labeled metabolites continued for 3 hr. Regional measurements of radioactivity from the PET scans were obtained using a stereotactic localization method that is independent of the appearance of the images.

A 3-compartment model describing the in vivo behavior of ^{18}F-SP and a parameter estimation technique were used to analyze the PET and arterial-blood data. This enables us to calculate 4 parameters: (1) the combined forward-rate constant, k_1', (equal to the product of an association rate and the maximum number of available binding sites; (2) the binding-site dissociation rate constant, k_{-1}; (3) the free fraction of ^{18}F-SP not specifically bound in tissue, f_2; (4) the regional permeability-surface-area product, PS, of the blood-brain barrier for SP.

We calculated the following data (mean ± SD) for the 10 subjects: f_1 = .047 ± .014 and f_2 = .0065 ± .0021. For left caudate, PS = .047 cc/sec ± .022; k_1' = .140 sec^{-1} ± .056; and k_{-1} = .00034 sec^{-1} ± .00074 and for the right caudate, PS = .045 ± .021; k_1' = .191 ± .054; k_{-1} = .00053 ± .00074. Repeated studies in selected subjects yielded similar results.

This nonsteady state method for estimating radioligand-receptor binding in vivo can be implemented in human subjects and, with careful attention to technical details, yields reproducible results.

58.4 DOPAMINE D_2 RECEPTOR LIGAND BINDING SUBUNIT DETECTED BY PHOTOAFFINITY LABELING. D.E. Grigoriadis, H.B. Niznik and P. Seeman. Dept. Pharmacology, University of Toronto, Toronto, Ontario, M5S 1A8.

The ligand binding subunit of the dopamine D_2 receptor from canine striatal membrane homogenates has been identified by a novel photoaffinity label. The compound [^{3}H]azido-N-methylspiperone ([^{3}H]AMS) was derived from the potent neuroleptic spiperone and developed as a specific D_2 photoaffinity probe. In the absence of light, [^{3}H]AMS bound reversibly and with high affinity (K_D = 70 pM) to sites which could be competitively inhibited by agonists and antagonists with the appropriate pharmacologic dopaminergic rank order profile and stereospecificity. The dissociation constants of all agonists and antagonists correlated well with those obtained with other [^{3}H]dopaminergic ligands. Pre-exposure of [^{3}H]AMS to UV light did not affect its ability to bind to D_2 dopamine receptors in a reversible and saturable manner with high affinity. Upon photolysis by direct irradiation under UV light, [^{3}H]AMS was found to covalently incorporate into a peptide of Mr 92,000 as assessed by SDS-PAGE. Minor peptides of 70,000 - 50,000 Da were observed under some conditions and were the result of proteolytic breakdown of the 92,000 Da protein. The presence of protease inhibitors decreased the occurrence of these minor labeled peptides. The incorporation of [^{3}H]AMS into canine striatal membranes was inhibited by the proper pharmacologic rank order of potencies for both agonists and antagonists identifying the major labeled peptide at 92,000 Da as the dopamine D_2 receptor.

The alkylating agent N-ethylmaleimide or the reducing agent dithiothreitol did not have any effect on the mobility of the 92,000 Da protein suggesting that the ligand binding subunit is composed of a single polypeptide chain. The photolabeled receptor could be deglycosylated by the enzyme neuraminidase suggesting that the glycoprotein nature of the D_2 receptor contains terminal sialic acid residues. Cleavage of the photolabeled protein at terminal sialic acid residues altered the mobility of the 92,000 Da peptide to 55,000 Da. The specificity of [^{3}H]AMS labeling of the 92,000 Da peptide suggests that this peptide is the ligand binding subunit of the canine striatal D_2 dopamine receptor. This ligand should prove to be a useful tool in the further elucidation of the molecular structure of dopamine receptors.

This work was supported by the Medical Research Council of Canada (MRC) and the Ontario Mental Health Foundation (OMHF). D.E.G. is supported by an OMHF Studentship. H.B.N. is a recipient of an MRC post-doctoral Fellowship.

58.5 **ANTI-AMINOSPIROPERIDOL ANTIBODIES WITH HIGH AFFINITY FOR SPIROPERIDOL.** **R. R. Luedtke*, M. Korner*, K. Neve, F. Rahhal* and P.B. Molinoff. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.**

Spiroperidol (SPD), a member of a structural class of neuroleptics called butyrophenones, is a high-affinity antagonist for the D2 subtype of dopamine receptor. An N-bromoacetyl derivative of aminospiroperidol (ASPD) was used to covalently couple ASPD to keyhole limpet hemocyanin. The hapten-protein conjugate was used to immunize white New Zealand rabbits. Rabbit antisera bound ^{3}H-SPD at nanomolar concentrations (10 to 30 nM), indicating that high-affinity antibodies for SPD had been elicited. Scatchard plots of the data from direct binding experiments were curved, indicating that a heterogeneous population of anti-ASPD antibodies had been elicited. BALB/c mice were also immunized and a panel of 16 monoclonal antibodies was selected based on the ability to bind ^{3}H-SPD with high affinity (K_d values from 10 to 100 nM). The majority of antibodies were IgG_1 although the IgM, IgG_3 and IgG_{2a} isotypes were also expressed. Each of the anti-ASPD antibodies had a kappa light chain. The binding site of each monoclonal antibody was characterized by inhibiting the binding of ^{3}H-SPD with ligands that are either structurally related to SPD (anilinoperidol, nitroperidol, haloperidol and pipamperone) or are selective antagonists for D2 receptors structurally different from the butyrophenones (domperidone, sulpiride and clebopride). Seven different reactivity patterns were observed. Several anti-ASPD antibodies bound only SPD with high affinity, while others bound only butyrophenones (SPD, haloperidol and pipamperone). One antibody (APSD 4-7) bound the D2 antagonists SPD and domperidone with high affinity (K_d values of 13.5 nM and 24.5 nM, respectively). However, the affinity of ASPD 4-7 for pipamperone (K_d of 3000 nM), a butyrophenone that is an antagonist for the S2 subtype of serotonin receptors, was 100- to 200-fold lower than for SPD or domperidone. ASPD 4-7 has been purified by affinity chromatography using a p-fluorophenethylamine-epoxy Sepharose column. The purified ASPD 4-7 antibody will be used to prepare anti-idiotypic antibodies that will be tested for cross-reactivity with membrane-associated D2 receptors. (Supported by U.S.P.H.S Grant No. NS 18591 and the Scottish Rite Schizophrenia Research Program.)

58.6 D-1 AND D-2 DOPAMINE RECEPTOR MECHANISM IN DOPAMINERGIC BEHAVIOR. W. C. Koller, R. O'Donnell* and G. Herbster*. Deparment of Neurology, Loyola Medical Center, Maywood, IL 60153

Dopamine (DA) receptors are classified into D-1 and D-2 subtypes. Most dopaminergic behaviors are thought to be mediated by D-2 DA receptors. We have investigated the selective D-1 DA agonist, SKF 38393 (SKF), alone and in combination with dopaminergic agonists to define the role of D-1 agonism in these behaviors. The administration of SKF (1 to 64 mg/kg) to rats resulted in occasional non-stereotypic sniffing and grooming which was also observed in placebo treated animals. SKF potentiated apomorphine (0.25 mg/kg) induced stereotypy in a dose-dependent manner (1-40 mg/kg) when the drugs were given concomitantly. SKF (1-20 mg/kg) enhanced and altered the behaviors of ciladopa (20 mg/kg), a partial DA agonist. Full agonist behaviors i.e. gnawing, were observed when 20 mg/kg of SKF was combined with ciladopa. In unilateral 6-OH DA substantia nigra lesioned rats SKF (1-10 mg/kg) with mixed D-2 agonists, PHNO or quinpirole, or mixed D-1/D-2 agonist, pergolide or levodopa, resulted in a potentiation of the number of contralateral rotations induced by these drugs. SKF (1-40 mg/kg) produced no change in locomotion, as measured by a Columbus Varimax Activity Monitor. Apomorphine produced a biphasic response with inhibition at low doses. SKF (10 mg/kg) in combination with apomorphine did not alter the inhibititory response but markedly enhanced the stimulatory effect. No changes in behavior were observed in rats challenged with apomorphine after being treated 21 days with SKF, PHNO, SKF plus PHNO, and placebo.

It is concluded that D-1 agonism is capable of augmenting dopaminergic behavior of both D-2 agonists and DA drugs with mixed D-1/D-2 activity. A combination of D-1 and D-2 agonism may represent optimal drug treatment for Parkinson's disease.

58.7 PHARMACOLOGICAL PROPERTIES OF THE PARTIAL DOPAMINE D-2 RECEPTOR AGONIST SDZ 208-912. M. Karobath, Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland.

SDZ 208-912 is an ergoline which interacts in binding assays in vitro with dopamine D-2 receptors (pKi 8.35), alpha-1 receptors (pKi 8.0), 5HT-1A receptors (pKi 8.09), alpha-2 receptors (pKi 7.72), 5HT-2 receptors (pKi 7.2), and dopamine D-1 receptors (pKi 7.0) with an affinity similar to that of haloperidol. The pharmacological actions of SDZ 208-912 in vivo are characterized by a predominance of simultaneous agonistic and antagonistic effects at dopamine D-2 receptors. Thus SDZ 208-912 is equipotent to haloperidol as antagonist of apomorphine (1 mg/kg s.c.) induced climbing behaviour in the mouse (ED 0.5 mg/kg p.o.) and of apomorphine (2 mg/kg i.v.) induced gnawing in the rat (100% inhibition at 0.02 mg/kg p.o.). SDZ 208-912 like classical neuroleptics strongly elevates dopamine turnover in striatum, and it induces the neuroleptic-like alterations in the rates of regional glucose utilization in several brain areas like the lateral habenula. In high doses SDZ 208-912 even induces catalepsy in rats (threshold dose 2 mg/kg p.o.). SDZ 208-912 has also potent dopamine D-2 agonistic actions in vivo. Thus orally administered SDZ 208-912 causes a long-lasting suppression of plasma prolactin in rats at dose levels similar to those blocking apomorphine effects in this species. In the same species after s.c. administration it is equipotent to bromocriptine as prolactine inhibitor. In rats with unilateral lesions of the S. nigra SDZ 208-912 induces contralateral circling behaviour at 1.0 mg/kg p.o. with immediate onset and long duration of action. These pharmacodynamic effects are compatible with the notion that SDZ 208-912 is a partial agonist with low intrinsic activity at dopamine D-2 receptors and that the thresholds for agonistic effects differ for dopamine D-2 receptors in different brain areas.

58.8 DOPAMINE (DA) D_2 RECEPTORS AND GUANINE NUCLEOTIDE BINDING (G) PROTEINS IN BENIGN AND MALIGNANT PROLACTIN (PRL)-SECRETING RAT PITUITARY TUMORS. R. Collu, C. Bouvier*, G. Lagacé*, Res. Unit Reprod. Devel. Biol., Pediat. Res. Center, Hôp. Ste-Justine and Univ. de Montréal, Montréal, Québec H3T 1C5.

Previous studies performed with an estrogen-induced PRL-secreting rat pituitary adenoma have allowed us to conclude that the minimal size for the binding site of normal D_2 receptors present in adenomatous mammotrophs is approximately 100,000 daltons when evaluated by radiation inactivation (RI), and that association with a GTP-binding subunit of a G protein increases the receptor size to 145,000 daltons and decreases its affinity for DA agonists (Bouvier et al., J. Neurochem., 47:1653, 1986). More recently, we have found that D_2 receptors present in high affinity form in two malignant PRL-secreting rat pituitary tumors, 7315a and MtTW15, which are resistant to the inhibitory actions of DA, are insensitive to guanine nucleotides but have similar minimal binding site sizes as normal D_2 receptors as evaluated by photoaffinity labeling. To further evaluate the structural differences existing between normal and tumoral D_2 receptors we have determined the target size (TS) of receptorial proteins present in adenomatous and malignant mammotrophs and we have looked for the presence of G proteins in the three types of preparations. For TS analysis, receptors were labeled by photoaffinity using [^{125}I]-N_3-NAPS. Labeled receptors were then exposed to increasing amounts of gamma rays using the Gammacell 220, and were then subjected to SDS-PAGE followed by autoradiography. The intensity of radioactivity bands was measured by scanning and found to decrease linearly with increased radiation exposure. TS was determined by calibration with proteins of known Mr. The presence of G proteins was evaluated by immunoblot using antibodies raised against Gi-alpha, Go-alpha and G-beta, and purified G proteins provided by Dr A.M. Spiegel. TS was found to be 93,000 for normal, 96,000 for 7315a and 163,000 for MtTW15 receptors. Gi-alpha was found to be present in all three preparations (ug/mg protein. Normal: 0.6; 7315a: 0.4; MtTW15: 0.2). Go-alpha was present in normal and MtTW15 but not in 7315a cells (Normal: 1.55; MtTW15: 0.48). G-beta was found in all three preparations (Normal: 1.43; 7315a: 1.71; MtTW15: 2.74). These results suggest the presence of an anomaly in the D_2 receptorial protein of MtTW15 cells (dimer?). They show furthermore the presence in a pituitary adenoma of the alpha subunits of Gi and Go as well as the common beta subunit which may be involved in the transduction of the DA inhibitory message controlling PRL release. The absence of the Go-alpha subunit in 7315a cells is an interesting finding and may indicate that DA-resistance in the two malignant PRL-secreting tumors is due to different molecular anomalies.

58.9 DOPAMINE INHIBITS PROLACTIN RELEASE AND cAMP GENERATION IN THE MMQ CELL, A HOMOGENEOUS PROLACTIN-SECRETING CELL LINE. A.M. Judd*, I.S. Login* and R.M. MacLeod. Departments of Internal Medicine and Neurology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Dopamine inhibits prolactin release from normal anterior pituitary cells. However, the pituitary cell population is heterogeneous and presently established prolactin-secreting cell lines do not express functional dopamine receptors, thus making the study of control mechanisms difficult. We determined that the prolactin and ACTH-secreting 7315a tumor has functional dopamine receptors and undertook to isolate from this tumor a homogeneous prolactin-secreting cell line possessing these receptors. The solid tumor was dispersed with collagenase and cultured in RPMI-1640 containing normal horse and calf serum. After 12 days in culture, two cell populations--one adherent, one nonadherent to plasticware--were obtained. In the nonadherent cells, but not in the adherent ones, dopamine inhibited prolactin release that was stimulated by maitotoxin, a calcium channel activator. A clonal cell line, MMQ, was derived from the nonadherent cells which secretes prolactin, but no detectable amounts of ACTH nor other pituitary hormones. Dopamine was found to inhibit (50-80%) maitotoxin-induced prolactin release from these cells. We studied cAMP levels in the MMQ cells. Vasoactive intestinal peptide (VIP), forskolin and cholera toxin each increased cellular cAMP levels, and dopamine (50-1000 nM) inhibited each response in a concentration-dependent manner as it does in normal pituitary cells. Haloperidol, a dopamine receptor antagonist, blocked these dopaminergic effects as did pretreatment of the cells with pertussis toxin, an inactivator of a receptor-associated GTP binding protein. Somatostatin (SRIF, 100 nM) also significantly attenuated (30%) forskolin- and VIP-stimulated cAMP generation, but to a lesser extent than the attenuation caused by 1 uM dopamine (50%). MMQ cells grow rapidly (dividing time less than 24 hours), and after more than a year in culture the effect of dopamine on prolactin release has not been altered. In conclusion, the MMQ cell line secretes only prolactin and contains dopamine receptors that are negatively coupled to adenylate cyclase by a GTP-binding protein. These cells also possess functional SRIF and VIP receptors. This homogeneous cell line appears to be a valuable tool for determining the mechanism(s) through which dopamine, VIP and SRIF regulate cellular function. [Supported by NIH Research Grants CA-07535 and CA-38228, and grants from the American Parkinson Disease Association.]

58.10 CHRONIC TREATMENT WITH FLUNARIZINE AND NIMODIPINE AFFECTS STRIATAL DOPAMINE RECOGNITION SITES. S. Govoni, M.R. Moresco*, S. Di Giovine*, A.Leggio* and M. Trabucchi^. Institute of Pharmacological Sciences, University of Milan and ^Chair of Toxicology, 2nd University of Rome, Italy.

Clinical and experimental observations suggest an interaction of calcium antagonists with dopaminergic transmission. In particular, the chronic treatment with flunarizine, a diphenylalkylamine calcium antagonist, induces extrapyramidal disturbances in old patients.

In addition, in in vitro experiments, flunarizine decreases dopamine release from rat striatal slices (Battaini et al., Naunyn Schmiedeberg's Arch. Pharmacol. 332:267-270, 1986). Moreover nimodipine, another calcium antagonist, belonging to a different chemical class (i.e. dihydropyridines), may reduce the synthesis of striatal dopamine when acutely injected in rats. On the basis of these data we have investigated whether these drugs may present direct actions on dopamine recognition sites either in vitro or after in vivo repeated treatment. Tritiated spiroperidol and tritiated SCH 23390 were used as selective dopaminergic ligands. Tritiated spiroperidol is displaced by flunarizine and verapamil with different potency (IC50 0.7 micromolar and 10 micromolar, respectively) whereas nimodipine and nitrendipine have no effect.

In contrast, the binding of tritiated SCH 23390, a selective D1 antagonist, is displaced by 36% and 52% by nimodipine and flunarizine (10 micromolar). The displacement by nimodipine can be observed at very low concentrations, it is maximal at 10 nM, then not increasing over 40% of the specific binding even at concentrations as high as 100 micromolar. The in vivo data further support the concept of an interaction of these drugs with dopaminergic transmission. In fact, the chronic treatment with flunarizine and nimodipine induces complex adaptative changes of dopamine receptors which differ in D1 and D2 receptors.

In particular both flunarizine and nimodipine produce an increase in the Bmax of haloperidol displaceable spiroperidol binding (+24% and +22% respectively) without effect on Kd. In contrast flunarizine, but not nimodipine, decreases the Bmax of tritiated SCH 23390 binding.

These data may explain, at least in part, the extrapyramidal side effects of flunarizine. In addition the results indicate that nimodipine may interact with dopaminergic transmission although with a mechanism different from that of flunarizine. Whether the two compounds alter also the functional state of other neurotransmitters remains to be determined.

58.11 EVIDENCE THAT TERMINAL DA-AUTORECEPTORS INHIBIT DA-RELEASE IN VIVO. A. Imperato* and G. Di Chiara. Inst. of Exp. Pharmacology and Toxicology, University of Cagliari, Italy.

Dopamine (DA) autoreceptors located on DA-terminals are believed to control in an inhibitory manner the release of transmitter DA. In spite of the number of studies devoted to clarifying this mechanism its actual significance for the control of DA-transmission in vivo remains uncertain. We have tested this possibility by studying the effects of DA-agonists and antagonists locally applied in the caudate on the in vivo release of DA estimated by brain dialysis in freely moving rats. Various DA-agonists active on D-2 receptors such as LY 171555, BHT 920, pergolide and apomorphine (0.1-10 /uM) reduced DA-release in a concentration dependent manner; DOPAC and HVA output was reduced only by high concentrations of the agonists. These effects were prevented by systemic administration of clebopride (0.5 mg/kg s.c.) a specific antagonist of D-2 receptors. (-)3PPP applied at concentration of 10 /uM stimulated DA-release and metabolism. Local application of DA-receptor antagonists (-) sulpiride and haloperidol (0.1-10 /uM) stimulated DA-release in a concentration dependent/manner. (+)Sulpiride, the isomer inactive on DA-receptors, failed to modifie DA-release up to concentrations of 10 /uM. The results are consistent with the possibility that DA-autoreceptors located on DA-terminals are operative in controlling DA-release also in vivo.

58.12 DO AUTORECEPTORS CONTROL DOPAMINE SYNTHESIS IN RAT FOREBRAIN? EFFECTS OF APORPHINE ANALOGS OF DOPAMINE. R.J. Baldessarini, J.L. Neumeyer, M.H. Teicher, N.S. Kula,* A. Campbell,* and E. Marsh.* Harvard Medical School; *Mailman Research Center, McLean Hospital, Belmont, MA 02178.

The significance of putative presynaptic autoreceptors at dopamine (DA) containing nerve terminals remains unclear. There is good evidence for the ability of such a mechanism to diminish neuronal release of DA. However, evidence for autoreceptor mediated control of tyrosine hydroxylation, the rate-limiting step in DA synthesis, is less secure. We prepared and evaluated several rigid, hydroxylated aporphine analogs of DA to assess stereoisomeric and hydroxy-substituent effects on DA receptors. Compounds assessed included the R(-) and S(+) enantiomers of N-n-propylnor-apomorphine (NPA, a catechol), and their 11-monohydroxy congener. The R(-) and S(+) isomers of these agents, respectively, showed behavioral evidence of enantiomerically selective agonism or antagonism of DA receptors in the intact rat. The R(-) agonist isomer of NPA appeared to diminish turnover of DA in rat forebrain in vivo (diminished HVA:DA ratio or decreased accumulation of DOPA after inhibiting its decarboxylation), based on liquid chromatographic assays with amperometric detection. These effects were not stereoselective, however, and the antagonist (+)NPA also inhibited turnover. Also, the effects of (-) and (+) N-propyl-11-hydroxynoraporphine to decrease or increase DA turnover, respectively, were not strong or consistently dose-dependent. However, after pretreatment with gamma-butyrolactone to diminish neurophysiological activity in DA neuronal projections, the agonist isomers inhibited the rise of DOPA after inhibiting its decarboxylation with NSD-1015. The latter effect was stereoselective and was inhibited by haloperidol. In in vitro studies of tyrosine hydroxylase activity (by production of $^{14}CO_2$ from 1-^{14}C-L-tyrosine) with a synaptosomal fraction of corpus striatum, the NPA isomers inhibited DA synthesis by a process that was neither stereoselective nor inhibited by D-1 or D-2 dopaminergic antagonists. The monohydroxyaporphines produced a weak and reserpine-sensitive effect that also was not influenced by neuroleptic agents. The effects of exogenous DA on synthesis in synaptosomes were not inhibited by DA receptor antagonists but were blocked by potent antagonists of DA transport. These results taken together suggest that end-product and postsynaptically mediated inhibition of tyrosine hydroxylation can be demonstrated readily, but that demonstration of effects mediated by putative presynaptic DA autoreceptors at DA nerve terminals depends on the experimental methods employed.

59.1 IN THE GUINEA PIG, HORSE, AND MONKEY ASTROCYTES ARE RESTRICTED TO VASCULARIZED PARTS OF THE RETINA. Jutta Schnitzer* (SPON: A.C. Rusoff). MPI für Hirnforschung, D-6000 Frankfurt/M. 71, F.R.G.

Until recently, it was generally believed that astrocytes occurred ubiquitously in the nerve fiber layer (NFL) of mammalian retinae. However, the rabbit retina has been shown to have a distribution of astrocytes restricted to the medullary rays (Schnitzer, J., J. Comp. Neurol. 240:128, 1985). Interestingly, this region is the only part of the rabbit retina that is vascularized.

In the present study the distribution of astrocytes has been investigated in the sparsely vascularized guinea pig and horse retina, and in the richly vascularized monkey retina. The guinea pig retina and optic nerve head lacked glial fibrillary acidic protein (GFAP) immunoreactivity; but GFAP-positive astrocytes were detectable in the myelinated part of the guinea pig optic nerve. However, the optic nerve head and adjacent regions of the retina contained vimentin-positive astrocytes. The restriction of astrocytes to the small vascularized retinal region immediately adjacent to the optic nerve head was confirmed in semithin sections. In the whole-mounted horse retina, GFAP-positive astrocytes were restricted to a narrow zone close to the optic nerve head. This is also the only region of the horse retina that is vascularized. Thus, as in the rabbit, in the guinea pig and horse retina astrocytes and blood vessels coexist in the NFL.

In the whole-mounted Old World Monkey (Cercopithecus aethiops) retina, GFAP-positive astrocytes were found ubiquitously in the NFL. The avascular foveal region and a small vascularized rim which only contains capillaries in the inner nuclear layer, lacked astrocytes. The cessation of blood vessels close to the fovea coincides with a reduction in astroglial cell density; and supports the hypothesis of a co-occurrence of astrocytes and intraretinal blood vessels, a feature which may be common to all mammals.

59.2 ASTROCYTES IN RAT OPTIC NERVE ARE GENERATED IN A SINGLE WAVE BEFORE OLIGODENDROCYTES. R.P. Skoff and C. Brown*. Dept. of Anatomy and Cell Biology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

To determine whether astrocytes (AS) in the rat optic nerve (ON) are generated in a single wave before oligodendrocytes (OLS) are formed or whether a second wave of AS formation also occurs after OLS, a series of ^{3}H-thymidine autoradiographic studies were undertaken. Our previous studies of gliogenesis in ON (Skoff,R.P., J. Comp. Neurol., 169: 291 & 313, 1976) indicated that OL formation tapers off sharply by 15 days postnatal (DPN). One set of animals was given daily injections of thymidine from 15 to 19 DPN and sacrificed at 26 DPN, one rat was injected daily from 15 to 19 DPN, 22 and 24 DPN, and another set injected every other daily beginning at 19 DPN and ending at 28 DPN. The rats were perfused, ONs embedded in Araldite and sectioned at 1um for autoradiography. Adjacent thin-thick sections were also prepared so that the type of labelled cells could be definitively determined at the ultrastructural level. Both the orbital and intracranial segments of the ON were examined. Approximately 40 sections were quantitated/animal. Most sections were exposed for 1 month. In ONs from 2 animals, adjacent sections were exposed for either 1 or 2 months.

In the rats injected at 15 DPN, the average no. of labelled cells per section is 7.8. In the rats injected at 19 DPN, the average is 1.1. This change in number indicates that proliferation drops off sharply between 15 and 19 DPN. Since the total no. of glia/section is a little over 300, the low no. of labelled cells shows that only a few percent of glia are generated after 15 DPN. No signifcant differences in the no. of labelled cells were found in the different regions of the nerve nor in the no. of labelled cells exposed for 1 vs 2 months.

At the light level, the vast majority (>90%) of the labelled cells appear to be OLS in different degrees of differentiation. A few labelled AS are present. Ultrastructurally, 30 labelled cells have been identified so far. With the exception of 1 cell, the labelled cells all appear to be OLS. The vast majority have the characteristic features of OLS including stacks of ER, clusters of ribosomes, an eccentric nucleus, etc. Our results indicate that glial proliferation after 15 DPN is minor and that a second wave of astrocytes are not generated after OL formation. In a recent study from Raff's group (Miller, R.H. etal, Dev. Biol., 111:35, 1985), the investigators claimed that type-2 astrocytes, which are akin to fibrous astrocytes, are generated after OLS in a second wave in rat ON. Since the injection schedules in both studies were the same, the discrepancy in results between these two studies might be explained by the method of fixation. Raff's group used mostly frozen sections at the light level while we utilized embedded tissue at light and em level. It is easy to confuse many OLS with AS at the light level, especially OLS which are differentiating at later stages of development, as they often exhibit a moderate density, modest cytoplasm and may be surrounded by AS processes, giving the impression that they may be AS. Indeed, some of the labelled cells we tentatively idetified as AS at the light level have turned out to be OLS at the em level in the adjacent thin section. This and our previous studies of gliogenesis showing AS are generated mostly before OLS has significant implications regarding the time of appearance and lineage of AS in postnatal ON.

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59.3 **DEVELOPMENT OF A RAPID BIOLOGICAL ASSAY FOR THE CELL-SURFACE LIGAND, ASTROTACTIN.** T. N. Stitt and M. E. Hatten, Dept. of Pharmacology, New York University Medical School, 550 First Avenue, New York, N. Y. 10016.

In the early postnatal mouse cerebellum, granule neurons migrate along Bergmann glia from the pial surface to their final position in the cortex. Use of an in vitro microculture system in which purified populations of granule neurons and cerebellar astroglia are co-cultured has allowed direct observation of glial-guided neuronal migration and has been used to identify an immune activity, named anti-astrotactin, which blocks neuron-glia interactions in culture (Edmondson et al., J. Cell Biol., in press). An initial step in the further purification and characterization of the astrotactin antigen has been the establishment of a simple and rapid biological assay based on such neuron-glial interactions. As the astrotactin activity is localized to the plasma membrane, granule neuron membranes were used as a probe to monitor astrotactin-dependent binding to a glioma cell line, G26-24, which has previously been shown to bind and differentiate in response to granule neurons.

Purified granule neurons from P7 mouse cerebellum were obtained following a step-gradient centrifugation in Percoll and preplating on polylysine-coated substrata. The granule cells were metabolically labelled with 35S-methionine (100 µCi/ml) for 4 hr and collected. Following incubation in 1 mM zinc chloride for 12 hr at 4°C, the cells were homogenized and a plasma membrane fraction was prepared by centrifugation in a two-phase Dextran-polyethylene glycol gradient. Labelled granule neuron membranes in Ca, Mg-free PBS were added to confluent monolayer cultures of G26-24 glioma cells in 24-well plates and incubated at 37°C. At time points of five minutes to 2 hr later, the wells were shaken vigorously on a rotary platform to remove non-adherent material. The medium and the cell layer were separately harvested and assayed for radioactivity by scintillation counting. By 1 hr of incubation, approximately 70% of the radiolabelled material was found in the cell layer fraction, indicating that the membranes had bound to the glioma monolayer. When the neuron membrane fraction had been pre-incubated in a 1:50 dilution of anti-astrotactin serum raised against cerebellar cells, however, the membrane binding was blocked and nearly all of the labelled material remained in the medium.

The binding of the granule neuron membranes is apparently not due to cell-cell interactions mediated by the adhesion molecules N-CAM or Ng-CAM. Absorption of the astrotactin antiserum with intact PC12 cells, which express both N-CAM and Ng-CAM on their cell surface, did not remove the blocking activity of the serum. The neuron membrane-glioma interaction, then, is dependent on a ligand neutralized specifically by anti-astrotactin. Studies are now being done to tailor this system for use as a blocking assay for other candidate antisera and as a competition assay for astrotactin-like activity in biochemical fractions of cerebellar cells.

These studies have been supported by NIH grant 15429.

59.4 TRANSFERRIN IN OLIGODENDROCYTES OF THE HUMAN CNS. E.S. Wargotz* and J.R. Connor. (SPON: R. Bohn) Depts. of Pathology and Physiology, Geo. Washington Univ. Sch. of Med. Washington DC 20037

Transferrin is an 80,000 MW glycoprotein. It is mainly synthesized in the liver, but an mRNA for transferrin (Tf) is found in a number of organs including the brain. In the brain, the mRNA for Tf is reportedly located in oligodendrocytes [Block et al., (1985) PNAS 88:6706]. Recently, we have reported that Tf is present in oligodendroyctes in the rat and mouse central nervous system. The purpose of this study is to extend our investigation of the cellular localization and distribution of CNS Tf to the human. Human CNS material was obtained at autopsy within 12 hrs post-mortem. Samples of tissue were taken from the precentral gyrus, cerebellum and upper cervical spinal cord and placed into a 10% buffered formalin overnight. The next day the tissue was either cut at 30µM thickness on a Vibratome or processed through 10% and 30% sucrose solution (in Tris buffered saline) and cut at 8 µm on a cryostat. The antisera in this study was either rabbit anti-human Tf (Polysciences) or goat-antihuman Tf (E-Y Labs). Both antisera had an optimal dilution range of 1:750-1:1000. Both the sections cut on the Vibratome and those from the cryostat were processed as follows: 3% H_2O_2, 5% DMSO, serum block, overnight incubation in primary antisera. The immunohistochemistry was accomplished using the Vectastain ABC kit with 3-3'diaminobenzidine as the chromogen. The results of the study demonstrate that oligodendrocytes in both gray and white matter of the human CNS contain Tf. The Tf-positive cells in the gray matter had small, round somata. The reaction product was confined to one area of the soma giving the immunolabeled cells a "cap-like" appearance similar to that seen in rats and mice. White matter immunoreactive cells generally had rectangular somata. The Tf-positive cells rarely had immunoreactive processes. This study extends our finding that Tf is present in oligodendrocytes to the human CNS. Furthermore, these data support the hypotheses that iron and Tf may play a major role in the maintenance of myelin and that oligodendrocytes are involved in regulating iron in the CNS.

[JRC is Supported by NS22671 and 2507 RR05359-23 (GWU)].

59.5 EXTRACELLULAR MATRIX PRODUCTION BY ASTROCYTES AND ITS RELATION TO NEURITE GROWTH. M.D. Ard and R.P. Bunge. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Astrocytes have previously been shown to produce the extracellular matrix (ECM) molecules heparan sulfate proteoglycan (HSPG) and laminin in vitro (Ard and Bunge, Soc. Neurosci. Abstr. 1986 12:394). Because certain components of ECM are promoters of neurite growth, and astrocytes provide a terrain for neurite growth within the developing CNS, we have asked whether there is a correlation between ECM production by astrocytes and their ability to influence neurite growth. Production of laminin and HSPG by astrocytes was studied by light microscopic (LM) immunocytochemistry and electron microscopy (EM) and was found to be regulated by culture conditions. Astrocytes grown on rat tail collagen in medium with serum for 5 days to 2 weeks exhibited ECM visible by LM and EM. On the other hand, astrocytes grown in defined medium produced no ECM detectable by these methods, and the amount of ECM in serum-containing cultures decreased rapidly upon conversion to defined medium. Long-term (4-6 weeks) growth in serum-containing medium also resulted in a decrease in the amount of HSPG and laminin. In all conditions studied, morphology of the cells changed from flat to stellate in parallel with the decline in HSPG and laminin.

The importance of serum in up-regulating ECM production may not be attributable to the fibronectin content of serum, since addition of 20 ug/ml of purified fibronectin to defined medium did not result in deposition of detectable HSPG or laminin, or change in morphology of the astrocytes.

When fetal rat dorsal root ganglion neurons were cultured on astrocytes, neurite growth was not correlated with ECM production by astrocytes nor with changes in their morphology. Neurites grew on astrocyte surfaces in preference to the rat tail collagen substratum. In serum-containing cultures at 5 days, the few layers of astrocytes present exhibited a small amount of ECM deposited on the substratum and between the cellular processes. Intercellular spaces were small. Neurites were found on the upper surface and between layers of astrocytic processes. After 11 days of culture with serum, there were several layers of astrocyte processes separated by large intercellular spaces or channels containing ECM. Neurites grew within these channels, though not apparently in relation to the ECM. In older (4-6 weeks) cultures in serum, neurites grew both on the upper surface of the astrocytes and between the many layers of astrocytic processes; little ECM was visible between astrocytic processes. In defined medium astrocytes were sparse, but their processes were seen to partially enclose bundles of neurites. Thus the capacity of astrocytes to interact with growing sensory ganglion neurites is not regulated in tandem with production of ECM components as examined in this study. (Supported by NIH NS09923 and a National Multiple Sclerosis Society Fellowship.)

59.6 CEREBELLAR ASTROGLIA SYNTHESIZE BASIC FIBROBLAST GROWTH FACTOR, A PROMOTER OF NEURITE EXTENSION BY GRANULE NEURONS IN VITRO. M.E. Hatten, M. Lynch*, J. Joseph-Silverstein*, D. Moscatelli* and D.B. Rifkin*. Departments of Pharmacology and Cell Biology and the Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016.

When grown in the absence of astroglial cells, purified mouse cerebellar granule neurons survive less than 16 hours and do not differentiate. We have found that basic fibroblast growth factor (bFGF) at low doses 1-25 ng/ml maintains the viability and promotes the differentiation of granule neurons grown under these conditions. This maintenance and differentive activity of bFGF was blocked by antibodies prepared against bFGF.

In mixed microcultures of cerebellar neurons and astroglial cells, the presence of bFGF (1-4 ng/ml) markedly increased the density of granule cell neurites. When affinity-purified antibodies against bFGF were added to these cultures, the amount of process extension by granule neurons was severely impaired. No differences in the pattern of the association of neurons with astroglial cells was seen; granule neurons were located along the arms of the astroglial cells in the microcultures. The inhibition of neurite outgrowth in the presence of anti-FGF antibodies was reversed by the addition of 20 ng/ml of exogenous bFGF.

Immunoprecipitation of ^{35}S-labeled cerebellar astroglial cells with affinity-purified anti-FGF antibodies revealed a single band at 18,000 daltons, the molecular weight of bFGF. These results indicated that glial cells synthesize bFGF and may be an endogenous source of bFGF in cerebellar cultures. Thus, astroglial cells may provide soluble factors needed for neuronal differentiation.
Supported by NIH grant NS 21097 (M.E.H.).

59.7 SCHWANN CELL MITOGEN IN MYELIN STIMULATED MACROPHAGE CONDITIONED MEDIUM MAY BE RELATED TO MYELIN BASIC PROTEIN Baichwal, R.R., Bigbee, J.W., DeVries G.H., Dept. of Biochem. and Mol. Biophys. and Anatomy, Virginia Commomwealth Univ., Richmond, VA 23298

Myelin phagocytosis by infiltrating macrophages during Wallerian degeneration may correlate with Schwann cell proliferation observed in the distal stumps of degenerating nerves. We have previously shown that exogenously added myelin-enriched fraction (MEF) and myelin stimulated macrophage conditioned medium (MCM) are mitogenic to cultured Schwann cells (SCs) (Baichwal et al, Soc. Neurosci. Abstr., Vol 12, p394, 1986). MEF from rat, bovine and human CNS were equally potent in stimulating the production of the mitogenic supernatant indicating a lack of species specificity. Supernatants obtained by the addition of CNS MEF were 2-3 times more mitogenic than those obtained from autologous PNS fractions. Sensitivity of MCM to heat and trypsin suggested that the mitogenic factor may be a polypeptide. CNS-derived supernatants are two to three fold more mitogenic than PNS-derived MCM which is similar to the ratio of myelin basic protein (MBP) in myelin of CNS and PNS. To further test the possibility that the mitogenic factor may be related to MBP, membrane fractions from the mutant mouse shiverer which lacks MBP and proteolipid protein (PLP) were used to produce MCM. Shiverer brain white matter was homogenized and fractionated on a 10-40% linear sucrose gradient to obtain axolemma-enriched fraction (AEF) and MEF as described previously (DeVries et al, J. Neurochem. 40, 1983). Membrane fractions from 29-31% sucrose region which showed highest specific activity for acetylcholinesterase were pooled as AEF. Fractions from 17-19% sucrose region of the gradient were pooled as MEF and lacked MBP and PLP but did contain the Wolfgram proteins when analyzed by SDS PAGE. Direct stimulation of SCs with AEF gave a labelling index (LI) of twenty fold over background while MEF produced only a two fold stimulation. However, SC cultures showed a LI of 0.86% (±0.23%) in response to AEF conditioned medium and 0.30% (±0.06%) in response to MCM, with background levels of stimulation being 0.62% (±0.37%). The lack of SC response to MCM derived from shiverer myelin may indicate that the mitogenic factor may be derived from MEF and may be related to MBP. (Supported by NS15408 and NS10821).

59.8 A NEUROBLASTOMA-DERIVED GROWTH FACTOR FOR OLIGODENDROCYTE PRECURSORS. S.F. Hunter*, M.F. Seidel* and J.E. Bottenstein, Dept. of Pharmacology, Marine Biomedical Institute and Dept. of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX 77550.

There is evidence that neurons and/or axons are influential in the appearance and development of oligodendrocytes (ODC) in vivo, although they have not been shown to be required for development of galactocerebroside-positive (GC^+) ODC in vitro. Chemically defined N4 medium which has been conditioned (CM) for 4 days by B104 rat CNS neuroblastoma cells possesses activity which promotes the proliferation of A2B5-positive ($A2B5^+$) ODC precursor cells in mechanically dissociated neonatal rat brain cultures. Cells are inoculated at 500,000 cells/mm^2 in chemically defined O3 medium on poly-D-lysine and fibronectin-modified culture dishes. O3 medium consists of Dulbecco's modified Eagle's medium (DME) supplemented with 15 μg/ml insulin, 1 μg/ml transferrin, 30 nM sodium selenite, and 10 ng/ml biotin. The activity in the CM is soluble in nature, lyophilizable, sensitive to 100°C or trypsin treatment, and is not ether-extractable. Increases in both $A2B5^+$ and GC^+ cells are observed at 4, 8, 12, and 20 days in vitro (DIV) when 33% B104 CM is added to O3 medium. At 8 DIV the mean densities of $A2B5^+$ cells are increased 4-fold and of GC^+ cells are increased 10-fold. These increases are even greater when compared to DME with 15% calf serum rather than our usual control: O3 medium with 33% unconditioned N4 medium. When considering all of the glial phenotypes present, the percentage of $A2B5^+$ and GC^+ cells in B104 CM treated cultures is about 3.5 times that of control cultures, and the percentage of glial fibrillary acidic protein-positive (GFA^+) Type I astrocytes is about half that of controls. Autoradiography (24 hr ^3H-thymidine pulse) combined with indirect immunofluorescence for these cell surface antigens reveals that 28% of $A2B5^+$ cells and 10% of GC^+ cells are labelled at 4 DIV while 7% and 0% respectively are labelled in control cultures. Mature, morphologically well-differentiated ODC are generally unlabelled, while the less differentiated $A2B5^+$ cells are labelled. Treatment of cells with CM factor partially purified by gel filtration chromatography also results in dose-dependent increases in the densities of $A2B5^+$ and GC^+ cells in O3 medium. Our results are consistent with the hypothesis that the major target of the growth factor is the ODC precursor cell. A similar CNS neuron-derived endogenous factor might be a signal involved in gliogenesis, glial turnover, and/or recovery from demyelinating injury. (Supported by NIH grant NS20375)

59.9 OLIGODENDROGLIAL STEM CELLS ARE INDUCED TO PROLIFERATE ON A "VELATE PROTOPLASMIC" LIKE CEREBELLAR ASTROGLIAL CELL CLONE. N. Delhaye-Bouchaud, F. Alliot and B. Pessac. Centre de Biologie Cellulaire CNRS, 94200 Ivry-sur-Seine, France.

We have previously reported the derivation from 8 PN day mouse cerebella of permanent clonal cell lines with astroglial properties which might be the in vitro counterparts of the Golgi-Bergmann epithelial cells and of the velate protoplasmic astrocytes (Alliot, F. and Pessac, B., Brain Res., 306:283-291, 1984). When neuronal cells from 15 embryonic day cerebella are seeded at low density on monolayers of these astroglial cell lines, 60-80 % survive after 5 days of coculture (Pessac, B. and Alliot, F., Soc. Neurosci. Abstr., vol. 12, Part 1, p. 397, 1986). To investigate whether the Golgi-Bergmann and the velate protoplasmic lines could support the survival of other cell types, single cells were dissociated from the ventral part of 18 embryonic day mouse medulla and were plated on layers of these astroglial clones. A few round cells could be seen on both layers during the first days of coculture. However, very rare foci of these cells appeared only on the velate protoplasmic layers after the 10th day of coculture. These cells proliferated very actively during 2-3 weeks, so that each focus had about 1.5×10^5 cells which were identified as oligodendrocytes by the presence of surface galactocerebrosides and cytoplasmic 2'-3' cyclic nucleotide 3' phosphodiesterase. Similar results were obtained when single cells from embryonic cerebella were plated on the velate protoplasmic astroglial layers. In contrast, when single cells from embryonic mouse medulla or cerebellum were plated on astrocyte-enriched passaged cultures from medulla, cerebellum or cerebral hemispheres, many foci of round cells rapidly appeared, which were composed of about 10^5 oligodendroglial cells.

Taken together, these data show that the ventral medulla and the cerebellum contain very rare stem cells that can actively proliferate in vitro for 15-20 generations with a doubling time of less than 18 hrs and which acquire markers specific for oligodendrocytes. The mitogenic signal responsible for the proliferation of this subset of very rare oligodendroglial stem cells is present in the cerebellar velate protoplasmic astroglial clone but apparently not in astrocyte-enriched cultures from various regions of the CNS.

59.10 EXPRESSION OF PROTO-ONCOGENES *fos* AND *myc* INCREASES AFTER INJURY TO THE FISH OPTIC NERVE: IN VITRO AND IN SITU STUDIES. C. Stein-Izsak, I. Cohen*, M.-F. Chesselet, M. Murray, and M. Schwartz. The Weizmann Institute of Science, Rehovot, Israel and Medical College of Pennsylvania, Philadelphia, Pennsylvania, USA.

CNS injury in a species capable of regeneration causes proliferation and differentiation of the surrounding non-neuronal cells, including the production of factors that appear to stimulate regeneration in mammalian and non-mammalian axons (Schwartz, M. et al., Science 228: 600, 1985). This supports the idea that activated non-neuronal cells might contribute to successful regeneration. Previously, we have shown that mRNA from non-neuronal cells of carp (Cyprimus carpio) optic nerve contains sequences homologous to the oncogenes, *fos* and *myc* (Stein-Izsak, C. et al., Proc. 16th Meet. Soc. Neurosci. 12:12, 1986). We now show that crush injury to the carp optic nerve results in increased proto-oncogene mRNA levels in the non-neuronal cells, evident 1 and 8 days post-operatively. RNA slot blot hybridizations with nick-translated *fos* and *myc* probes showed 2-4 fold increases in *myc* expression 8 days after nerve crush and up to 10-fold increases in *fos* expression, as measured by scanning densitometry of the slot-blot autoradiograms. This increase in proto-oncogene expression in the non-neuronal cells seems not to reflect a general increase in all mRNA species, as hybridization with a tubulin probe showed no increases at the same times.

We used in situ hybridization with single-stranded RNA probes to visualize cells expressing *fos* and *myc* mRNA in the fish optic nerve. The hybridization signal, indicated by silver grains, was much greater in nerve 1 and 8 days post-crush than in intact nerves. More grains were distributed over the distal than over the proximal stump. The cellular distribution of *fos* and *myc* mRNA also appeared to be different, providing a hint as to their possible role in the non-neuronal cell response to injury.

59.11 EFFECTS OF L-GLUTAMATE ON ION TRANSPORT PROCESSES AND SWELLING IN PRIMARY ASTROCYTE CULTURES. H.K. Kimelberg and S. Pang*, Div. of Neurosurgery, Albany Medical College, Albany, N.Y. 12208.

L-glutamate is known to be taken up avidly by astrocytes both in situ and in vitro, and in vitro such uptake is known to be Na^+, and perhaps Cl^- dependent. Application of L-glutamate is also known to cause astroglial swelling in situ and is released during pathological states when astroglial swelling also occurs. Such swelling may have deleterious consequences. The effects of L-glutamate on volume and ion transport in astrocytes is thus of importance and we are studying these processes in primary astrocyte cultures from neonatal rat brain to elucidate potential mechanisms. 10^{-5} to 10^{-4} M L-glutamate directly causes Na^+ dependent depolarization of cells in such cultures (Bowman & Kimelberg, Nature 311:656-659, 1984) and we have found that, in the presence of ouabain, L-glutamate causes cell swelling and increased uptake of $^{22}Na^+$ and $^{36}Cl^-$. In the absence of ouabain L-glutamate causes increased uptake of $^{86}Rb^+$ (used as a K^+ analog), which is also Na^+ dependent as well as being completely ouabain-sensitive. The initial uptake of 10^{-4}M [^{3}H] L-glutamate is not blocked by 1mM ouabain added at the same time as [^{3}H] L-glutamate, but when measured over 10 min. the uptake of 10^{-4}M [^{3}H] glutamate is inhibited by about 50%. We ascribe this to dissipation of the inwardly directed transmembrane Na^+ gradient. Na^+ dependent uptake (95-97% of total [^{3}H] glutamate uptake) of 10^{-4}M [^{3}H] L-glutamate is inhibited by SITS (46%), DL aspartic acid β hydroxamate (63%), β-methyl DL aspartate (77%), DL threo β hydroxy aspartic acid (86%) (values in parentheses refer to % inhibition and concentrations of all inhibitors was 1mM). 1mM DL threo β hydroxy aspartic acid also inhibited 10^{-4}M L-glutamate-stimulated $^{22}Na^+$ uptake. These data are consistent with electrogenic uptake of L-glutamate due to a Na:glutamate ratio of > 1 and that, in the presence of ouabain, intracellular accumulation of Na^+ and Cl^-, which is presumably being co-transported, leads to cell swelling. We have attempted to directly estimate the ratio of increased $^{22}Na^+$ uptake to [^{3}H] L-glutamate taken up. At 10^{-5} to 10^{-4}M L-glutamate we have found ^{22}Na:[^{3}H] glutamate ratios of 2 to 3. These studies were done in the presence of ouabain to eliminate outward pumping of Na^+ but considerable error in the estimation of $^{22}Na^+$ uptake led to some variation in this ratio which appeared to be at least 2. In the absence of ouabain there is clearly stimulation of (Na+K) pump activity as evidenced by increased uptake of $^{86}Rb^+$. Under some pathological conditions we propose that the (Na+K) pump is inhibited and that uptake of Na^+ and Cl^- associated with glutamate uptake and the lessened capacity to pump out accumulated Na^+ is partly responsible for the astroglial swelling observed. (Supported by grant NS23750 from NINCDS).

59.12 EFFECTS OF PENTYLENETETRAZOL (PTZ) ON ION TRANSPORT PROCESSES OF ASTROCYTES IN PRIMARY CULTURE. H. Steve White*, Sandra Reinhold*, Sien Yao Chow* and Dixon M. Woodbury. Dept. of Pharmacology and Toxicology, College of Pharmacy, Univ. of Utah, S.L.C., UT 84112.

In view of the role that has been implicated for astrocytes in regulating extracellular fluid potassium concentration ($[K^+]_o$) during increased neuronal firing and the fact that PTZ increases K^+ flux across epithelial cell types, the aims of the present study were to identify and characterize the effects of PTZ on astrocyte ion transport processes. The effect of PTZ on radioiodide ($^{125}I^-$) and radio-chloride ($^{36}Cl^-$) distribution, Na^+/K^+-ATPase activity and total cell K^+ was determined in mature primary astrocyte cultures derived from the cortices of newborn rats and mice.

Cells were preincubated for 1 hour in a HEPES-balanced salt solution (122 mM NaCl, 4.2 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM HEPES, 20 mM $NaHCO_3^-$, 10 mM glucose; pH 7.4) and then exposed to various concentrations of PTZ (1.0 to 10 mM) for 60 min. Under these conditions, PTZ reduced the steady-state content of $^{125}I^-$ and $^{36}Cl^-$ in cultured astrocytes in a dose-dependent manner. PTZ (10 mM) decreased the $^{125}I^-$ content from 319.9 ± 19.8 to 218.2 ± 14.3 DPM/mg protein and the $^{36}Cl^-$ content from 1407.0 ± 129.3 to 1141.6 ± 76.6 DPM/mg protein in control vs treated cells, respectively. This effect of PTZ to decrease $^{125}I^-$ and $^{36}Cl^-$ transport was attenuated when the $[Na^+]_o$ was reduced from 144 mM to 20 mM. For example, at 20 mM $[Na^+]_o$ the total $^{125}I^-$ and $^{36}Cl^-$ of control cells ($^{125}I^-$, 268.4 ± 43.3 DPM/mg protein; $^{36}Cl^-$, 1229 ± 116 DPM/mg protein) was not significantly different from that of PTZ-treated cells ($^{125}I^-$, 211.4 ± 14.3 DPM/mg protein; $^{36}Cl^-$, 1253.7 ± 74.3 DPM/mg protein). Similar results with respect to total $^{36}Cl^-$ were observed when the $[K^+]_o$ was reduced from 4.2 to 1.2 mM. PTZ (1 to 10 mM) inhibited Na^+/K^+-ATPase in a concentration-dependent manner. This action of PTZ reduced the total K^+ content of astrocytes in culture (1.20 ± 0.12 vs 0.96 ± 0.07 meq/mg protein for control vs PTZ-treated cells, respectively) and depolarized the astrocytic membrane.

The present findings demonstrate that PTZ inhibits anion transport into primary astrocytes maintained in tissue culture by blocking Na^+/K^+-mediated anion cotransport. Furthermore, PTZ inhibits Na^+/K^+-ATPase, decreases intracellular K^+, and causes membrane depolarization. Thus, in addition to its action on neurons, the data presented herein demonstrate that PTZ affects ion transport processes of astrocytes in a manner that may contribute to the convulsive action of this agent. Supported by Grants from the Epilepsy Foundation of America and from the NIH (1-R01-NS22200).

60.1 THE ULTRASHORT-LOOP FEEDBACK EFFECT OF CENTRALLY INJECTED GROWTH HORMONE-RELEASING FACTOR TO SUPPRESS PULSATILE GROWTH HORMONE SECRETION IS SITE SPECIFIC TO THE ARCUATE NUCLEUS. M.D. Lumpkin* (SPON: C. Colton), Department of Physiology and Biophysics, Georgetown Univ. School of Medicine, Washington, D.C. 20007

Previously it was shown that intracerebroventricular injection of growth hormone-releasing factor (GRF) would suppress GH release by a postulated mechanism of ultrashort-loop feedback whereby endogenously released or exogenously administered GRF could act as an inhibitor to the further secretion of GRF from its neurons [Endocrinology 116: 2070, 1985]. I next sought to determine whether the site at which centrally administered GRF produced this GH suppression did, in fact, correspond to the locus of GRF cell bodies in the hypothalamic arcuate nucleus (ARC) or, as another possibility, to somatostatin (SS) somata in the anterior periventricular region (PV). In order to examine this question directly in vivo, 23 gauge stainless steel cannulae were implanted stereotaxically into the bilateral ARC or bilateral PV of adult male rats. After a recovery period of 7-10 days, synthetic rat GRF (1-43, Peninsula) at a dose of 10 ng in 0.5 ul of normal saline was microinjected bilaterally over a period of 60 seconds into the ARC of conscious, unrestrained male rats through 30 gauge insertion cannulae. Microinjections into tissue sites commenced immediately after withdrawing a preinjection blood sample at 1000 h. Control animals received 0.5 ul of saline alone into these two sites. Sequential blood sampling every 15 min for 6 h between 1000 and 1600 h revealed that GRF infused into the ARC essentially abolished the 2 periods of peak releases of radioimmunoassayable GH that occurred predominantly between 1030-1130 h and 1400-1500 h in these experiments. For example, mean plasma GH $\pm$ SEM (ng/ml) at 1100 h: ARC GRF-treated (n=8), 14.6 $\pm$ 4.0 vs control (n=7), 68.0 $\pm$ 23.2 ($p<.025$). Mean plasma GH $\pm$ SEM at 1430 h: ARC GRF-treated, 13.4 $\pm$ 3.5 vs control, 135.0 $\pm$ 58.1 ($p<.025$). The overall mean for plasma GH levels between 1000-1600 h in the ARC GRF-injected group was 14.6 $\pm$ 7.4 vs 56.0 $\pm$ 9.4 ng/ml for controls ($p<.0001$). In contrast, injection of GRF into the bilateral PV produced no significant change in episodic GH secretion between 1000 and 1600 h when compared to saline-treated controls. The inhibition of pulsatile GH release by GRF injected into the ARC appeared to be specific to GH controlling elements since prolactin levels were not significantly changed. These findings suggest that the "ultrashort-loop feedback" (autoregulation) of centrally injected GRF to suppress pulsatile GH secretion is mediated to a significant degree by inhibition of neurosecretion from ARC GRF neurons rather than by stimulation of PV somatostatinergic neurons.

(Supported by NIH grant RO1-NS23036)

60.2 COEXISTENCE OF GROWTH HORMONE RELEASING FACTOR AND VASOTOCIN IN THE HYPOTHALAMO - HYPOPHYSEAL SYSTEM OF THE FROG. F. Vandesande and S. Marivoet. Lab. of Neuroendocrinology, K.U.L. Zoological Inst. B - 3000 LEUVEN (Belgium).

Rabbit anti human pancreatic growth hormone releasing factor (h.p.GRF 1-44) antisera were prepared by immunizing two rabbits with h.p.GRF 1-44 conjugated to thyroglobulin using glutaraldehyde as coupling reagent. Mouse monoclonal anti rat hypothalamic growth hormone releasing factor (r.h.GRF 1-10) antibodies were prepared using synthetic r.h.GRF 1-10 conjugated to thyroglobulin with the carbodiimide method. Two groups of adult male and female frogs (Rana temporaria) were studied. The first group was killed in January, the second in July. Serial 5 µm sections of the brains were stained using the rabbit anti h.p.GRF 1-44 antisera, mouse monoclonal anti r.h.GRF 1-10 antibodies and rabbit anti vasotocin (VT), anti mesotocin (MT), anti somatostatin (SRIF) and anti corticotrophin releasing factor (CRF) antisera in combination with a single and double PAP technique.

Single immunocytochemical staining revealed a GRF - like immunoreactivity in the magnocellular preoptic nuclei, the internal and external zone of the median eminence and in the neural lobe. No colocalisation of GRF with MT, SRIF and CRF could be detected. However, double staining with as well the polyclonal as the monoclonal anti GRF antibodies and anti VT revealed the presence of three celpopulations. The first was only positive for GRF, the second was only positive for VT and the third showed both GRF and VT - like immunoreactivity.

In the internal and external zone of the median eminence and in the neural lobe, double staining also resulted in three populations of nerve fibres. No difference between the two groups of animals was observed. Our results strongly suggest a coexistence between GRF and VT in the frog .pp

60.3 MECHANISMS OF IMPAIRED GROWTH HORMONE (GH) SECRETION IN GENETICALLY OBESE ZUCKER RATS: ROLES OF GH-RELEASING FACTOR AND SOMATOSTATIN. G.S. Tannenbaum, M. Lapointe*, W. Gurd* and J.A. Finkelstein. McGill University and Montreal Children's Hospital, Montreal, Quebec H3H 1P3, and Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 45267.

Growth hormone secretion is markedly blunted in obesity; however, the mechanism(s) mediating this response remains to be elucidated. In the present study, we examined the involvement of the two hypothalamic GH-regulatory peptides, GH-releasing factor (GRF) and somatostatin (SRIF), using the genetically obese male Zucker rat. Spontaneous six-hour GH secretory profiles obtained from freely-moving chronically cannulated rats revealed a significant suppression of pulsatile GH secretion in obese (655 $\pm$ 13 gm bw) animals compared to their lean (397 $\pm$ 8 gm bw) littermates (mean 6-h plasma GH level; 4.7 $\pm$ 1.0 vs. 16.1 $\pm$ 4.1 ng/ml; $P < 0.02$). The plasma GH response to an intravenous bolus of 1 µg rGRF(1-29)NH_2 (kindly provided by Dr. P. Brazeau), administered during peak and trough periods of the GH rhythm, was significantly attenuated in obese rats at peak (87.8 $\pm$ 25.7 vs. 192.4 $\pm$ 28.9 ng/ml; $P < 0.02$) but not trough (56.1 $\pm$ 20.3 vs. 43.4 $\pm$ 12.7 ng/ml) times. Moreover, both pituitary wet weight (10.3 $\pm$ 0.46 vs. 11.9 $\pm$ 0.49 mg; $P < 0.05$) and pituitary GH concentration (29.2 $\pm$ 2.1 vs. 42.5 $\pm$ 3.3 µg/mg wet wt; $P < 0.01$) were reduced in the obese group. Measurement of hypothalamic GRF immunoreactivity using a specific radioimmunoassay for rGRF revealed a significant ($P < 0.02$) reduction in mediobasal hypothalamic GRF concentration in obese rats (30.2 $\pm$ 2.9 pg/mg wet wt) compared to lean controls (47.4 $\pm$ 5.0 pg/mg wet wt), although no significant difference was observed in hypothalamic SRIF concentration. In contrast, peripheral SRIF immunoreactive levels were significantly elevated in both the stomach (1.38 $\pm$ 0.09 vs. 1.05 $\pm$ 0.07 ng/mg wet wt; $P < 0.01$) and pancreas (0.94 $\pm$ 0.13 vs. 0.58 $\pm$ 0.06 ng/mg wet wt; $P < 0.01$) of obese rats. These results demonstrate that the genetically obese Zucker rat exhibits (1) marked impairment in both spontaneous and GRF-induced GH release, (2) significant reduction in pituitary GH concentration, (3) depressed hypothalamic GRF concentration and (4) elevated gastric and pancreatic SRIF levels. The findings suggest that the defect in pituitary GH secretion observed in obesity may be due, at least partially, to insufficient stimulation by hypothalamic GRF concomitant with exposure to increased circulating levels of SRIF.

60.4 FACILITATION BY PENTOBARBITAL OF GROWTH HORMONE SECRETION IN HAMSTERS: THE INVOLVEMENT OF ENDOGENOUS SOMATOSTATIN. K.T.Borer, Dept.of Kinesiology,Univ.of Michigan,Ann Arbor, MI 48109 and A.G. Amedor*, Dept.of Physiology, Southern Illinois Univ.,Carbondale,IL 62901.

Growth hormone (GH) release is under antagonistic influence of facilitatory GH-releasing hormone (GHRH) and of inhibitory somatostatin (SRIF). In the rat, both GHRH and SRIF appear to be released in a pulsatile manner.A SRIF pulse induces a GH trough and prevents exogenous GHRH from eliciting a GH pulse, while at the time of SRIF trough, a GHRH pulse triggers GH release (Tannenbaum & Ling, Endocrinol.,1984,115:1952). Sodium pentobarbital facilitates GH release in the rat (Howard & Martin, Endocrinol.,1971,88:497) presumably by suppressing the release of endogenous somatostatin.

We tested the hypothesis that a morning pulse of SRIF accounted for a GH trough between 0900 and 1130 h in mature female hamsters (Borer et al., Endocrinol.,1986,118:844). An intracardiac catheter was implanted through the right jugular vein 24 h before the experiment. Animals received 3 i.v. injectione: 1 ml of anti-SRIF serum or NRS at 0830 h; 0.5 ml of heparinized saline or of sodium pentobarbital (42 mg/kg) at 0945 h; and 0.3-0.4 ml of GHRH (5ng/g) or saline at 1015 h. Blood samples were collected sequentially at 5 min (first two) and at 10 min intervals (next 10) following the third injection. A blood replacement was given after each blood removal. GH determinations were made with a homologous RIA for hamster GH (Borer et al.,Neuroendocrinol.,1982,35:349). Spontaneous GH pulses were examined in CONTROL group (n=8:1.NRS, 2.saline, 3.saline). Possible presence of SRIF antagonism of GHRH was examined in GHRH group (n=7: 1.NRS, 2.saline, 3.GHRH). A possible suppression of SRIF by sodium pentobarbital was examined in B-GHRH group (n=7:1. NRS, 2.sodium pentobarbital, 3.GHRH). Finally, a possible presence of a SRIF pulse was examined in AS-GHRH group (n=7: 1.anti-SRIF serum, 2.saline, 3.GHRH).

A small GH pulse followed the third injection in control hamsters followed by a large endogenous GH pulse after 1130 h. A 3.5-times higher pulse of GH followed the third injection in GHRH group. GHRH elicited a GH pulse that was 2.4 times (AS-GHRH group) and 4.1 times (B-GHRH group) greater than in GHRH group.

We conclude that (1) SRIF is released between 0900 and 1130 h in mature female hamsters and contributes to suppression of GH release; (2) endogenous SRIF reduces the capacity of GHRH to elicit GH release at that time of day; and (3) sodium pentobarbital may facilitate GH release by suppressing SRIF release or action rather than through elicitation of endogenous GHRH release.

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60.5 **HIERARCHIC CONTROL OF CALCIUM ION SIGNALLING IN SINGLE PITUITARY SOMATOTROPES: POTENT INHIBITION OF CALCIUM CHANNELS BY SOMATOSTATIN (SRIF) DOMINATES OVER GHRH-INDUCED CALCIUM MOBILIZATION.** R.W. Holl*, M.O. Thorner, G.L. Mandell*, J.A. Sullivan*, Y.N. Sinha* and D.A. Leong*. (SPON: T. Johns), Department of Internal Medicine, University of Virginia, Charlottesville, VA 22908.

The generation of intracellular signals activated by opposing (stimulatory and inhibitory) receptors can be studied together in the somatotrope. We recently described an endogenous oscillatory pattern of cytosolic calcium transients associated with spontaneous secretory activity in somatotropes (Prog. Ann. Meet. Endoc. Soc., 1987, Abstract #117). SRIF abolished this oscillatory activity and markedly inhibited GH secretion. GHRH stimulated calcium ion mobilization and promoted GH secretion. Thus SRIF and GHRH exert opposing actions in the somatotrope. The outcome of combined GHRH/SRIF treatment was determined by measuring calcium ions with the fura-2/AM method. A reverse hemolytic plaque assay (RHPA) was used to quantitate hormone secretion in single pituitary cells obtained from male Sprague-Dawley rats (125-250 gm). Spontaneous calcium oscillations were abolished, within 20 seconds, and ambient calcium levels were reduced, when calcium was withdrawn from the extracellular medium. This effect upon calcium mobilization was mimiced exactly by treatment with 1 nM SRIF, even when a source of extracellular calcium was provided, suggesting that SRIF inhibits calcium influx. Despite a 10-fold molar excess of GHRH (10 nM) over SRIF (1 nM), co-treatment with GHRH/SRIF caused cytosolic calcium levels to decrease in the same manner observed with SRIF alone. Parallel effects on GH release were measured by RHPA in the same single cells. Thus calcium mobilization is potently abolished by inhibitory input (SRIF receptor) by a mechanism that completely overrides stimulatory input (GHRH receptor). The signalling pathways for these opposing receptors must therefore converge at a common locus prior to calcium mobilization. These findings demonstrate a novel hierarchic mechanism governing opposing receptors and signal transduction in the somatotrope.

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60.6 INFLUENCE OF THE GONADS ON CIRCADIAN CORTISOL SECRETION IN RHESUS MACAQUES. C.J. Smith* and R.L. Norman, Dept. of Cell Biology and Anatomy, Texas Tech University Health Sciences Center, Lubbock, Tx. 79430

Circadian/ultradian patterns of plasma cortisol were assessed in adult ovariectomized (OVX) rhesus macaques and compared with cortisol patterns in gonadally intact females during the late follicular (day 9-10) and mid-luteal (day 21-22) phases of the menstrual cycle. Beginning at 0800h, blood samples were obtained every 15 min for 24 h via an indwelling catheter. Plasma concentrations of cortisol, estrogen (E_2), and progesterone (P) were determined using specific RIAs. For purposes of data analyses, group cortisol measurements were collapsed across hourly intervals. Diurnal variations in absolute cortisol levels were analyzed separately for each group with a single-factor repeated measures ANOVA. Cortisol differences between the 3 gonadal states were evaluated using split-plot ANOVA and Tukey's t-tests. Additional estimates of change in cortisol secretion were evaluated using the relative change from the daily mean as a reference. The pulse frequency of cortisol release was determined for each subject utilizing PULSAR computer program.

A diurnal pattern of cortisol secretion was documented in all 3 groups and characterized by a progressive rise during early morning hours (0300-0600) followed by a decline of short duration and a peculiar elevation which persisted from 0900-1400 h. Within all groups, cortisol reached a nadir in the early evening hours. Significant differences between the follicular and luteal groups were not discerned. However, plasma cortisol concentrations in the OVX group were significantly depressed when levels in the gonadally intact females reached their zenith. In addition, the daily mean level of cortisol was reduced nearly 50% by OVX. When cortisol changes were assessed using the percentage change from the daily mean as an index, group differences disappeared. No statistical differences in ultradian characteristics of cortisol secretion between the 3 female groups throughout the sampling period were found.

When data in the intact female groups were combined and compared to those previously obtained from gonadally intact adult male macaques, similar 24-h patterns of cortisol secretion were documented. Surprisingly, amplitude changes in cortisol concentrations after OVX were temporally and quantitatively similar to those in orchidectomized (ORCH) males. In both sexes, circadian patterns of cortisol secretion were reduced/absent after gonadectomy. These results indicate that gonadal steroids have a significant effect on hypothalamic-pituitary-adrenocortical function.

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60.7 CONSTANT LIGHT AND DARK AFFECT THE CIRCADIAN RHYTHM OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS. R. L. Moldow*, A. J. Kastin, M. V. Graf* and A. J. Fischman*. Seton Hall University, S. Orange, NJ 07079 and Veteran Administration Medical Center and Tulane University, School of Medicine, New Orleans, LA 70146

The effect of constant light and constant dark on the circadian rhythm of the concentrations of hypothalamic corticotropin releasing factor like immunoreactivity (CRF-LI), plasma ACTH, and corticosterone was investigated. Groups of rats were maintained under normal light-dark (LD), constant light (CL), or constant dark (CD) conditions for 10 days. Rats were then killed over a 24 hour time period and hypothalamic CRF-LI, plasma ACTH, and corticosterone concentrations were determined by RIA. Under normal light-dark conditions, hypothalamic CRF-LI concentrations exhibited significant decreases at 1700 h and 0200 h that coincided with peaks in plasma ACTH and corticosterone concentrations. In rats housed under CD conditions for 10 days, higher hypothalamic CRF-LI concentrations were detected at night than during the early part of the day. These relatively high hypothalamic CRF-LI concentrations coincided with relatively low plasma ACTH concentrations. The timing of peak plasma ACTH concentrations was slightly phase-shifted, and the amplitude was markedly attenuated compared to levels of rats housed under normal light-dark conditions. The rats exposed to CD continued to demonstrate higher plasma corticosterone concentrations in the pm than in the am. The peak in plasma corticosterone coincided with the peak in plasma ACTH concentrations; however, the amplitude was normal. In rats maintained in CL for 10 days, a decrease in hypothalamic CRF-LI concentrations at 2000 h coincided with a peak in plasma ACTH. The peak in plasma ACTH concentrations was not associated with a peak in plasma corticosterone concentrations. The rhythm of plasma corticosterone concentrations was dramatically attenuated and phase-shifted. Together, these findings indicate that alterations of normal light-dark conditions result in changes in the circadian variation in hypothalamic CRF-LI, plasma ACTH, and corticosterone levels. Changes in the circadian rhythm of plasma ACTH concentrations were related to changes in the rhythm of hypothalamic CRF-LI, but an apparent dissociation between the pituitary and adrenal rhythms was observed.

60.8 ULTRASTRUCTURAL EVIDENCE FOR CORTICOTROPIN RELEASING HORMONE (CRH) SYNAPSES WITHIN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN) OF THE RAT. A.J. Silverman. Dept. of Anat. & Cell Biology, Columbia University, N.Y., N.Y. 10032.

Immunocytochemical studies have shown a large population of CRH neurons resides within the PVN (Swanson et al, Neuroendocrinology 36:165) and that these cells form the major if not sole source of CRH terminals on the hypophysial portal vessels (Bruhn et al, Endocrinology 114:57). We have now examined the ultrastructural characteristics of these neurons and the connections that they make within the PVN. CRH was detected in vibratome sections through the adult male rat PVN as previously reported (Hou-Yu et al, Neurosci. Absts.217.10, (86) except that the detergent was 0.02% saponin and the DAB reaction product was silver-gold intensified (Liposits, Neurosci. Lett. 31:7). In these normal animals, CRH neurons were characterized by heterochromatic nuclei which were frequently indented, an extensive Golgi apparatus and several stacks of rough endoplasmic reticulum. The antibody used reacted primarily with the numerous neurosecretory granules. These cells and their dendrites were richly innervated as has been described previously (Liposits & Paull, Peptides 6:1021). Numerous CRH positive synapses were also found within the PVN, concentrated but not confined to the periventricular zone. The presynaptic axonal elements contained, in addition to CRH positive granules, other, smaller dense core-granules and numerous clear, round 'synaptic' vesicles that accumulated in the active zone. The synapses were characterized by well defined synaptic clefts and pre and post-synaptic membrane specializations. To date the majority of CRH terminals were found on non-identified dendrites. However, as suggested by light microscopic images of CRH axonal baskets around PVN neurons (Hou-Yu, unpub.), occasional perikarya contacted by several CRH terminals have also been observed. Although most CRH axons innervated non-CRH structures within the PVN, instances of axo-somatic connections between CRH elements have also been identified. It is not known whether all the CRH terminals have their origin with the PVN but these current observations provide evidence that CRH could act locally within the hypothalamus to modulate neurosecretory or autonomic activity. (USPHS grant AM 37205).

60.9 GLUCOCORTICOID RECEPTOR IN MANGOCELLULAR NEUROSECRETORY CELLS. J.Z. Kiss[1]*, J.A.M. Van Eekelen[2]*, J.M.H.M. Reul[2]*, H.M. Westphal[3]* and E.R. de Kloet[2]* (SPON: J.-J. Dreifuss). Institute of Histology and Embryology, Univ. of Geneva Medical School, Switzerland[1], Rudolf Magnus Institute, Univ. of Utrecht, The Netherlands[2], Institute for Molecular Biology and Tumorresearch, Marburg, Federal Republic of Germany[3].

It has previously been shown, that glucocorticoids inhibit vasopressin (VP) release from the hypothalamic magnocellular system. However, the mechanism and the site of this action remained controversial. There is evidence, for example, that the effect of glucocorticoids might be mediated by increased plasma volume or by action on the peripheral osmo-receptor.

Here we report that glucocorticoid receptor (GR) in fact is present in magnocellular neurosecretory cells of the rat. In vitro ligand displacement experiment suggests that these are the type II "feed back" type receptors as defined by Reul and de Kloet (Endocrinology 1985, 117:2505). Using a monoclonal antibody against purified liver GR, immunostaining was found in magnocellular neurosecretory neurons in the SON but not in magnocellular neurons located in the paraventricular nucleus. Immunoreactive cells seem to concentrate in ventral parts of the SON where VP cells were previously shown to be located. At the ultrastructural level GR immunoreactivity has been found in the cell nucleus as well as in various cytoplasmic compartments. One to two weeks after bilateral adrenalectomy, there was a substantial decrease of immunostaining in magnocellular neurons. Administration of synthetic glucocorticoids (RU 28362 or dexamethasone) induced a robust increase in the intensity of immunostaining in cell nuclei of neurosecretory cells. The presence of GR in the SON suggest that glucocorticoids may affect vasopressin synthesis or/and secretion through direct action in magnocellular neuron.

60.10 INVOLVEMENT OF THE PARAVENTRICULAR NUCLEUS IN THE ACTH SECRETORY RESPONSE TO CENTRAL NICOTINIC CHOLINERGIC STIMULATION. S.G. Matta*, H.S. Beyer* and B.M. Sharp* (SPON: S. Nicol). Minneapolis Med. Res. Fdn. and Dept. of Med., Hennepin Cty. Med. Ctr. and Univ. of Minnesota, Mpls, MN 55415.

Nicotine is a potent stimulus for the secretion of ACTH from the pituitary in the rat. We have previously demonstrated that injection of nicotine into the hypothalamic region of the third ventricle elevated rat plasma ACTH to levels comparable to those seen when nicotine was administered iv. To identify specific brain nuclei that are involved in this central action of nicotine (N), permanent bilateral intracerebral nuclear (ICN) cannulae were used for the direct injection of nicotine into the hypothalamic paraventricular nucleus (PVN). The PVN was targeted because it contains binding sites for ^{125}I-α bungarotoxin and its neuropil contains ^{3}H-nicotine sites. On separate days, rats fitted with chronic jugular cannulae received N 0.5, 1.0 or 10.0 ug or an equivalent volume of buffer (B) into each PVN. ACTH values (pg/ml) are mean ± sem; comparisons of integrated response areas (N vs. B) were by ANOVA. Nicotine significantly elevated (B = N 0.5<N 1.0<N 10.0, p <.05) plasma ACTH, yielding peak levels similar to the maximal responses to iv or ip N:

	N	0 min	3 min	7 min	15 min
Buffer	18	25±5	27±7	33±7	43±10
N 0.5 ug	9	50±11	142±66	163±60	150±50
N 1.0 ug	15	38±7	164±51	211±76	225±78
N 10.0 ug	17	28±5	449±76	726±155	481±120

To determine whether mecamylamine (Mec) is an effective antagonist of N-induced ACTH secretion, Mec 2 mg/kg BW was delivered ip 10 min prior to N 0.25 or 1.0 mg/kg BW ip. Mec significantly inhibited the ACTH responses to both doses of N (B/B 24±6; Mec/B 31±12; B/N 1.0, 633±52; B/N 0.25, 468±79; Mec/N 1.0, 110±24*; Mec/N 0.25, 23±8*; *p<.01 compared to respective B/N). Subsequent studies with ICN Mec were conducted to determine whether the PVN mediates the effect of peripherally administered nicotine. B or Mec 10 ug were injected into PVN 10 min prior to the iv injection of saline (S) or N 0.03 mg/kg BW. Analysis of response areas (pg·min/ml) indicates that Mec effected a partial, but not significant blockade of the ACTH response to peripheral Nic: Mec/N 5904±1359; B/N 8326±1199; B/S 1855±339; Mec/S 1659±377. Thus, the PVN is directly responsive to relatively high doses of N; the activation of additional central site(s) appears to be necessary for the maximal ACTH response to peripherally administered nicotine. (Supported by NIDA DA03977)

60.11 BIOSYNTHESIS OF PRO-OPIOMELANOCORTIN IN THE RAT INTERMEDIATE PITUITARY IS GLUCOCORTICOID-SENSITIVE FOLLOWING DENERVATION. M.A. Seger*, J.A.M. van Eekelen*, J.Z. Kiss*, H.H.M. van Tol*, J.P.H.Burbach* and E.R. de Kloet* (SPON: Tj. van Wimersma Greidanus). Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, The Netherlands.

Deafferentation of the rat anterior hypothalamus by a Halasz knife cut is known to sever also the neural input to the neural and intermediate lobes (NILs) of the pituitary gland. One week after performing the lesion in male (150-180g) rats, the animals were sacrificed and the NILs were incubated in vitro for 24h with tritiated amino acids to compare their POMC-synthesizing capacity with sham-operated controls. A general increase (2 to 4-fold vs control, $p<0.05$) of incorporation of ^{3}H into HPLC-analyzed POMC-peptides was observed, with no concommitant change in the content of the NILs. The induction of the glucocorticoid receptor (GR) in the NILs by denervation (see also Antakly, T., Science 229: 277, 1985) was visualized by immunocytochemistry. In order to test whether the induced GR was functional, the experiment was repeated in lesioned adrenalectomized (ADX) rats chronically treated with corticosterone (100 mg pellets of CORT, giving 23 ug% in plasma) and in the appropriate control groups (6 animals each). The result of the in vitro biosynthesis study showed that ADX attenuated the lesion-induced increase in POMC-peptide labelling (2 to 5-fold decrease from sham-lesioned CORT-replaced rats, $p<0.05$) and CORT-replacement reversed the effect. This suggests that IL POMC gene expression becomes sensitive to CORT after anterior hypothalamic deafferentation.

The POMC mRNA levels in the IL of rats from the 4 groups were measured by dot-blot analysis at 3 weeks post-lesion, when GR induction was maximal. The changes in IL POMC mRNA paralleled the changes in incorporation of label into POMC peptides observed in the in vitro biosynthesis study. It is concluded that:
1. anterior hypothalamic lesions induce GR in the IL which participate in the regulation of POMC gene expression;
2. CORT stimulation of GR enhances biosynthesis of POMC peptides in the IL, in contrast to its blocking role in the anterior lobe;
3. these findings may partially explain the lesion-induced increase in POMC biosynthesis.

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61.1 RESERPINE TREATMENT INDUCES AN ENHANCED SENSITIVITY OF ADENYLATE CYCLASE TO DOPAMINE STIMULATION WITHOUT CHANGING THE PROPERTIES OF ^{3}H-SCH 23390 SPECIFIC BINDING. C. Missale, P. Liberini*, E. Nisoli*, M. Memo, A.Rossi, P.F. Spano. Inst. Pharm. Exp. Ther., School of Medicine, University of Brescia, Brescia, Italy and Farmitalia C. Erba, R.& D., CNS Line, Milano Italy

Behavioral studies have shown that reserpine treatment in rats causes an increased sensitivity of both D-1 and D-2 receptors to stimulation by selective agonists.

The present study shows that the DAergic behavioral supersensitivity induced by reserpine does not correlate with the kinetic properties of ^{3}H-SCH 23390 binding sites but is reflected by an enhanced activity of DA-stimulated adenylate cyclase (AC).

Male SD rats were given a single injection of reserpine (5 mg/Kg ip) and then sacrificied 2 and 20h after treatment. D1 $_{3}$DA receptor function was tested by measuring the kinetics of ^{3}H-SCH 23390 binding sites and the ability of the selective D-1 receptor agonist SKF 82526 to stimulate AC activity. We found that acute treatment with reserpine significantly increased the sensitivity of striatal AC activity to SKF 82526 stimulation. Maximally effective concentrations of SKF 82526-stimulated striatal AC activity by 100% in saline group, and 155% in striatum from rats pretreated 2h and 20h respectively with reserpine before killing. The IC-50 calculated from the dose-response curves did not differ significantly among the three groups. The function of D-1 DA recognition sites was also measured by ^{3}H-SCH 23390 binding assay. We found that 2 h and 20 h following reserpine treatment the total amount (Bmax) of H-SCH 23390 specific binding decreased by 35% with no change in the apparent dissociation constant (Kd). In a separate experiments, saturation analysis of ^{3}H-SCH 23390 specific binding to striatum from reserpine and saline treated animals were conducted after preincubating the membrane preparations with 250 nM DA. Preincubation of striatal membranes from reserpinized rats reversed the treatment-induced loss of ^{3}H-SCH 23390 binding sites, Bmax values for ^{3}H-SCH 23390 binding to striata from saline-and reserpine-treated rats were 680 fmol/mg prot and 660 fmol/mg prot, respectively. Irrespective for the mechanism by which DA preincubation reverses an apparent loss of ^{3}H-SCH 23390 binding sites, our data indicate that reserpine treatment does not alter either number or affinity of D-1 recognition sites.

61.2 **DIFFERENTIAL REGULATION OF DOPAMINE RECEPTORS IN CAUDATE-PUTAMEN AND NUCLEUS ACCUMBENS FOLLOWING CHRONIC ADMINISTRATION OF RESERPINE.**

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Mapping of dopamine (DA) receptors in rat brain has indicated that D1 and D2 receptors are distributed independently (Boyson et al, J. Neurosci.6:3177, 1986). For example, the relative ratio of D1/D2 receptors in the lateral parts of the caudate-putamen (CPu) is less than in the medial CPu and nucleus accumbens septi (NAS). There also appear to be regional differences in the response to depletion of DA from the striatum (Marshall et al., this vol.) and in the response to chronic treatment with neuroletpics (Boyson et al, Soc. Neurosci. Abstr., 1986). The present experiments were designed to determine if administration of reserpine could produce selective regional changes in DA receptors or in the two principal subtypes of DA receptors.

Male, Sprague-Dawley rats (180 g at first drug treatment) were given reserpine (2.5 mg/kg I.P. first dose, 1 mg/kg s.c. thereafter) or saline (1 ml/kg) on two successive days and then at weekly intervals for two weeks (n=6) or 8 weeks (n=4). Animals were given i.p. injections of sucrose/saline for 4 days following the first injection of reserpine. After 2 weeks or 8 weeks, the animals were killed and the distribution of DA D2 ([3H]spiroperidol) and D1 ([3H]SCH 23390) receptors was determined autoradiographically.

There were regional differences in the denisty of DA receptors apparent with the short-term (2 weeks) and longer-term (2 months) experiments. After 2 weeks the density of D2 receptors had increased in all regions of the rostral CPu (lateral, central, medial; 40-60%) and NAS (90%). At more caudal levels the increase was restricted to the lateral CPu (25%). The density of D1 receptors was increased in all regions of the striatum (CPu and NAS), but the increase was significant only in the NAS (25%) within the rostral striatum and in the caudal CPu (40%).

After 2 months of reserpine treamtment the increase in the density of D2 receptors apparent after 2 weeks of treatment with reserpine had disappeared in the CPu, and had decreased in magnitude in the NAS (25% increase over saline control). Additionally, the increase in the density of D1 receptors in the CPu apparent after 2 weeks of reserpine treatment had been reversed such that the density of receptors was decreased. A 20% decrease as compared to controls was observed in all regions except the NAS, where there was a 35% increase as compared to matched controls. (USPHS GM 34781)

61.3 REPEATED ADMINISTRATION OF (-) SULPIRIDE AND SCH 23390 DIFFERENTIALLY UP-REGULATE D-1 AND D-2 DOPAMINE RECEPTOR FUNCTION IN RAT MESOSTRIATAL AREAS AND PITUITARY BUT NOT IN CORTICAL-LIMBIC BRAIN REGIONS. E. Nisoli*, M. Memo, M. Pizzi*, C. Missale, M.O. Carruba*, A. Forgione and P.F. Spano. Inst. Pharm. Exp. Ther., School of Medicine University of Brescia, Brescia, Italy

We have previously found that repeated administrations of haloperidol, a mixed D-1 and D-2 dopamine (DA) receptor antagonist, to rats for 21 days induced supersensitivity of both classes of central DA receptors. In the present study, we examined the possible functional modifications of both D-1 and D-2 DA receptor subtypes following repeated administration of DA antagonists that act selectively on a single class of DA receptors. The functional state of D-1 and D-2 DA receptors in particular was evaluated by measuring SKF 82526-stimulated and bromocriptine-inhibited adenylate cyclase activity in different brain regions of rats treated with saline, SCH 23390, or (-)sulpiride for 3 or 5 weeks. The results indicate that chronic blockade of D-1 DA receptors in striatum, nucleus accumbens, and substantia nigra by SCH 23390 induced up-regulation of the D-1 receptors without changing the functional activity of D-2 receptors. Likewise, chronic blockade of D-2 DA receptors by (-)sulpiride caused up-regulation of D-2 but not D-1 DA receptors in striatum, nucleus accumbens, substantia nigra and pituitary. We also examined the function of the DA receptor modulating striatal DA release. Treatment with either (-)sulpiride or SCH 23390 caused a decrease in the sensitivity of DA autoreceptor to inhibit DA release. SCH 23390 or (-)sulpiride did not modify the functional activity of either D-1 or D-2 DA receptors located in frontal cortex and hippocampus. In conclusion, these results indicate that chronic treatment with selective D-1 or D-2 DA receptor blockers induces a receptor-specific up-regulation which involves the DA receptors located in the nigrostriatal system and pituitary but not those in the limbic-cortical areas. These data may be of interest in understanding the development of extrapyramidal disordes following repeated administrations of neuroleptic drugs.

61.4 SELECTIVE DOWN REGULATION OF D_1 DOPAMINERGIC MEDIATED ROTATIONAL BEHAVIOR IN SUPERSENSITIVE MICE. James Winkler, Kathleen Callison*, Stephen Cass* and Benjamin Weiss, Division of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of Pennsylvania/EPPI, Philadelphia, PA. 19129

Although the existence of different subtypes of dopaminergic receptors has been recognized for a number of years, it is still unclear what specific behavioral functions each of these receptors subserves. We have addressed this question by attempting to selectively down regulate D_1 or D_2 responses by administering mice selective D_1 or D_2 agonists chronically via Alzet minipumps.

Mice were initially rendered supersensitive to dopamine agonists by making unilateral 6-hydroxydopamine-induced lesions of the corpus striatum. They were then chronically implanted either with the D_1 agonist SKF 38393 or the D_2 agonist LY 171555 (Quinpirole). The concentration of drugs in the pumps were adjusted to deliver a calculated dose of 0.48 umole/hr. for SKF 38393 and 0.0625 umoles/hr. for LY 171555. Spontaneous rotational behavior as well as their response to acute challenge doses of SKF 38393 (40 umole/kg, s.c.) or LY 171555 (2.5 umole/kg, s.c.) were then determined.

Continuous exposure to SKF 38393 produced spontaneous rotations that began within one hr. after the implant. These spontaneous rotations stopped by 5 hrs. after implantation and did not reoccur throughout the two-week period of exposure. At the time spontaneous rotations ceased, the mice were totally unresponsive to an acute challenge injection of SKF 38393. This down regulation in the response to the D_1 agonist was relatively specific since no inhibition of rotational behavior was seen in response to the acute administration of LY 171555.

Mice chronically implanted with LY 171555 also showed marked spontaneous rotations beginning within one hr. after the implant. However, in contrast to the results with SKF 38393, relatively little diminution of the spontaneous rotations was seen during the two week period of exposure. Moreover, there was little change in response to acute challenges with either the D_1 or D_2 agonist.

These results suggest that D_1 and D_2 mediated events respond differently to chronic activation of their respective receptors, and demonstrate that one can produce selective and long term alterations in specific subtypes of dopaminergic receptors. Selective alterations of dopaminergic responses may provide the tools necessary for distinguishing the behaviors mediated by the different subtypes of dopaminergic receptors.

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61.5 REGULATION OF MESOLIMBIC DOPAMINE RECEPTORS BY CYCLO(LEUCYL-GLYCYL). J.Z. Fields, L.R. Meyerson, J.M. Lee, F. DeLeon Jones and R.F. Ritzmann. Research Services, Hines VA Hospital, Hines, IL 60141, American Cyanamid Co., Mahwah, NJ 07430 and Olive View Medical Center, Los Angeles, CA 91342-1495.

According to the dopamine (DA) hypothesis of schizophrenia, suppression of dopaminergic (DAergic) neurotransmission in the mesolimbic DA tract should be therapeutic. Accordingly, we have been studying a family of peptides related to prolyl-leucyl-glycinamide (PLG, MIF) that appear to be capable of just such a suppression. After male Sprague-Dawley rats were pretreated on day 1 with cyclo(leucyl-glycyl)("CLG")(s.c., 8 mg/kg), there was a decrease (30 to 60%)($p<0.05$) in amphetamine (1.5 mg/kg, i.p.) induced locomotion on day 5. Activity was automatically recorded for 20 min using the radiofrequency field capability of Stoelting Electronic Activity Monitors. Under these conditions, the incidence of oral stereotypies, e.g. sniffing, was less than 1%, thus insuring that locomotion was not suppressed by stereotypic behaviors that can emerge at higher doses of DA mimetics.

Increasing the number of injections from one on day 1 to one each on days 1 to 4 did not alter the outcome. Altering CLG levels by either increasing or decreasing the dose (range 2 to 24 mg/kg) decreased the down-regulatory effect of CLG. This "inverted-U" shaped dose response curve is typical for a number of the pharmacological actions of CLG and related peptides.

At 8 mg/kg CLG there is an increase in apomorphine-induced stereotypy and an increase in the affinity and density at the higher affinity subsite observed in curves for dopamine inhibition of (^{3}H)spiroperidol binding to striatal membranes. This suggests that regulation of nigrostriatal D2 DA receptors (DA-R) may be unlike regulation of mesolimbic D2 DA-R. Hypothalamic DA-R, as measured by apomorphine-induced hypothermia and D2 DA-R, were down-regulated, the data matching effects of CLG on mesolimbic DA-R but opposite to effects on striatal DA-R.

CLG also down-regulated mesolimbic DA-R in a model of mesolimbic supersensitivity. Haloperidol (1 mg/kg, s.c.) injected daily for 21 days to male Swiss Webster mice induced a hyperlocomotion response seen following a challenge dose of apomorphine (1 mg/kg, i.p.) 48 hr following the last dose of haloperidol. Co-administration for 21 days of CLG (2 mg/kg, s.c.) completely suppressed the development of this supersensitivity.

61.6 STRESS-INDUCED ALTERATIONS IN CORTICAL SPIRODECANONE RECEPTORS. A.J. Friedhoff, H.R. Rosengarten, E.A. Stone and M. Egawa*, Dept. Psychiat., New York Univ. Sch. Med., New York, NY 10016.

Chronic stress is known to produce increases in the responsiveness of brain dopaminergic receptors. In a previous study designed to shed further light on this phenomenon we reported that chronic restraint stress alters the properties of spiroperidol binding sites (defined with (+)-butaclamol) in the cerebral cortex such that the B_{max} was increased and the K_D was reduced (Friedhoff, Rosengarten & Stone, Abstr. Soc. Neurosci. 12:192, 1986). Spiroperidol in the presence of a serotenergic antagonist, is believed to bind to both spirodecanone and D2 receptors in the cortex. Our previous study did not distinguish between these two receptors. The present study was designed therefore to determine which of these receptors is affected by stress.

Rats were subjected to repeated restraint stress (2 hrs/day for 10 days) or to control conditions (daily weighing) and were killed 15-20 hrs after the last stress. [^{3}H]Spiroperidol binding (0.1-0.8 nM) was measured in frontal cortical membranes using (+)- or (-)-butaclamol (100 nM) as the displacing agent. Specific spirodecanone receptor binding was defined as the difference between total and (-)-butaclamol-displaced spiroperidol binding and was found to comprise 10-20% of the total binding. Specific D2 receptor binding was defined as the difference between (+)- and (-)-butaclamol-displaced spiroperidol binding and was found to be 10% of the total. Because of the low amount of specific binding to these sites the data from 5 rats were pooled for each Scatchard determination. A total of 15 control and stressed rats were run.

It was found that stress caused an increased density and reduced affinity of spirodecanone receptors (B_{max} (fmol/mg prot.): control, 35.3 $\pm$ 9.4; stress, 107 $\pm$ 20.7, $p < .05$; K_D (nM): control, 0.28 $\pm$ 0.08; stress, 1.03 $\pm$ 0.18, $p = .05$). The effect of stress on cortical D2 receptors could not be assessed because the low counts obtained precluded accurate Scatchard analysis.

The above preliminary findings indicate therefore that at least part of the alteration in spiroperidol binding in the cortex after stress results from changes in spirodecanone receptors. Other authors have shown that these receptors are also increased in density in the cortex after repeated treatment with antidepressant drugs (Pol. J. Pharmacol. Pharm. 37:317, 1985). While a function for spirodecanone receptors in dopaminergic neurotransmission is still not definitely established it appears from these findings that they have a role in adaptive physiological changes in the CNS. (Supported in part by grants MH08618 and MH22768).

61.7 **BRIEF EXPOSURE TO MUSCARINIC AGONIST DECREASES MUSCARINIC RECEPTOR DENSITY IN PRIMARY CULTURES OF CORTICOSTRIATAL CELLS.** **C. Eva,[a] E. Genazzani[a] and E. Costa[b]. [a]Istituto di Farmacologia e Terapia Sperimentale, University of Turin, Italy and [b]FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Medical Center, Washington, D.C. 20007.**

Regulation of muscarinic cholinergic receptors was studied in primary cultures of cerebral corticostriatal cells prepared from neonatal rats. Muscarinic recognition sites, determined by measuring the binding of the muscarinic radioligands ^{3}H-quinuclidinyl benzylate (^{3}H-QNB) and ^{3}H-N-methylscopolamine (^{3}H-NMS), were detected after 5 days of culture and markedly increased in the subsequent days, reaching a plateau at the 12th day, and decreasing thereafter. In these cultures, stimulation of muscarinic receptors by specific agonists activated two mechanisms of signal transduction: the stimulation of phosphionositide breakdown and the inhibition of adenylate cyclase. At the 10th day of culture carbachol stimulated ^{3}H-inositol-1-phosphate formation by 330% with an EC_{50} of 30 uM. Oxotremorine inhibited by 20% the forskolin-stimulated adenylate cyclase with an IC_{50} of 20 uM. Rapid receptor-mediated regulation of cell membrane surface receptors occurred upon contact with muscarinic agonists. Exposure of these cultures to 1 mM carbachol at 37°C resulted in a significant reduction in the specific binding of the hydrophobic ligand ^{3}H-NMS. The reduction was observed already after 5 min of pretreatment and increased up to 70% after 30 min. The biochemical intracellular mechanisms involved in the regulation of muscarinic cholinergic receptors and of the receptor-coupled transducer systems in these cultures will be discussed in terms of dose dependency and metabotropic mechanisms involved.

61.8 RHYTHMIC CHANGES IN RAT HYPOTHALAMIC ^{3}H-IMIPRAMINE BINDING AND 5HT UPTAKE SITES: POSSIBLE BIOCHEMICAL CORRELATE WITH ANTIDEPRESSANT ACTION. N. Brunello, A.C. Rovescalli*, M. Riva*, R. Galimberti* and G. Racagni°, Center of Neuropharmacology, University of Milan and °Institute of Pharmacology, University of Pavia, Italy.

Seasonal rhythmicity in the occurrence of acute depressive episode, as well as the therapeutic efficacy of light exposure have been documented. These observations suggest the possible involvement of the pineal gland or other biological oscillators in the pathophysiology of severe depressive illness. Therefore we have performed studies with the aim to clarify whether different light-dark cycle schedule may induce changes in the biochemical targets of antidepressants in the rat central nervous system. In particular we have investigated the effect of short day (LD 8:16) or long day (LD 14:10) photoperiods on different biochemical parameters of serotonergic neurons. A significant increase in the density of ^{3}H-imipramine (^{3}H-IMI) binding and in the Vmax of ^{3}H-5HT uptake was found in the hypothalamus of LD 8:16 with respect to LD 14:10 exposed rats. These modifications seem to be limited to serotonergic presynaptic sites, since no difference was found in the kinetic properties of postsynaptic 5HT receptors. Moreover at the moment of the sacrifice (6 hrs from the beginning of lighting), no change was found in 5HT metabolism in the hypothalami and pineals of rats exposed to the 2 different photoperiods. No analogous modification was observed, in the same conditions, in the cerebral cortex, thus indicating that a seasonal rhythm of ^{3}H-IMI binding sites and 5HT uptake possibly exists only in certain brain areas, such as the hypothalamus (which receives direct visual stimuli). A similar increase in ^{3}H-IMI binding and 5HT uptake was present in the hypothalamus of rats accustomed to a light-dark reverted cycle (DL 10:14) and sacrificed 6 hrs after the stopping of lighting in comparison to rats exposed to normal LD 14:10 cycles and sacrificed 6 hrs after the beginning of lighting. Therefore a circadian modification of the serotonergic presynaptic sites studied seem to be also present and related to dark-light exposure. Since the pineal gland of different animal species, including the rat, contains remarkable concentrations of 5-methoxytryptoline, a compound able to inhibit ^{3}H-IMI binding and 5HT uptake, the involvement of this structure in the periodic changes observed could be postulated.

61.9 **ANTIDEPRESSANT EFFECTS ON SECOND MESSENGER SYSTEMS IN RAT BRAIN: RELATIONSHIP TO EFFECTS ON RECEPTOR NUMBER AND DESENSITIZATION.** M.E.Newman and B.Lerer (SPON: H.Goldman). Jerusalem Mental Health Center, P.O.B. 140, Jerusalem, Israel.

Chronic administration of desipramine (DMI, 10 mg/kg i.p. for 3 wk) or chronic electroconvulsive shock (ECS) each resulted in a significant reduction in the degree of stimulation of cyclic AMP formation induced by noradrenaline, isoproterenol, forskolin, and the adenosine analog 2-chloro-adenosine in rat cerebral cortical slices. In cortical membrane preparations from similarly treated animals, stimulation of adenylate cyclase by GppNHp, which acts at the N protein of the enzyme, and by forskolin was increased compared to controls, while less significant increases were also obtained with manganese or sodium fluoride activation. In membranes from caudate nucleus, however, chronic DMI did not affect the degree of forskolin activation, while in cerebellar membranes an increase comparable to that seen in cortical membranes was obtained. In hippocampal membranes, forskolin-stimulated activity was increased after chronic DMI treatment, but was unaltered by chronic ECS.

These results provide partial support for the hypothesis that down-regulation of beta-adrenoceptors by chronic antidepressant treatment, an effect observed in cortex and hippocampus but not in caudate nucleus, leads in turn to increased post-receptor mediated stimulation of adenylate cyclase when membranes are prepared from these brain areas.

In cortical slices from animals which received chronic ECS, the inositol phosphate (IP_1) response to noradrenaline was increased compared to controls, but the responses to carbachol or serotonin were unchanged. The response to carbachol in hippocampal slices, however, was decreased in animals which had received chronic ECS compared to controls. Chronic DMI resulted in an increase in the noradrenaline response and a decrease in the serotonin response in cortical slices, with no change in the response to carbachol. These effects in general parallel the changes in receptor number induced by antidepressant treatment.

Chronic treatment of animals with the antimanic drug lithium (0.4% LiCl in food for 3 wk), which does not down-regulate beta-adrenoceptor number, resulted in decreased cyclic AMP responses to noradrenaline, isoproterenol and forskolin in both slice and membrane preparations from cortex. Acute LiCl in vivo (4 m eq/kg s.c. 24 hr before sacrifice) potentiated the IP_1 response to noradrenaline only, while chronic Li reduced this response but had no effect on basal IP_1 levels or the responses to other agents.

61.10 DIFFERENTIAL MODULATION OF BENZODIAZEPINE RECEPTOR BINDING BY ETHANOL IN LS AND SS MICE. LG Miller, DJ Greenblatt*, JG Barnhill*, RI Shader*. Div. Clinical Pharmacology, Tufts-New England Medical Ctr., Boston, MA 02111 and Depts. Medicine and Pharmacology, LSU Medical Ctr., New Orleans, LA 70112.

The LS and SS lines of mice were initially selected based on differential loss of the righting reflex("sleep time") in response to ethanol. Subsequent studies indicate that these lines differ in response to a wide range of hypnotics, and that alterations in GABAergic function may acount for these differences. Studies using brain membrane preparations in vitro indicated no differences in benzodiazepine receptor binding in the 2 lines. Since these techniques may not reflect binding characteristics in vivo, we examined benzodiazepine receptor binding using [3H]-Ro15-1788 binding in vivo and modulation of binding by ethanol, pentobarbital, and stress. Receptor binding was increased in LS compared to SS mice in cortex and hippocampus (cortex 850 fmol/g vs 650 fmol/g; hippocampus 986 fmol/g vs 709 fmol/g). There were no differences in binding in hypothalamus, cerebellum, or pons-medulla, and no differences in nonspecific binding in any brain region. Apparent affinity at the receptor for clonazepam was similar in LS and SS mice(IC50 21 ng/g vs 19 ng/g). Pentobarbital administration(30 mg/kg i.p.) resulted in increases in binding in all brain regions in both lines, similar to results reported with CFW mice, with a trend toward greater increases in SS compared to LS mice. Defeat stress also led to similar increases in cortex, cerebellum, and hypothalamus in both lines, with an increase also present in pons-medulla in LS mice. Administration of ethanol, 1 gm/kg i.p., with sacrifice after 1 hour, caused significant increases in binding in cortex, cerebellum, and hypothalamus of LS mice. A further increment which did not achieve significance occurred in all brain regions after administration of 2 gm/kg. In contrast, marked increases in binding occurred in all brain regions after administration of ethanol, 1 gm/kg, to SS mice. Further increases occurred in all regions after 2 gm/kg, and were significant in cortex, hypothalamus, and pons-medulla. Benzodiazepine receptor binding in vivo is increased in cortex and hippocampus in LS compared to SS mice, apparently due to an increase in receptor number. Increases in binding induced by stress and pentobarbital are similar in the two lines. After acute ethanol administration, changes in LS mice are modest compared to the substantial increases in all brain regions in SS mice. These data indicate that LS and SS mice differ both in baseline benzodiazepine receptor number and in ethanol modulation of benzodiazepine receptor binding. This finding may contribute to the differential response to ethanol in these lines.

61.11 NEUROPEPTIDE Y RECEPTORS COUPLED TO G PROTEINS: BIOCHEMICAL AND PHYSIOLOGICAL EVIDENCE. M.W. Walker* and R.J. Miller (SPON: S.P. Grossman). Univ. of Chicago, Chicago, Il 60637.

Neuropeptide Y (NPY) is a 36 amino acid neurotransmitter widely distributed throughout the central and peripheral nervous systems. NPY modulates a broad range of physiological processes, from sexual behavior to intestinal ion transport. We have previously shown that $I^{125}(Tyr^1)$-NPY binds with high affinity to receptor sites in rat brain synaptosomes. A survey of various brain regions using10 pM I^{125}-NPY revealed the greatest amount of displaceable binding in both hypothalamus (11 fmol/mg) and hippocampus (6 fmol/mg), and the lowest in cortex (2 fmol/mg) and cerebellum (1 fmol/mg). High affinity receptor sites were also seen in homogenates of dorsal root ganglion (DRG) cells cultured from neonatal rats, but not on F11 (undifferentiated and differentiated) or PC-12 (differentiated) neuronal cell lines, or on HSWP fibroblast cells.

Scatchard analysis of rat forebrain synaptosomes at 25 °C indicated the presence of two binding sites with Kd's of 30 pM (Bmax = 20 fmol/mg) and 3 nM NPY (Bmax = 300 fmol/mg). When synaptosomes were incubated for one hour with 0.1 mM GMPP(NH)P, approximately 50% of the low affinity sites were lost, suggesting that at least some of these sites are coupled to a G-protein. There was no apparent change in Kd. This seems to contrast with several other well-studied systems, such as the B-adrenergic and muscarinic receptors, in which GTP and its analogs induce a change in Kd rather than Bmax. When synaptosomes were washed free of GMPP(NH)P, resuspended into 10 uM GTP for 20 minutes, and resuspended again into standard binding buffer, the concentration of binding sites returned to control levels. The loss of binding sites is therefore reversible on a fairly rapid time scale and may reflect a shift to an extremely low affinity state which cannot be detected over the range of the binding assay.

Scatchard analysis of DRG cell homogenates similarly indicated the presence of two binding sites with Kd's of 40 pM (Bmax = 100 fmol/mg) and 5 nM NPY (Bmax = 2000 fmol/mg). When homogenates were incubated for one hour with 0.1 mM GMPP(NH)P, approximately 80% of the low affinity binding sites were lost without any apparent change in Kd, again indicating that at least some of these sites are coupled to a G-protein. Viable intact DRG cells containing metabolic concentrations of GTP would then be expected to display a low concentration of low affinity binding sites relative to membrane preparations. Indeed, Scatchard analysis of binding to intact cells revealed one major site with a Kd of 0.7 nM NPY and a binding capacity about 15% as great as that seen in homogenates (Bmax = 300 fmol/mg). Furthermore, when 0.1 nM I^{125}-NPY was competitively displaced from whole cell binding sites with unlabelled NPY, a biphasic displacement curve was obtained with IC_{50}'s of 0.3 nM (70%) and 0.4 uM NPY (30%). The data thereby suggest that GTP and analogs stabilize an extremely low affinity state of the NPY receptor.

We have demonstrated electrophysiologically that NPY inhibits inward Ca^{+2} current in DRG cells (see D. Ewald, et. al., in this volume). The effect was abolished when the cells were previously incubated with pertussis toxin, which covalently modifies Gi and Go. The inhibition was restored, however, when purified Go was added to pertussis toxin treated cells, indicating that NPY receptors must be linked to G-proteins in order to modulate current. Thus, we have shown that G-proteins modulate not only NPY receptor binding but also physiological events distal to receptor/ligand interaction in DRG cells.

62.1 **DIPS IN CROSS-CORRELOGRAMS ARE POOR INDICATORS OF INHIBITION BETWEEN ON AND OFF SYSTEMS IN CAT LATERAL GENICULATE NUCLEUS.** D. N. Mastronarde. Dept. of MCD Biology, University of Colorado, Boulder, CO 80309.

Cross-correlograms are a major tool for studying the connections between neurons. However, they can be difficult to interpret if the two cells being recorded have inputs whose activity is itself correlated. A dip in a correlogram, which is usually considered a sign that one cell inhibits the other, can be particularly ambiguous. Here, in the context of a study of inhibition between ON and OFF systems in the cat LGN, I report two reasons why the correlogram dip is a poor indicator of inhibition, and two potential solutions to this problem. I recorded overlapping retinal ganglion cell – LGN cell pairs of opposite center sign (e.g., an OFF ganglion cell and an ON LGN cell). The correlogram for such a pair generally shows that the LGN cell has a dip in firing rate after spikes from the ganglion cell. This dip does not necessarily imply that the ganglion cell inhibited the LGN cell, for two reasons. *1) Retinal correlated firing.* A similar dip appears in the correlogram between an ON and an OFF ganglion cell, which tend not to fire at about the same time. Thus, an ON LGN cell has a reduced firing rate after spikes from an OFF ganglion cell simply because the LGN cell's ON excitatory input(s) fire at a lower rate at those times. *2) Temporal summation in the LGN cell.* Retinal input spikes are less effective in firing an LGN cell, the longer the interval since the last input spike. ON ganglion cell spikes occurring for 30 to 50 ms after firings of an OFF ganglion cell do, in fact, tend to have longer interspike intervals and should thus be less effective in firing an ON LGN cell. Thus the dip in an OFF ganglion cell – ON LGN cell correlogram can be deeper and much longer than the dip in the correlogram between the OFF ganglion cell and the LGN cell's ON-center input, again without implying inhibition by the OFF ganglion cell.

One way to demonstrate inhibition is by recording simultaneously from an LGN cell, its excitatory input, and a ganglion cell of opposite sign; the analysis of one such case will be shown. Another source of evidence for inhibition may be a protracted elevation in LGN cell firing rate following a dip, which may reflect a post-inhibitory rebound. ON–OFF retinogeniculate correlograms often showed such overshoots; many overshoots appeared to be too large to arise either from inter-ganglion cell correlations or from temporal summation effects. Taking such unaccountably large overshoots as evidence for inhibition, the incidence of inhibition can be tentatively assessed for various combinations of ganglion cell and LGN cell types. In conclusion, when there is correlated firing in the afferent input, inhibition cannot be reliably detected from dips in simple correlograms; post-dip overshoots may provide an indicator for inhibition; but, more complex recordings and analysis are needed for conclusive evidence.

62.2 SHAPE AND ARRANGEMENT OF 'ON' AND 'OFF' MODULES IN CAT DORSAL LATERAL GENICULATE NUCLEUS. N. Berman, A. Naporn and B.R. Payne. Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129 and Dept. of Anatomy, Boston University, School of Medicine, Boston, MA 02118.

On and off center neurons in the cat lateral geniculate nucleus are segregated into modules oriented parallel to projection columns, i.e. perpendicular to laminar borders (Naporn, Berman & Payne, '85). To learn more about the shape and arrangement of these modules, we made microelectrode penetrations approximately parallel to lines of projection, lines of isoelevation, or lines of isoazimuth relative to the map of the visual field in the nucleus. We also analyzed a large sample of units according to laminar location.

Although the modules have the form of columns arranged perpendicular to laminar borders, these columns appear to have sloping sides, since at the top of layer A, on cells outnumber off cells by 2.7 to 1 while at the bottom of layer A off cells outnumber on cells by 1.3 to 1. A similar result is seen in layer A1, where on cells outnumber off cells by 3.3 to 1 at the top, and only by 1.3 to 1 at the bottom. These results are in good agreement with Bowling and Wieniawa-Narkiewicz ('85). In addition, in the sample of units recorded in layers A and A1, on center cells account for 60% of the total, while in layer C, off center cells account for 66% of the total.

In tracks which travel parallel to isoelevation or isoazimuth lines, on and off center cells are arranged in alternating clusters. In layers A and A1, ON clusters are larger than OFF cluters. Along the isoelevation lines, ON clusters average 506μm in width while OFF clusters average 303μm in width. Along the isoazimuth lines, ON clusters average 281μm in width while OFF clusters average 176μm in width. These data show that both types of modules are larger along representations of isoelevation (which run mediolaterally) than along representations of isoazimuth (which run rostrocaudally). Furthermore, along these axes adjacent units (within 50μm) sometimes have receptive field centers which do not overlap. These jumps in receptive field position are commonly associated with changes in receptive field center type, suggesting that the ON and OFF modules represent discrete regions of the visual field.

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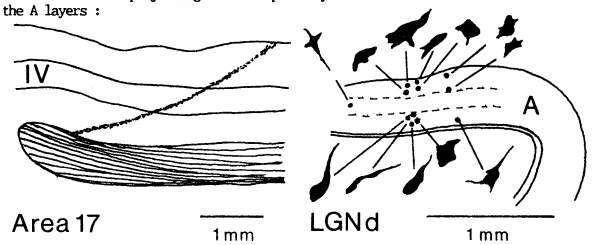
62.3 RECTILINEAR DEPOSITS OF TRANSPORTABLE LABELS : EVIDENCE FOR DEPTH DEPENDENT GENICULO-CORTICAL PROJECTIONS FROM SINGLE LAYERS IN THE LATERAL GENICULATE NUCLEUS (LGNd) OF THE CAT. Douglas B. Bowling, Department of Medical Physiology and The Lions Sight Centre, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

In the A layers of the cat geniculate X, Y and On and Off signals from the retina are processed within mixed pools of cells that also serve as immediate precursors to cortical cells with new receptive field properties (e.g., orientation, direction selectivity, and ocular dominance). The sublaminar organization of the A layers as evidenced by the depth distributions of X, Y and On and Off afferents (Bowling & Michael, 1980,1984) and by X, Y and On and Off target cells (Bowling & Wieniawa-Narkiewicz, 1986) is likely to provide insight into the functional relationships between these fundamentally different visual signals and their roles in the creation of cortical receptive fields.

To examine the sublaminar and topographic features of the geniculo-cortical projection, I have developed a mechanical technique for depositing transportable labels (e.g. HRP or latex microspheres) in thin (40-100um) and continuous straight lines several millimeters in length. When made obliquely across cortical layers in area 17, the position of the label changes continuously in depth and retinotopy along the length of the deposit. Retrograde transport of the label results in an effective transformation from depth in the cortex to retinotopic position in the geniculate. Initial results support the recent finding of Humphrey et al (1985) that X-like cells projecting to the top of layer IV tend to be near the borders of the A layers :



The geniculo-cortical projections appear to reflect the sublaminar organization of the X, Y and On and Off cells in the A layers. Functional correlates of this organization are systematic differences in the amplitude and timing of X-on and X-off responses through the depths of the layers (Bowling & Wieniawa-Narkiewicz, 1987). Thus, the projections indicate that these sublaminar differences in the physiological responses to light from geniculate cells are preserved in the cortical relay and may play a role in the creation of cortical receptive field properties.

Bowling & Michael (1980) Nature 286, 899-902; (1984) J. Neurosc. 4, 198-216; Bowling & Wieniawa-Narkiewicz (1986) J. Physiol. 375, 561-572; (1987) J. Physiol. 390; Humphrey, Sur, Uhlrich & Sherman (1985) J. Comp. Neurol. 233, 159-189

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62.4 RETINOTOPIC DISTRIBUTION OF NON-LINEAR INTERACTIONS OF RETINAL AND AFFERENT EYE POSITION/MOVEMENT SIGNALS IN CAT LGN_d. R. Lal and M.J. Friedlander Neurobiology Research Center and Dept. of Physiology & Biophysics, University of Alabama at Birmingham.

In previous studies we have shown that passive eye movement in anesthetized cats affects the visual response of neurons in the dorsal lateral geniculate nucleus (LGN_d). In the present study, we quantitatively analyzed a) the relative effects of eye position/movement signals on the spontaneous and visually elicited activity of LGN_d neurons and b) the probability and relative strength of such effects with respect to retinotopy. Activity of single neurons in lamina A of the left LGN_d of anesthetized, paralyzed cats were recorded extracellularly with micropipettes. Neurons were classified as X- or Y- based on a battery of electrophysiological tests. Computer generated visual stimuli were presented through the right (contralateral) eye. Visual stimuli consisted of either drifting sinusoidal gratings at twice threshold contrast and optimal spatial and temporal frequency, or a square-wave modulated circular spot of appropriate contrast sign and size for the neuron's receptive field center at twice threshold contrast. Retinal input was prevented from the left (ipsilateral) eye by placement of an opaque contact lens and intravitreal TTX injection. The movement of the left eye was induced by complementary tension applied to the tendons of insertion of the medial and lateral retus muscles. The neuronal responses on 30-100 trials of identical visual stimuli at different eye positions or times of eye movement relative to the visual stimulus onset were collected in a randomized and interleaved fashion. Of 143 neurons for which the eye position effect was tested, 57 had a statistically significant (predominantly inhibitory) effect on visual response. None of the (35/143) neurons which were also tested for effects of eye position on spontaneous activity showed a significant effect. The ratio of the effect on visual response to the effect on spontaneous activity (non-linearity index) for most of these neurons was $\geq$ 3.0. Of 78 neurons for which the effects of eye movement were tested, 42 had significant (predominantly facilitatory) effects on visual response. However, only 9 of these neurons also had a significant effect on spontaneous activity. The non-linearity index for most of these (36/46) neurons was also $\geq$ 3.0. The magnitude (percent response change) and likelihood (percent of neurons tested which had effect) of an eye position effect increased with receptive field eccentricity while similar measures for the eye movement effect decreased with receptive field eccentricity. A model incorporating the spatial activity profile in the geniculostriate system of these effects will be presented.

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62.5 **RELATIONSHIP BETWEEN RESPONSE LATENCY AND AMPLITUDE FOR GANGLION AND GENICULATE X- AND Y-CELLS IN THE CAT.** A.K. Sestokas, S. Lehmkuhle and K.E. Kratz. Dept. of Neurosurgery, University of Maryland School of Medicine, Baltimore, MD 21201; School of Optometry, University of of Missouri - St. Louis, St. Louis, MO 63121; Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

We have previously reported that visual response latencies of X- and Y-cells vary systematically as a function of stimulus spatial frequency and contrast (Sestokas, A.K., Vis.Res. 26:1041, 1986; Sestokas, A.K., Lehmkuhle, S. and Kratz, K.E., Vis. Res. in press). In general, shorter visual latencies are obtained with those stimuli that elicit larger responses. Hence, changes in latency as a function of stimulus condition could be due to concomitant changes in response amplitude. Alternatively, changes in latency and amplitude could be due to a direct influence of the stimulus on each of these parameters.

The purpose of this study was to examine the extent to which response latencies of ganglion and geniculate X- and Y-cells are coupled to response amplitudes. We measured the responses of ganglion and geniculate X- and Y-cells on a trial by trial basis during repeated stimulation with grating stimuli. Gratings of fixed spatial frequency and contrast were presented once per second for 100 msec in blocks of 24 trials. Stimulus spatial frequency and contrast were varied across blocks of trials. Correlations between response latencies and amplitudes calculated across stimulus conditions generally fell between -.5 and -.6 for ganglion cells, and between -.4 and -.5 for geniculate cells. Correlations between latencies and amplitudes calculated within stimulus conditions generally fell between -.2 and -.5 for all groups of cells. In general these results indicate that latency and amplitude are not tightly coupled response parameters. Therefore, for ganglion and geniculate cells, changes in visual latency that accompany changes in spatial frequency or contrast of the stimulus are not trivial consequences of response amplitude.

(Supported by PHS grant EY06517-02)

62.6 **FUNCTIONAL ROLE OF GABAERGIC CIRCUITRY IN THE LGN.** R.N. Holdefer, D.W. Godwin*, Y. Zhang*, and T.T. Norton. Depts. of Physiological Optics and Psychology, University of Alabama at Birmingham, Birmingham, AL 35294.

In a previous study (Holdefer et al., 1985) we described GABA-immunoreactive neurons and neuropil in the LGN of the tree shrew (Tupaia belangeri). At the ultrastructural level, labelled F2 profiles were often seen postsynaptic to retinal terminals and presynaptic to conventional dendrites.

The functional significance of GABAergic circuits was investigated with microiontophoretic application of the GABA antagonist bicuculline in the LGN of anesthetized tree shrews (NO_2/halothane). Although bicuculline increased the maintained discharge and the tonic component of the relay cells' response to briefly presented visual stimuli, there was a more significant effect on the visually driven peak firing ($p=.001$) and the duration of the phasic response ($p<.01$) of the cells.

This greater effect on visually-driven peak firing as compared to maintained discharge suggested that bicuculline application might change the signal-to-noise ratio for cells in the LGN. Receiver operating characteristic (ROC) curves were derived from interspike interval data using maintained discharge as "noise" and visually-driven activity as the "signal" (Wilson et al., submitted). Preliminary ROC curve analyses suggest that when the noise level is very low (4 cells, maintained discharge < 2.0 spikes/sec) bicuculline application can increase or decrease the discrimination of a visual signal from maintained discharge. Under moderate to high noise conditions (3 cells), bicuculline increased the detectability of a visual stimulus, and this effect was generally reversible during the postdrug control recordings from the cell.

Contrast sensitivity functions (CSFs) to a drifting, sinewave grating were also obtained before, during, and after bicuculline application. There was a significant increase in contrast sensitivity for the two lowest spatial frequencies tested (0.0625 c/deg and 0.0935 c/deg; $p<.01$) and no significant change in sensitivity at the higher spatial frequencies. CSFs taken during the postdrug condition showed that these effects were completely reversible. An analysis of the data using the difference of Gaussians model is continuing to determine whether this effect is due to changes in the surround, center, or both mechanisms.

These results demonstrate that in tree shrew, as in cat, there is some alteration of the receptive-field properties of relay cells by GABAergic processes. Our results are also consistent with a gating of visual information in which GABAergic synapses may regulate the signal-to-noise ratio in the LGN.

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62.7 DENDRITIC CURRENT FLOW IN X CELLS IN THE CAT'S LATERAL GENICULATE NUCLEUS: A COMPARISON OF RELAY CELLS AND INTERNEURONS. Stewart A. Bloomfield and S. Murray Sherman, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, N.Y. 11794.

Despite a large variability in dendritic architecture, relay X and Y cells in the cat's lateral geniculate nucleus are electrically compact, each having an electrotonic length (L) of about 1 (Bloomfield et al. J. Physiol. 383:653, 1987). Thus even synaptic innervation located most distally in the dendritic arbor will have a major influence on somatic and axonal responses. Geniculate interneurons have receptive field properties nearly identical to those of relay X cells, but dendritic morphology differs dramatically between these two cell types (Hamos et al. Nature 317:618, 1985). Dendrites of interneurons contain synaptic terminals in the form of clustered appendages that are both pre- and postsynaptic, and these cells also have conventional synaptic outputs from a locally ramifying axon. In addition to these morphological differences, we now describe differences in passive cable properties between relay X cells and interneurons. We made morphological measurements from 2 relay X cells and 2 interneurons after labeling them intracellularly with HRP, and we then used steady-state cable theory to determine the passive current flow from activation of a single synaptic input in their dendritic arbors.

As expected, the relay X cells were electrically compact, with L values of about 0.9. Thus even the most distal synaptic inputs will reach the soma with attenuation of only 40-50%. In addition, synaptic current applied to any segment within a single branch can propagate effectively throughout all portions of the arbor emanating from the same primary dendrite. We conclude that, for relay X cells, all synaptic information can be integrated at the soma to influence responsiveness of the axon, which represents the sole output of the cell. In contrast, the dendritic arbors of interneurons cover more than 3 electrotonic lengths. Distally generated synaptic potentials will thus be attenuated by more than 90% before reaching the soma, and attentuation less than 50% is possible only for synapses located within the proximal 1/3 of the dendritic arbor. Consequently, soma and axon responses may reflect only those inputs onto proximal dendritic shafts. Inputs onto distal dendritic shafts, as well as inputs onto clustered appendages, will have little effect on the soma, but may instead control the output of the presynaptic dendritic terminals. Furthermore, the more distally located dendritic segments may be electrically isolated from each other. Thus, much of the dendritic arbor may be comprised of autonomous functional subunits capable of both receiving and transmitting synaptic information.

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62.8 STIMULATION OF THE BRAINSTEM RETICULAR FORMATION DIFFERENTIALLY AFFECTS TWO GROUPS OF X-CELLS IN THE CAT LATERAL GENICULATE NUCLEUS A.L. Humphrey. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA 15261.

Two physiologically distinct groups of X-cells reside in the A-laminae of the cat lateral geniculate nucleus (LGN)[1,2]. Referred to as lagged (X_L) and non-lagged (X_N) X-cells, they respond differently to a small flashing spot centered in their receptive field. At spot onset, X_N-cells give a transient discharge, while X_L-cells show a strong inhibitory dip in firing. At spot offset, cell discharge decays rapidly among X_N-cells, but many X_L-cells display a transient "offset discharge." These two groups also differ morphologically[3], and they may be differentially contacted, and thus influenced by, intrageniculate and perigeniculate (PGN) interneurons.

Inputs to the LGN from the brainstem reticular formation (BRF) facilitate retinogeniculate transmission, probably by inhibiting intrageniculate and PGN interneurons. Given the likely involvement of these interneurons with X_L- and X_N-cells, I have investigated the effects of BRF stimulation on X-cells. Electrical stimuli (10ms train of 4 pulses) were delivered to the region of the pontine reticular formation while recording extracellularly from 7 X_N-cells and 4 X_L-cells in the LGN of barbiturate anesthetized cats.

When the animal viewed a uniformly illuminated screen, stimulating the BRF alone had a strong effect on the baseline firing of X_L-cells, but not on X_N-cells. The X_L-cells responded with a strong transient discharge, which peaked at 95ms, lasted about 150ms, and attained a maximum firing level (~100sp/s) up to nine times higher than that of baseline firing. The X_N-cells gave only a negligible response to BRF stimulation alone.

When BRF stimuli preceded by 100ms the turning on of a small spot in a cell's receptive field, the two X-cell groups were also differentially affected. BRF stimulation among X_N-cells increased their discharge rates significantly above control levels throughout the period of spot onset. BRF stimulation among X_L-cells elicited a transient discharge which replaced the inhibitory dip normally seen in these cells. However, the BRF-induced transient in the X_L-cells was short-lived (~160ms); following the transient, the sustained discharge dropped to control levels and the offset discharge was unaffected.

These results indicate that X_L- and X_N-cells differ not only in their visual response properties and morphologies, but they are differentially affected by inputs from the brainstem reticular formation. Possible mechanisms underlying these differences will be considered. Supported by USPHS grant EY06459.

[1]Mastronarde (1987) J. Neurophysiol. 57:357-380.
[2]Humphrey and Weller (1985) Soc. Neurosci. Abstr. 11:318.
[3]Weller and Humphrey (1985) Soc. Neurosci. Abstr. 11:318.

62.9 SUSTAINED (X) CELLS IN THE CAT LATERAL GENICULATE NUCLEUS RESPOND TO RATES OF LUMINANCE CHANGE. P. Heggelund, H.E. Karlsen*, G. Flugsrud*. Dept. of Neurophysiol., Univ. of Oslo, N-0162 Oslo, Norway.

Single cells in the lateral geniculate nucleus (LGN) respond strongly when the luminance on the receptive field is changed. We have studied how the rate of luminance change of a stationary test spot influences the response pattern of sustained (X) LGN cells. The spot size was adjusted so it approximately filled the receptive field center. The spot was surrounded by a contiguous annulus of fixed luminance (12 cd/m2). The spot luminance was modulated linearly (triangular or trapezoid luminance functions) or sinusoidally in time between 0.4 log units below to 0.4 log units above the surround luminance. Modulation frequencies between 0.1 and 400 Hz were used.

The cells responded to rate of luminance change and not to stimulus contrast. To the linear stimulus functions, in which the contrast was continuously changed, the cells responded to the constant rate of luminance change with a constant rate of action potentials at stimulus frequencies below 4 Hz. On-center cells responded when the luminance was increased, off-center cells when the luminance was decreased. This constant firing level increased monotonically with the stimulus frequency. At stimulus frequencies above 4 Hz an initial overshoot occurred. This transient became more pronounced as the frequency increased. Thus, the response gradually approached the typical pattern seen to a step function. To sinusoidal stimuli at low temporal frequencies there was a considerable negative phase shift of the response. At 1 Hz the average phase shift of the response maximum of the cells was -92 deg when the delay caused by response latency (25 deg) was subtracted.

We concluded that the X-cells perform a differentiation-like operation in the time domain. At low temporal frequencies (<4 Hz) the response approximates the first time derivative. Previous studies (e.g. Movshon et al., J. Physiol., 1978, 283, p. 101) have shown that it is at such low frequencies that most striate cortex cells operate, indicating that the LGN cell properties at such frequencies may be essential for the cortical processing. (Supported by NAVF).

62.10 **THE TEMPORAL PROPERTIES OF THE MACAQUE MAGNO AND PARVO PATHWAYS IN THE FACE OF CHANGING LEVELS OF RETINAL ILLUMINATION.** K. Purpura*, E. Kaplan and R.M. Shapley, The Rockefeller University, N.Y.,N.Y., 10021.

Psychophysical studies (deLange,1958; Kelly,1972) have demonstrated the importance of the temporal frequency of a stimulus in determining its visibility at different background levels of retinal illumination. At high temporal frequencies (for instance, 20 hz) and low spatial frequencies (1 c/deg), contrast sensitivity is proportional to background level. At low temporal frequencies (1 hz) and low spatial frequencies, contrast sensitivity does not depend on the background and is constant.

In an effort to understand the neuronal basis of these psychophysical results, we studied the magnocellular and parvocellular pathways of macaque monkeys which were anesthesized with urethane and paralyzed with flaxedil. We recorded responses of neurons in the lateral geniculate nucleus (LGN) and their retinal afferents (M and P cells) to luminance gratings at several temporal frequencies at various levels of retinal illumination. The drifting sinusoidal gratings were produced on a white CRT. Mean luminance was controlled by neutral density filters, and the monkeys were fitted with contact lenses with artificial pupils.

Our preliminary data indicate that the responses of visual neurons of both the magno and parvo pathways resemble the human psychophysical temporal frequency/light adaptation behavior. At high temporal frequencies, the contrast gains (the slope of the initial linear portions of the response-contrast functions) of these neurons varied proportionally with mean retinal illumination, while at lower frequencies the contrast gain was less influenced by the mean light level. The qualitative similarity of this behavior between parvo and magno cells is somewhat surprising given their different light adaptation behavior in general (Purpura *et al.*,1986). However, we have also examined the temporal tuning of these neurons over several light levels and found them to change in qualitatively similar ways. This suggests that the temporal properties of neurons in the parvocellular and magnocellular pathways may be established by the temporal filtering properties and light adaptation behavior of the photoreceptors or other, distal neurons.

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62.11 **THE PROCESSING OF COLOR AND LUMINANCE INFORMATION IN MONKEYS: 1. PSYCHOPHYSICS.** E.R. Charles*, N.K. Logothetis, A.C. Hurlbert*, P.H. Schiller (SPON: R.Held). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA

Luminance and chrominance information are thought to be separately conveyed by parallel channels in the primate visual system. Psychophysical experiments in humans show that motion, depth, and pattern perceptions are degraded at isoluminance (IL), when only chromatic cues provide contour information. These results suggest that the chrominance channel is only weakly responsive to motion, depth, and pattern cues. We report on a set of psychophysical experiments designed to study visual perception at IL in old-world monkeys, the results of which we have correlated with physiological recordings from broad-band and color-opponent cells in the monkey visual system (see abstract by Hurlbert et al. in this vol.).

We have determined IL values for four rhesus monkeys using an adaptation of the flicker photometric technique. Each monkey was trained on a visual discrimination task that required him to fixate on a central point at the start of each trial, then to saccade to a target that appeared as one of eight peripheral stimuli equidistant from the fixation point. The stimuli were checkerboard patterns in which the colors of adjacent squares were counter-flickered between red and green at a rate of 30 cps. The target differed from the non-targets only in the size of its squares. The red-green luminance ratios were varied between trials. On control tasks, the monkey discriminated between different-sized checkerboards of the same color with checkers of different luminances. All visual stimuli were generated by an Adage image processor and presented on a color monitor with linearized guns.

IL values were identified as the red-green luminance ratios at which the monkey's percentage of correct responses fell to near chance from near 100 at extreme ratios. We have compared these IL values with the red-green luminance ratios at which monkey perceptions of motion and stereoscopic depth are impaired. The results of our control tasks suggest that the low luminance contrast of the red-green IL stimuli is not responsible for the observed impairment in visual perception.

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62.12 **THE PROCESSING OF COLOR AND LUMINANCE INFORMATION IN MONKEYS: 2. PHYSIOLOGY.** A.C. Hurlbert*, N.K. Logothetis, E.R. Charles, P.H. Schiller (SPON: S.Mann). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA

Psychophysical experiments in humans show that at isoluminance, visual perceptions of motion, depth, and form are impaired. It has been suggested that this impairment is due to the different ways in which the primate color-opponent and broad-band channels process chrominance and luminance information. In particular, it has been proposed that at isoluminance, the broad-band channel is effectively inactivated. To test these suggestions, we have studied the physiological properties of color-opponent cells (in parvocellular LGN -- PLGN) and broad-band cells (in magnocellular LGN -- MLGN) and compared them with our psychophysical data on monkey visual perception at isoluminant (see abstract by Charles et al. in this volume).

The properties of LGN cells in anesthetized, paralyzed rhesus monkeys were studied using three methods: single-unit recordings, multiple-unit recordings, and evoked potentials. In each study, a small portion of the retina (the center of the receptive field under study) was stimulated by a red-green flickering light, generated by an LED or an Adage image processing system. Counter-flickering center-surround stimuli designed to mimic the theoretically optimal stimuli for PLGN and MLGN cells were also used. The rate of the red-green light exchange was varied from 1 to 40 Hz, and the red-green luminance ratio was systematically varied to find maximal and minimal responses at each electrode site. These optimal responses were determined by on-line computer analysis of spike frequencies.

Our results show that for most LGN cells (excluding highly color-selective cells) there is a red green luminance ratio at which an equal and minimal response occurs to each light exchange. This balanced response is generally of a lower magnitude in PLGN than in MLGN. The balancing red-green luminance ratio varied widely among cells, especially in PLGN. Thus, at the LGN level, it seems that there is no single isoluminance value for either the broad-band or color-opponent channel that correlates with our behaviorally observed effects at isoluminance.

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62.13 BINOCULAR INTERACTIONS IN THE LATERAL GENICULATE NUCLEUS OF THE ALERT MONKEY. C.E. Schroeder, C.E. Tenke, M.A. Kraut*, J.C. Arezzo and H.G. Vaughan, Jr. Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY.[1]

It has been hypothesized that the pattern of fine lamination in the parvocellular division of the macaque lateral geniculate nucleus (LGN) may have evolved to support binocular interactions. While both excitatory and inhibitory binocular interactions in cat LGN are widely reported, studies of macaque LGN have demonstrated only weak, binocular inhibition, confined to the magnocellular laminae. In the present study we have re-evaluated this issue using procedures which allow simultaneous recording of activity in multiple laminae.

Flash-evoked field potentials and concomitant multiunit activity were recorded from LGN in 3 alert, monkeys (m. Fascicularis),using 16 channel, multicontact electrodes with contact impedances of approximately 300 kOhm (at 1000Hz) and intercontact spacing of either 75 or 150 uM. At least 2 laminae were sampled in each electrode position and responses to binocular and monocular stimulation were recorded. Prominent oscillatory potentials and multiunit responses were observed in all laminae, but both the response duration and oscillation frequency differed between parvo- and magnocellular regions. Binocular interactions were noted in every electrode penetration, and in all laminae. The most common type was binocular inhibition. One form observed was a 15-70% reduction in the peak amplitude of multiunit responses during binocular, as opposed to monocular stimulation of the dominant eye for the lamina under study. This effect was seen in all laminae, but was more prominent in magno- than in parvo-cellular layers. A second form of binocular inhibition, limited to the ipsilateral laminae in our current data, is a 10-30% reduction in multiunit activity, below its baseline level, with stimulation of the contra-lateral eye. This effect is coincident with excitatory responses in adjacent, contralaterally innervated laminae. Finally, on many occasions, we noted activity suggestive of excitatory modulation by the nondominant eye.

Our results are consistent with earlier work in that, binocular interactions were found to be more prominent in the magnocellular laminae. However, contrary to previous studies, we also observed clear binocular interactions in the parvocellular layers. In addition, the magnitude and complexity of binocular interactions were greater than previously reported, with evidence that they differ in ipsilaterally vs. contralaterally innervated laminae. These data, together with the growing body of evidence from experiments in the cat, underscore the notion that the mammalian LGN is an important site of binocular interaction.

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LEARNING AND MEMORY: ANATOMY I

63.1 : CRITICAL RE-EVALUATION OF THE CONSOLIDATION HYPOTHESIS OF AMNESIA. N. J. Cohen* (SPON: F.H. Baker). Cognitive Neuropsychology Lab., Johns Hopkins University, Baltimore, MD 21218.

An early and influential view of the nature of the memory impairment in at least some forms of amnesia postulates a failure of memory consolidation. One version of this idea holds that amnesia resulting from medial temporal-lobe damage impairs processes that normally operate during the time after learning to permit the formation and/or retrieval of stable memory representations (Squire & Cohen, 1984; Squire, Cohen, & Nadel, 1985). The major pieces of evidence offered in support of this view are that medial temporal-lobe amnesia is associated with an abnormally rapid rate of forgetting and that such patients have temporally-limited retrograde amnesia (RA). The present paper reconsiders these two lines of evidence and concludes that neither provides any special support for the consolidation view.

Abnormally rapid forgetting: Extensive work with the patient H.M., profoundly amnesic since sustaining bilateral resection of medial temporal-lobe structures in 1953, failed to find rapid forgetting: Across multiple testing occasions when H.M. was given extra time to process the to-be-learned material so that his memory performance could be equated to the level of normal subjects at a time soon after learning, he showed a largely normal rate of forgetting for the material over the subsequent 3-7 days (Freed, Cohen, & Corkin, 1986). More damaging, however, is that the present paper shows that even if rapid forgetting were a reliable feature of medial temporal-lobe amnesia, it would provide no special evidence for a consolidation view of this amnesia. A simple quantitative model is offered to show how rapid forgetting could be produced equally well by an impairment in encoding, retrieval, or consolidation processes.

Temporally-limited RA. Also cited in support of the consolidation view is the existence of RA's that impair all memories acquired during the 1-3 year period prior to the onset of amnesia regardless of the content of the memories. Yet, the present paper discusses two patients whose RA's are temporally defined in the manner just described but cover time periods extending to 16 and 40 years. The graded RA's associated with Korsakoff's disease are similarly temporally defined for a period encompassing decades. Accordingly, the present paper suggests the necessity of a principled justification for the claim that the brief (or limited) temporally-defined RA reflects a disruption of consolidation processes but that the extensive form does not.

63.2 LEARNING AND FORGETTING DURING AND AFTER POSTTRAUMATIC AMNESIA IN HEAD INJURED PATIENTS. H.S. Levin, W.M. High, Jr.* and H.M. Eisenberg*. Neuropsychology Lab., Div. Neurosurgery, University of Texas Medical Branch, Galveston, TX 77550.

Following previous work implicating rapid decay of memory in a patient with bihippocampal lesions (Huppert and Piercy, Cortex, 15:385, 1979) and the postulated association between posttraumatic amnesia (PTA) and temporal lobe injury, we compared the rate of forgetting in head injured patients studied during PTA (n=13) to the retentive capacity of head trauma patients studied after resolution of PTA (n=18) and normal controls (n=18). The patients studied during PTA had more severe impairment of consciousness at the time of hospital admission and were tested after longer intervals since injury as compared to the head injured patients examined after their PTA had cleared. We modified the recognition memory procedure developed by Huppert and Piercy in which slides were presented initially for longer durations to the patients with presumed memory deficit. In contrast to the 120 color slides of pictures utilized by Huppert and Piercy, we presented 66 slides initially to the head injured patients. Total durations of presentation were 8 seconds for patients in PTA, 2 seconds for patients tested after PTA resolution and 1 to 1.5 seconds in controls. A duplicate series of these slides were divided into three sets of 22 slides (targets) for testing recognition memory at 10 minutes, 2 hours and 32 hours later. Each recognition test involved discrimination of the target slides from 22 slides which were not shown previously. Nearly one-fifth of the injured patients studied were unable to attain a criterion of initial learning (d'=2.0 or about 70% correct) over a 10 minute interval despite a prolonged exposure duration. Analysis disclosed that patients in PTA exhibited greater forgetting by 32 hours as compared to head injured patients out of PTA. Subgroups of 8 head injured patients matched according to severity of injury disclosed significant differences in forgetting at 2 hours and 32 hours despite comparable initial learning at 10 minutes following presentation.

Our findings indicate that PTA following head injury is associated with a defect in acquisition of pictorial information unless it is presented at exposure durations which exceed the times given to normal controls by a factor as great as 8. Our finding of rapid forgetting during PTA is compatible with the decay of memory exhibited by patient H.M. following bilateral hippocampal excisions. Although further investigation of head injured patients with focal lesions is indicated, our results offer preliminary support for the postulation that PTA is a form of temporal lobe memory disorder.

63.3 **MONKEY BEATS MAN IN EFFICIENCY OF MNEMONIC RETRIEVAL.** Jeffrey D. Lewine, Robert W. Doty, Sherri Provencal*, and Robert Astur*. University of Rochester, Rochester, New York, 14642.

To determine whether the temporal characteristics of mnemonic retrieval are similar in man and monkey, 4 Macacca nemestrina and 4 students were trained and tested on Sternberg's serial probe recognition task (Science, 153, 1966). Basically, subjects had to distinguish positive (target) from negative (non-target) probes as rapidly as possible, classifying them by means of differential manual responses. The probes were selected from an inventory of images of 100 multi-colored patterns. At the beginning of each test set, subjects viewed 1-6 target patterns. Each target set was then followed by several classification trials. Within a given experimental session (consisting of several test sets), a particular positive probe (+P) occurred in only one test set while a particular negative probe (-P) often occurred in two test sets. Also, patterns that served as positive probes in one session occurred as negative probes in other sessions and vice-versa. This served to insure that subjects made probe classifications based upon the relevant target information and not upon a novel vs repeat strategy, or previous pattern-response associations. The abilities of human subjects and macaques to correctly discriminate between +P and -P trials were, as measured in terms of "detectability", not significantly different [$F(1,7)=2.88$, $P>.05$], three macaques demonstrating overall detectability values equivalent to, or greater than, those of two of the human subjects! Both human and macaque subjects typically classified probes in < 600 msec, the average time required to evaluate probes increasing as a linear function of the number of relevant targets that had to be remembered. However, increasing the number of relevant targets, on average, increased probe-evaluation-time by only 8 msec/target (range 5-11 msec/target) for the monkeys but 23 msec/target (range 17-31 msec/target) for the human subjects. These values are significantly different, [$F(1,7)= 15.24$, $P<.01$]. The reaction time data suggest that, when the number of targets does not exceed the "immediate memory span", both species utilize serial strategies to access the target information, but macaques can do so at 3X the rate of man! The most parsimonious explanation is that the smaller temporal increment for macaques simply reflects shorter neural paths in a smaller brain. However, the possibility that probe evaluation by human subjects involves additional processing stages not utilized by the macaques cannot be ruled out. Nevertheless, despite the macaque's lack of the neocortical elaboration characteristic of the human brain, the data clearly demonstrate that some mnemonic processes in macaques may be more efficient than those in man. (Supported by USPHS Fellowship 5-F31-MH09117 to JDL and NINCDS Grant NS20052 to RWD.)

63.4 NORMAL LEARNING SET FORMATION IN MONKEYS WITH COMBINED ABLATION OF THE AMYGDALOID COMPLEX AND HIPPOCAMPAL FORMATION. E. A. Murray. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892

Monkeys with combined ablations of the amygdaloid complex and hippocampal formation (AH) are severely impaired in both visual and tactile object recognition, and have been shown to exhibit a pattern of impaired and spared learning abilities that is similar to the pattern observed in patients with global anterograde amnesia. In an effort to understand the spared learning abilities of amnesic monkeys, monkeys with AH ablations were compared with unoperated controls on their rate of acquisition of a learning set. Since learning set formation is traditionally viewed as a relatively "advanced" function, it was hypothesized that this phenomenon would be dependent upon the medial temporal lobe limbic structures and that monkeys with AH ablations would be unable to form a learning set. To avoid penalizing the monkeys with AH ablations for their marked memory impairment, the monkeys (M. mulatta) were trained on a version of discrimination learning that had earlier been reported to yield normal scores for monkeys with AH ablations (Malamut et al., Behav. Neurosci. (1984) 98: 759-769.) All monkeys were trained on 10 consecutive sets of concurrent object discrimination problems. Each set consisted of 20 different pairs of objects, with one item of the pair arbitrarily designated as positive (baited) and the other negative (unbaited). Within a day's test session, each pair of objects was presented for discrimination, one at a time, at intervals of 20 seconds, until all 20 pairs had been used. This procedure was repeated at 24-hr. intervals with the order of the presentation of the pairs remaining constant, but the left-right position of reward following a pseudorandom order, until the monkeys reached a criterion of 90 correct responses in 100 trials. When criterion had been attained on the first 20 discriminations, the monkeys were trained in the same manner on a second set of 20 concurrent discriminations, and so on, until all 10 such sets consisting of 20 problems each had been learned.

Monkeys with AH ablations learned the first two sets of concurrent discriminations at the same rate as the controls (AH, $\bar{X}$=12.4 trials; CON, $\bar{X}$=7.8 trials), a finding that confirms the results of the earlier study by Malamut et al. Furthermore, the two groups did not differ in their rate of acquisition of the last two sets of 20 concurrent discriminations (AH, $\bar{X}$=5.1; CON, $\bar{X}$=4.7), and both groups learned the last two sets significantly faster that the first two sets (paired t-test, AH, $t=3.20$, 3df, $p<.025$; CON, $t=2.36$, 5df, $p<.05$). The results thus show that learning set formation can take place at a normal rate even in the absence of the medial temporal lobe limbic structures and therefore suggest that this process involves the retention of procedures or skills as opposed to the retention of specific facts or events.

63.5 TOPOGRAPHIC ORGANIZATION OF THE ENTORHINAL PROJECTION TO THE DENTATE GYRUS IN THE MONKEY. D.G. Amaral, M.P. Witter* and G.W. Van Hoesen. The Salk Institute, La Jolla, CA 92037, Dept. Anatomy Vrije Universiteit, Amsterdam, The Netherlands, and Univ. of Iowa, Iowa City, IA 52242.

Human clinicopathological studies and experimental primate studies have clearly indicated that the hippocampal formation plays a central role in the consolidation of sensory information into memory. It is thus of some importance to determine from which cortical regions the hippocampal formation receives its sensory inputs and how this information is conveyed to each of its distinct cytoarchitectonic fields. The monkey entorhinal cortex (EC) receives direct inputs from several polysensory associational regions of the frontal, temporal, insular and cingulate cortices. The entorhinal cortex, in turn, gives rise to the major input (the perforant pathway - PP) to the dentate gyrus which is the route through which the hippocampus proper receives most of its cortical input. The organization of the perforant pathway has been extensively studied in the cat and rat and is topographically organized such that a lateral-to-medial axis of PP origin in EC corresponds to a longitudinal or dorsal-to-ventral axis of termination in the DG. Since the topography of the primate PP has not yet been examined, we studied the organization of this pathway in two species of macaque monkeys with both retrograde and anterograde tracing techniques. Injections of retrograde tracers (WGA-HRP, Fast Blue or Diamidino Yellow) were placed at different positions along the anteroposterior extent of the DG in 10 monkeys. A cocktail of tritiated amino acids (TAA) was injected into the entorhinal cortex of 16 additional animals. Injections of retrograde tracers aimed at the most anterior portion of the DG results in a rostrocaudally oriented band of labeled cells that occupies the medial 1/3 of the EC. Injections that were placed at more posterior levels result in progressively more laterally located longitudinal zones of labeled cells. The TAA experiments confirmed this topographic organization and indicated that the pattern of PP termination in the molecular layer is not as laminated as in the rat brain. We have concluded that the origins of the projection from the entorhinal cortex to different rostrocaudal levels of the dentate gyrus are organized in obliquely oriented, longitudinal bands that more or less parallel the anterolateral-to-posteromedial axis in EC. These bands are oriented perpendicular, however, to the major axis of cytoarchitectonic differentiation of the EC and cut across most of its fields. Recent studies in the cat and monkey indicate that different regions of the entorhinal cortex receive distinct complements of afferents. Therefore, the described organization of the PP may indicate that functionally different types of information are transmitted to different levels of the DG. The bearing of this topographic organization on the function of the primate hippocampal formation will be discussed.

63.6 SUCCESSFUL PERFORMANCE BY MONKEYS WITH HIPPOCAMPAL LESIONS ON PIAGET'S A$\bar{B}$ TASK. Adele Diamond, Dept. of Psychology, Washington University, St. Louis, MO 63130, Stuart Zola-Morgan and Larry R. Squire, VA Medical Center, San Diego, CA 92161 & Dept. of Psychiatry, UCSD, La Jolla, CA 92093.

Piaget's A$\bar{B}$ task is a well-known marker of developmental change between 7½-12 months in human infants. In the task, the subject watches as a reward is hidden in 1 of 2 identical wells side by side. A brief delay follows after which the subject is allowed to reach. The reward remains in the same location until the subject is correct twice in a row; then location of reward is reversed and the procedure repeated. Human infants of 7½-9 months make "the A$\bar{B}$ error" at delays of 2-5 sec (infants 10 months or older succeed at delays this brief). Subjects making "the A$\bar{B}$ error" reach correctly at the first hiding place, or when hiding is repeated where they have just reached correctly on the previous trial. Their errors are confined to reversals and to trials where the hiding is in the same place as the previous trial and the subject was wrong on the previous trial. Performance on A$\bar{B}$ has been linked to dorsolateral prefrontal cortex by work in adult monkeys (Diamond & Goldman-Rakic, Neurosci. Absts., 1983) and infant monkeys (Diamond & Goldman-Rakic, Neurosci. Absts., 1986). E.g., monkeys with prefrontal lesions err at delays of 2-5 sec, showing the same A$\bar{B}$ error pattern as human infants.

Because interpretations of A$\bar{B}$ stress its memory requirements, we have assessed performance on A$\bar{B}$ in monkeys following damage to the hippocampus. Six cynomologus monkeys (3 with bilateral lesions of the hippocampus [H] & 3 unoperated controls [C]) were tested. All 6 monkeys performed correctly on A$\bar{B}$ at delays of 2-5 sec ($\bar{x}$ at 2 & 5 sec combined: H = 95% correct; C = 91%). At delays of 10 sec & 15 sec, performance of monkeys with hippocampal lesions progressively declined ($\bar{x}$ at 10 sec: H = 84%; C = 90%; $\bar{x}$ at 15 sec: H = 79%; C = 92%). H monkeys deteriorated equally across all types of trials; never showing the pattern of performance characteristic of human infants and of monkeys with prefrontal lesions (success on repeat trials following correct reaches; failure on reversals & on repeat trials following errors). H monkeys did have a memory impairment, as they performed poorly on Delayed Non-Match to Sample both before and after A$\bar{B}$ testing.

Accordingly, the memory impairment associated with hippocampal damage cannot explain failure on A$\bar{B}$. Prefrontal ablation but not hippocampal ablation produces errors on A$\bar{B}$ at 2-5 sec, the delays at which 7½-9 month old human infants fail. In addition, the pattern of those errors over trials does not appear to be simply a result of poor recall as H monkeys never show this pattern, even at longer delays, when their errors begin to appear. Success on A$\bar{B}$, therefore, seems to depend specifically on normal function of dorsolateral prefrontal cortex.

63.7 METABOLIC ACTIVITY IN THE THALAMUS AND MAMMILLARY BODIES OF THE MONKEY DURING SPATIAL MEMORY PERFORMANCE.
H. R. Friedman, J. Janas*, and P.S. Goldman-Rakic. Sec. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06515.

The 2-DG technique is unique in its capacity to provide a whole-brain record of functional activity during experimental manipulations in animals. We have been using this method to investigate the metabolic profile underlying cognitive behavior in the monkey and previously reported that specific regions of the hippocampus were activated in tasks requiring working-memory (Friedman & Goldman-Rakic, Soc. Neurosci. p. 460, 1985). We have now extended this work to several diencephalic nuclei that have been implicated in the neural circuitry of memory.

Seven male rhesus monkeys were trained on one of two spatial working-memory (WM) tasks: delayed spatial response or delayed spatial alternation, both of which require monkeys to remember specific spatial events from the preceding trials. Metabolic activity in this WM group was compared with activity in six male rhesus monkeys trained in one of two control paradigms which did not involve working-memory (the NWM group): 1) a visual pattern discrimination task which presented the same problem on every trial, or 2) a control task with similar sensory and motor components as the other tasks but with no overt mnemonic requirement.

For the final behavioral test, monkeys received an i.v. injection of ^{14}C-2-DG (100 µCi/kg) and were tested for 45 min. Local cerebral glucose utilization (LCGU) was measured using a computer based image-analysis system. Nissl-stained sections were superimposed on adjacent autoradiograph images to identify specific brain regions. A linear regression model was used to factor out individual variability in overall metabolism on the basis of the LCGU of the medial geniculate body (MGB). The mean LCGU in the MGB was nearly identical for both groups as would be expected from the fact that the white background noise was kept constant across conditions.

We examined activity in the mammillary bodies (MB), in the anteroventral (AV) and anteromedial (AM) thalamic nuclei, in the dorsal and in the ventral magnocellular (MDmc) and in the parvocellular MD (MDpc). Activity in the AV, AM, the MDpc and the ventral MDmc averaged about 15% higher in the WM group and was significantly different from the NWM group ($p<.05$). Activity in the dorsal MDmc did not significantly differ as a function of working memory. Further, no group differences were evident in the activity of the MB.

These data are congruent with previous findings which, for example, have shown that MD lesions impair performance on spatial memory tasks (Isseroff et al. Br.Res., **232**, 97-113,1982) and that MD neurons respond selectively during such tasks (Fuster & Alexander, Br.Res.,**61**,79-91). Moreover, the metabolically more active AV, AM, MDpc and the ventral MDmc all map topographically onto the very prefrontal cortical areas (Goldman-Rakic and Porrino, J comp Neurol., **242**, 535-560, 1985) that mediate behavior on the same tasks. These findings spotlight the AV, AM, the MDpc and the ventral MDmc as important components of a neural system dedicated to spatial working memory.

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63.8 IMPAIRMENTS IN VISUAL SEARCH FOLLOWING DIENCEPHALIC LESIONS IN MONKEYS.
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Both the mammillary and mediodorsal nuclei of the diencephalon have been reported to be involved in memory functions in human and nonhuman primates. Lesions of these nuclei in monkeys have previously been reported to affect short-term memory capacity (Neurosci. Abstr., 1985, 11, 460) and increase susceptibility to proactive interference (Neurosci. Abstr., 1981, 7, 649). The present study was designed to investigate the effects of these lesions on visual search behavior since it was reasoned that an impairment in short-term memory may also affect "normal" search capacity.

In order to test this hypothesis, 11 cynomolgus (M. fascicularis) monkeys were trained on two different visual search tasks. Of the 11 animals, three monkeys had bilateral lesions of the mammillary nuclei, four monkeys had bilateral lesions of the mediodorsal nuclei, and four monkeys served as operated controls.

In the first task, every other foodwell in a test board containing 8 foodwells was baited. All 8 foodwells were covered either with identical white Plexiglas plaques (uncued paradigm) or with 8 plaques of different color (cued paradigm). The animal's task was to displace one of the 8 plaques on each trial until the four bits of food reward were discovered. The displaced plaques were replaced at the end of each trial with a 5-sec. intertrial interval.

In the second task, only one of the foodwells in the 8-choice test board was initially baited and covered. Once the reward was retrieved, a second foodwell was baited and covered on the next trial with the first foodwell remaining covered but not baited. After the second reward was located, a third foodwell was baited/covered and so on for each of the other foodwells (in a pseudo-random) order) with the earlier response sites remaining covered but not baited. An erroneous response to a previous reward site ended the trial, the plaque was replaced during a 5-sec. intertrial interval, and that trial was repeated. The plaques were either of different color (cued paradigm) or all painted white (uncued version).

In the first task, the monkeys with mammillary body or mediodorsal thalamic lesions did not differ from the controls in performance in either the cued or uncued paradigms. In the second task, however, the experimental animals were markedly impaired (with double the no. of errors) relative to the controls in the uncued paradigm, but again, were not impaired when color cues were used.

Thus, despite their memory handicap, monkeys with diencephalic lesions seem to be impaired in visual search only in the limited case in which the location of the reward is constantly changing and the search has to be reinitiated each time a reward site is discovered without the assistance of any salient cues. These results are consistent with previous findings of spatial/cognitive impairments in monkeys following lesions of the diencephalon.

63.9 FRONTAL AND HIPPOCAMPAL MECHANISMS IN CONDITIONAL AND NON-CONDITIONAL SPATIAL LEARNING BY MONKEYS.
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In the first experiment 6 monkeys (Macaca fascicularis) with fornix transection, 6 with bilateral sulcus principalis ablation, and 6 control monkeys, learned either spatial-visual conditional discriminations or visual-spatial conditional discriminations. In spatial-visual conditional discriminations the position of a pair of visual stimuli, to the monkey's left or right, indicated which of the visual stimuli was the correct (rewarded) one. In visual-spatial conditional discriminations the visual stimuli indicated, irrespective of their own spatial position, whether reward was to be found on the monkey's left, or on the right. The animals with sulcus principalis ablation were impaired in both tasks. In both tasks also, the animals with fornix transection were more severely impaired than the animals with sulcus principalis ablation. Within each of the two operated groups, the degree of impairment was equal in the two tasks, in proportion to the difficulty of the tasks for control animals.

Experiment 2 showed that neither of the operated groups was impaired in non-conditional visual learning. Experiment 3 examined non-conditional spatial learning. Here the fornix-transected group were impaired but the group with sulcus principalis ablations were normal. The contrast between impaired conditional spatial learning (Experiment 1) and unimpaired non-conditional spatial learning (Experiment 3) was significant for the group with sulcus principalis ablations, even when the impairments were assessed proportionally to task difficulty for control animals. For the fornix-transected animals the impairment in non-conditional spatial learning was as severe, in proportion to task difficulty, as the impairment in conditional spatial learning.

From these and other findings it is apparent that fornix transection produces a general impairment in learning about personal space, while sulcus principalis ablation selectively impairs conditional spatial learning. These impairments can be related, more generally, to the role of the hippocampal system in personal memory and the role of the frontal lobe in learning conditional relationships.

63.10 THE EFFECTS OF SEPARATE AND COMBINED LESIONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND THE FORNIX ON RECOGNITION MEMORY.
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Recognition memory in monkeys is served by both an amygdalo-thalamic and a hippocampo-thalamic circuit. Lesions of either circuit alone, whether at the level of the limbic structures themselves, their efferent paths (the amygdalofugal pathways and fornix), or their thalamic targets (the magnocellular part of the medial dorsal nucleus, MDmc, and the anterior nuclei of the thalamus, AntN), result in only a mild impairment in recognition memory. By contrast, combined damage to the two limbo-thalamic circuits at any level results in a severe loss (Mishkin, Phil. Trans. R. Soc. Lond., B292:1982). The amygdala (A) and the hippocampal formation (H) have both direct and indirect routes to the thalamus. Thus, the H projects via the fornix both directly to the AntN and indirectly via the mamillary body (MB). Similarly, the A projects both directly to the MD and indirectly via the bed nucleus of the stria terminalis (BNST). Thus, the MB and the BNST appear to occupy comparable anatomical positions within the two parallel limbo-thalamic circuits. It was previously demonstrated that the MB contributes to recognition memory in the monkey. In the present investigation we sought to determine whether the BNST also contributes.

Monkeys were trained preoperatively on a visual recognition memory task (delayed nonmatching-to-sample, DNMS) requiring memory of single objects for 10 sec. After achieving the criterion of 90% correct responses in 100 trials, the monkeys received removals of the BNST alone or in combination with a fornix transection. They were retrained on the task postoperatively and then given a performance test in which their memory was tested further by increasing the delays (up to 120 sec) between the sample presentation and the retention test and by increasing the number of items to be remembered (up to 10 objects).

Scores of these monkeys were compared with scores of monkeys with fornix transection alone which had been subjects in an earlier experiment. All animals relearned DNMS without difficulty. Preliminary results on the perfomance test indicate that the animals with the BNST lesions alone, like those with fornix lesions alone perform on average at better than 92% accuracy across the increased delays and list lengths. By comparison, two animals with the combined lesion obtained an average score of only 84% on the performance test. These results are consistent with the idea that the BNST participates in recognition memory and that its function in the amygdalo-thalamic circuit could be comparable to that of the MB in the hippocampo-thalamic circuit.

64.1 EPILEPTOGENESIS PRODUCED BY REPEATED INTRAPERITONEAL ADMINISTRATIONS OF NALOXONE IN INTACT CATS. L.Rocha*, R. Gutiérrez*, F. Pellicer* and A. Fernández-Guardiola. División de Investigación en Neurociencias, Instituto Mexicano de Psiquiatría, Calz. México-Xochimilco 101, Tlalpan, C.P. 14370, México, D.F., and Facultad de Psicología, UNAM.

It has been described (Fernández-Guardiola et al., Kindling 3, Raven Press. J. Wada (Ed) pp.157, 1986) that amygdaloid kindling can be developed in a shortened fashion ("massed kindling") in "Encephale isolé" preparation and that naloxone administrations during this process shortens the time needed to produce generalized convulsive seizures (GCS) in comparison with the massed kindling alone. Naloxone administered alone, i.e. without kindling trials, was also able to produce epileptic activity; this activity was facilitated with intermittent photic stimulation (IPS) in the same preparation. In order to investigate the effects of the repeated i.p. administrations of naloxone and those of sensory stimulation in freely moving cats, we conducted experiments in 11 adult cats divided in 3 groups: a) naloxone alone group (n=3) to which 8 mg/kg of naloxone were i.p. injected every 15 minutes until the first (GCS) was produced, b) the second group (n=5) had the same naloxone schedule plus photo-acoustic stimulation (IPS: pulses of 10 µs with 1500,000 candles at 25 cm in front of the animal; acoustic stimuli: pulses of 90 ms at 4000 Hz and 95 dB) for 1 minute at 1, 3, 10, and 15 Hz after the drug administration, c) the control group (n=3) was stimulated in the same manner but 1 ml saline was i.p. injected instead. Progressive behavioral changes were found in both experimental groups that consisted of drowsiness with the first doses and subsequent doses produced myoclonias and clonic-tonic convulsive seizures without postictal relaxation in all the animals. All the animals in the naloxone alone group presented "status epilepticus". The photoacoustically stimulated experimental group developed a wide variety of behavioral manifestations that were presented more often than the naloxone alone group, but only 2 animals presented "status epilepticus". The control group did not show noticeable behavioral changes. We conclude that the repeated naloxone administrations produce behavioral changes that resemble those produced by electrical amygdaloid kindling (Goddard et al., Exp. Neurol. 25:295, 1969; Wada & Sato, Neurology 24:565, 1974) and that sensory stimulation drives and synchronizes the multisynaptic central activity in the animals under the effects of naloxone, thus facilitating the apparition of epileptic behavioral manifestations. These results suggest the inhibitory role of endogenous opioids in epileptogenesis.

64.2 ANTAGONISM OF THE ANTICONVULSANT ACTIONS OF U50,488 BY THE SELECTIVE KAPPA OPIOID RECEPTOR ANTAGONIST NOR-BINALTORPHIMINE. F.C. Tortella, E. Echevarria, J.W. Holaday, A.W. Lipowski, A.E. Takemori and P.S. Portoghese, Neuropharm. Br., Div. of N.P., Walter Reed Army Inst. Res., Washington, DC 20307 and Depts. Med. Chem. and Pharmacol., Univ. of Minnesota, Minneapolis, MN 55455.

The highly selective kappa opioid receptor analgesic, U50,488 (trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide), has been shown to possess potent and efficacious anticonvulsant activity in rats (Tortella et al., JPET 237, 1986). The anticonvulsant actions of U50,488 are long-lasting, present following i.c.v. or s.c. administration, and antagonized by moderate (1.0 mg/kg) to high (10 mg/kg) doses of naloxone. This latter finding prompted the suggestion that the anticonvulsant actions of U50,488 are mediated by non-mu (probably kappa) opioid receptor subtypes. Recently, two bivalent ligands, Binaltorphamine and nor-Binaltorphamine (nor-BNI), have been reported to possess potent and selective antagonist activity for the kappa opioid binding site (Portoghese et al., Life Sci. 40, 1987). Using the supramaximal electroshock (MES) model, the purpose of the present study was to verify the kappa receptor mechanism of action for the anticonvulsant effects of U50,488 and to evaluate the in vivo selectivity of nor-BNI for kappa opioid receptors.

Male S.D. rats (225-275 g; n=10/grp) were pretreated with either saline (i.c.v. or s.c.) or the antagonist nor-BNI. Nor-BNI was administered i.c.v. (25 nmol; -30 min) or s.c. (2 mg/kg; -60 min). The rats were subsequently challenged with i.c.v. or s.c. saline (control groups), s.c. administered U50,488 (10-160 mg/kg), or i.c.v. injections of the selective mu opioid agonist DAGO (Try-D-Ala-Gly-NMe-Phe-Gly-ol)(0.275-2.2 nmol). Thirty minutes later, convulsions were induced via transauricular MES (2 sec at 60 Hz, 50 mA).

The anticonvulsant ED50's for the opioid agonists U50,488 and DAGO were 18.0 (10.7-26.9) mg/kg and 0.43 (0.23-0.81) nmol, respectively. Nor-BNI antagonized the anticonvulsant effects of U50,488, increasing the anticonvulsant ED50's for U50,488 to 42.0 (27.8-62.2) mg/kg following i.c.v., and 80.0 (41.2-155.5) mg/kg following s.c., nor-BNI pretreatment. In contrast, pretreatment with i.c.v. or s.c. nor-BNI failed to antagonize the anticonvulsant effects of DAGO (ED50's = 0.59 [0.27-1.30] and 0.69 [0.25-1.95] nmol, respectively). Importantly, nor-BNI, when given at these doses and routes of injection, had no apparent effect on overt behavior or MES convulsions.

We conclude that the anticonvulsant effects of U50,488 are mediated by kappa opioid receptors. Furthermore, Since nor-BNI antagonized the effects of the kappa selective opioid U50,488, but not those of the mu selective opioid DAGO, the results of this study have confirmed that nor-BNI represents a novel class of highly selective kappa opioid receptor antagonists.

64.3 EVIDENCE THAT OPIOID RECEPTORS MEDIATE THE ANALGESIC EFFECT OF MORPHINE INJECTED INTO THE AREA TEMPESTAS IN RATS. M.Massotti and A.D'Amore., Lab. di Farmacologia, Istituto Superiore di Sanita', 00161- Roma, Italy.

Area Tempestas is a forebrain area involved in the genesis and propagation of clonic convulsions due to bicuculline (Piredda and Gale, Nature, 317: 623, 1985) and morphine sulfate (MS) (Foote and Gale, 16th Ann.Meet., Soc.for Neurosci., Wash., D.C., 1986, Abst.n.25.6). Unilateral infusion of 0.5-20 ng/rat of MS into this area also enhances the licking reaction time to the hot plate stimulus of the contralateral forelimb in rats (Massotti, 16th Ann.Meet., Soc.for Neurosci., Wash., D.C., 1986, Abst.n.215.6). The question arises if the latter is a true analgesic effect or is related to other actions of the drug such as motor rigidity or local anesthesia.

In rats, deep cannulae were stereotaxically implanted into this area (A-P, +4.0mm from bregma; M-L, ±3.5mm from longitudinal suture; D-V, -6.5mm from dura) under pentobarbital anesthesia. Drugs were delivered at the speed rate of 60 nl/min for 2 min, then animals were tested various times after the end of the infusion. The hot plate (55.6 C) test and the test of the crossing of ipsilateral feets (Boissier and Simon, Therapie, 18:1257, 1963) were carried out to assess analgesia and catalepsy, respectively. The effects were separately recorded in right and left limbs.

Bilateral infusion of MS (20ng/rat) enhances the licking reaction time of both forelimbs (>180 sec). Subsequent unilateral infusion of naltrexone (4 ng/rat) reduces, already at the 1st min, the response in the contralateral forelimb, attaining the same values observed in intact rats (12±2 sec). In addition, unilateral infusion of chlorpromazine into this area (20-1000ng/rat) does not consistently modify the response to the hot plate stimulus.

When animals are tested for catalepsy after unilateral MS, they maintain the imposed position of the contralateral feets only (>100±20 sec) at the doses of 100 ng/rat but not after 50 ng/rat.

Present data suggest that contralateral analgesia induced by unilateral MS is an opioid-mediated phenomenon. This is confirmed by preliminary experiments showing that analgesic effect elicited by MS into this area undergoes to tolerance after a 5 days regimen of treatment.

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64.4 DYNORPHIN INHIBITORY EFFECTS ON CUSPID TOOTH DENTINE-EVOKED RESPONSES RECORDED IN THE ALVEOLAR NERVE OF THE DOG. M.F. Pacheco, J.F. Fernández*, S.H. Dueñas* and J.M. Briseño*. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Apdo. Postal 199, Colima, Col. 28000 México.

This study was undertook in order to investigate the electrophysiological mechanisms underlying the analgesic effectiveness of topically applied dynorphin observed on human dental pulp (Pacheco, M.F. and Briseño J.M., Proc. West. Pharmacol. Soc. 29: 175, 1986). In 10 pentobarbital-anesthetized dogs, three wire cuff-electrodes placed around the alveolar nerve were used to record evoked potentials elicited by electrical stimulation (50 µsec pulses) of exposed cuspid (canine) dentine. Electrical stimulation of dentine evoked compose potentials in the alveolar nerve, corresponding to discharges from both Aδ- and C-fibers (as characterized by conduction velocity measurements). Whereas the topical application of 5 µL of either saline (0.9%) or distilled water, over the exposed dentine, didn't change any parameter of the recorded potentials, the application of 5 µL of (1,2,5 or 10 mM) dynorphin 1-13 or dynorphin 1-17 produced, equipotent and dose dependent, inhibitory effects on the evoked potentials recorded. Dynorphin effects followed a temporal sequence: Aδ-fibers discharges were totally inhibited after 1-3 min of the opioid application, and recovered after 10-18 min; Activity from C-fibers was totally inhibited after 3-4 min of the peptide application, recovering after 7-10 min. The onset as well as the duration of the dynorphin effects were dependent on the dose used. These observations suggest that the deep dental analgesia elicited by dynorphin is exerted over specific receptors in dental pulp. The temporal sequence of the dynorphin inhibitory effects suggest a different location for Aδ- C-fibers, perhaps Aδ-fibers are located in dentine, and C-fibers in vascular pulp of the tooth.

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64.5 **DIFFERENTIAL DEVELOPMENT OF TOLERANCE TO ANALGESIA, RESPIRATORY DEPRESSION AND HORMONE RELEASE IN A MORPHINE INFUSION MODEL.** G.S.F. Ling, R. Simantov* and G.W. Pasternak [SPON: J. Posner], Cotzias Laboratory of Neuro-Oncology, Memorial Sloan Kettering Cancer Center and Cornell U. Medical College, NY, NY 10021.

Tolerance develops to almost all of morphine's actions, including both analgesia and respiratory depression. Recent studies from our laboratory suggest that analgesia and respiratory depression are mediated through different subtypes of mu receptors: mu_1 and mu_2, respectively. Similarly, different receptors classes mediate prolactin and growth hormone release. In view of the different receptor mechanisms involved with these opiate actions, we have compared their rate of tolerance development using a continuous intravenous morphine infusion model. In the infusion model, analgesic actions peaked at approximately 2 h after which they declined due to the development of tolerance, almost reaching baseline values by 8 h. Over a series of doses (10 to 50 μg/kg/min), the peak latency demonstrated a simple dose-response relationship, increasing linearly with the log of the dose. In addition to the gradual fall in tailflick latencies with continued infusions over 8 h, analgesic tolerance was also demonstrated following bolus injections of morphine (5 mg/kg, iv). Using the bolus approach, we found that tolerance progressed with continued morphine infusions even after the infusions no longer elicted any analgesic response. Thus, the development of tolerance is not dependent upon the the continued presence of analgesia. Unlike analgesia, no significant tolerance to morphine's respiratory depressant actions, determined by arterial blood gases, was observed over 10 h at a morphine dose of 50 μg/kg/min. Previous studies have implied a mu_1 mechanism of action for prolactin, but not growth hormone release. Rats infused with morphine (25 μg/kg/min) for 8 hours demonstrated significant tolerance to analgesia and the release of prolactin, but not growth hormone. These studies suggest that the development of tolerance is related to the receptor subtype mediating the response. Tolerance develops quickly to mu_1 actions, such as analgesia and prolactin release, but far more slowly to the two non-mu_1 actions we examined, respiratory depression and growth hormone release. This differential rate of tolerance development may correspond to a lower therapeutic index of opiates in tolerant subjects. These studies also demonstate the usefulness of a continous intravenous infusion model which permits the study of any drug at any dose without the excessive fluctuations in blood levels seen with pelletting techniques.

64.6 INTERMITTENT NALOXONE ATTENUATES PHYSICAL DEPENDENCE ON METHADONE IN RHESUS MONKEYS. J.H. Krystal,* M. Walker,* G.R. Heninger, (SPON: S. Stine) Dept. Psychiatry, Yale Univ. Sch. of Medicine, New Haven, CT 06508.

Recently introduced treatment strategies for opiate dependency have suggested that opiate antagonists, such as naltrexone, or mixed agonist-antagonists, such as buprenorphine, may shorten or attenuate the opiate abstinence syndrome in patients treated with methadone. In addition, there is data from studies in monkeys and rats indicating that coadministration of opiate agonists and antagonists reduces physical dependency. In order to more fully evaluate this phenomenon in primates, the ability of intermittent opiate antagonist administration to attenuate the degree of opiate withdrawal elicited by naloxone challenge was investigated in monkeys who received daily methadone (METH) injections.

METHODS: The behavior of individually-housed rhesus monkeys (n=8) was rated daily via video, during the 20 minutes before and 40 minutes after noon injections. The baseline period consisted of a rating day without drug administration, a saline day (SAL, i.m. at noon), and a naloxone day (NAL, 0.5 mg/kg i.m. at noon). During the treatment period, monkeys received daily METH injections (2.0 mg/kg i.m.) for 14 days under 2 treatment schedules: Tx I: METH daily at 8:00 a.m. and SAL, i.m., daily at noon; Tx II: METH daily at 8:00 a.m., and SAL alternating daily with NAL (0.5 mg/kg i.m. daily at noon). Each monkey received both treatments in a balanced order separated by approximately one month. On day 15, monkeys received a single injection of NAL (0.5 mg/kg, i.m.) but not methadone.

RESULTS: Naloxone produced no behavioral signs of opiate withdrawal in opiate-naive monkeys (repeated measures ANOVA, $p = 0.4$) or in monkeys that completed Tx I or Tx II approximately one month earlier ($p = 0.4$). Naloxone challenge in monkeys receiving methadone produced behavioral signs of opiate withdrawal including retching, lying on side, "wet dog" shakes, posturing, sterotypy, body jerks and piloerection. During Tx II, naloxone elicted a mild opiate withdrawal syndrome ($p = 0.001$) after two days of methadone which did not significantly change in severity during the 7 naloxone challenges included in the 14 day treatment ($p = 0.4$). Monkeys in Tx I exhibited a more severe opioid abstinence syndrome after naloxone on day 15 which was significantly larger than the day 15 withdrawal syndrome after Tx II ($p = 0.001$) or the mild naloxone precipitated withdrawal observed during days 1-14 of Tx II ($p = 0.03$). The day 15 withdrawal syndrome after Tx II was also less severe than the mild naloxone-elicited withdrawal observed during days 1-14 of Tx II ($p = 0.04$).

DISCUSSION: Since the 1/2 life of i.m. naloxone is 1-4 hrs, while the 1/2 life of i.m. methadone is 15-24 hrs, this study provides evidence that a short intermittent blockade of opiate receptors by naloxone is sufficient to attenuate the development of physical dependency on opiates.

64.7 DIFFERENTIAL REGULATION OF AGONIST BINDING TO OPIOID RECEPTOR TYPES BY GUANYL NUCLEOTIDES: EFFECTS OF CHRONIC MORPHINE TREATMENT. L.L. Werling, S.R. Brown*, and B.M.Cox. Uniformed Services University, Bethesda, Maryland 20814-4799

We have previously reported that subsets of opioid receptors with low affinity for agonists can be identified by labeling them with [^{3}H]antagonist and measuring agonist competition over a wide range of agonist concentrations in the presence of sodium (Werling et al., Molec. Pharmacol. 30:90-95, 1986). We have now used this technique to investigate the effects of guanyl nucleotides on opioid agonist affinity at mu, delta, and kappa sites in guinea pig cortical membranes. Saturation curves for [^{3}H]diprenorphine ([^{3}H]DIP) binding to each of the three opioid receptor types in the presence and absence of GTP indicated that the affinity of antagonist for mu, delta, and kappa opioid receptors was not affected by the guanyl nucleotide. In the presence of the appropriate site-selective blocking agents, we displaced [^{3}H]DIP binding to mu receptors with Tyr-D-Ala-Gly-(Me)Phe-Glyol (DAGO), binding to delta receptors with D-Ser^2-D-Leu^5-Enkephalin-Thr (DSLET), and binding to kappa receptors with U50488H. Maximal inhibitory effects of GTP were achieved at 100 μM (added twice during the course of incubation). In the absence of GTP, DAGO displaced [^{3}H]DIP in a monophasic manner with a K_D of about 30 nM, consistent with labeling of a single site. Addition of GTP (100 μM) produced a biphasic displacement pattern, with a high affinity site of about 60 nM and a lower affinity site of about 1 - 2 μM. In cortical tissue taken from guinea pigs which had been made tolerant to morphine (1.7 mg/kg/hr for 6 days), only one affinity state of the mu receptor could be reliably identified in the presence or absence of GTP. The K_D of DAGO for this site in the absence of guanyl nucleotide was about 70 nM, and was only marginally increased in the presence of GTP. These findings suggest that in the tolerant state, guanyl nucleotides may be less capable of regulating the interaction of a mu opioid with its receptor. Two agonist affinity states of the delta receptor could be identified in the absence of any added guanyl nucleotide with K_D values for DSLET of about 5 nM and 600 nM. The proportions of sites in each state were approximately equal. In the presence of GTP, two sites were detectable with K_D values for DSLET of 26 nM and 3.2 μM, in approximately equal proportions. After chronic morphine treatment, there was no apparent change in the ability of GTP to regulate DSLET binding to delta receptors. U50488H displaced [^{3}H]DIP binding to kappa receptors with a K_D of about 15 nM in the presence or absence of added GTP. Studies are currently underway utilizing non-hydrolyzable guanyl nucleotide analogues to provide further characterization of forms of the receptor-G protein-guanyl nucleotide complex that are being labeled in our assay system. (This work has been supported by NIDA.)

64.8 THE EFFECTS OF CHRONIC OPIOID EXPOSURE ON THE 7315c PITUITARY CELLS: A USEFUL MODEL IN THE STUDY OF TOLERANCE. P.S. Puttfarcken and B.M. Cox. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

At least three time-dependent cellular processes appear to occur after chronic exposure of delta opiate receptor containing NG108-15 cells to opiate drugs. Law et al. (Molec. Pharmacol. 24:413-424, 1983) have demonstrated etorphine-induced desensitization of inhibition of adenylyl cyclase (AC) and opiate receptor down-regulation. An increase in AC activity above baseline levels after withdrawal of the chronically administered agonist has also been reported (Sharma et al., Proc. Natl. Acad. Sci. USA 72:590-594, 1975). Although the NG108-15 cells provide a useful model to study development of tolerance, it may be more clinically relevant to study the effects of chronic opioid exposure at the mu opioid receptor, since most addictive narcotics have preferential affinity for mu receptors. We now report the effects of chronic opioid exposure on 7315c pituitary tumor cells which contain a homogeneous population of mu opioid receptors (Puttfarcken et al., Molec. Pharmacol. 30:81-89, 1986). Opiate activation of these receptors inhibits AC. The cells (2.5 x 10^7 cells/25 ml DMEM) were plated on 75cm^2 tissue culture flasks precoated with Vitrogen 100, a sterile solution of bovine dermal collagen which facilitated cell attachment. AC activity was measured according to the method of Cote et al. (Endocrinology 110:812-819, 1982). In untreated flasks, AC activity progressively decreased. After a one-day incubation the enzyme activity could not be detected since the cells no longer remained viable. AC activity remained stable for at least 18 days when the 7315c cells were plated on Vitrogen-coated 75 cm^2 flasks. In preliminary experiments we measured the ability of the opioid agonist, D-Ala-D-Leu-Enkephalin (DADLE), to inhibit AC activity after 1-, 3-, 5-, 9-, 24-, or 48-hour exposure to 10 μM morphine compared to activity in untreated cells incubated for the same period. After approximately 5 hours of chronic morphine exposure (10 μM), DADLE was no longer able to inhibit AC activity in a dose-dependent manner. Membranes from control cells incubated in a drug-free medium did not lose their sensitivity to DADLE. [^{3}H]-Radioligand binding studies are in progress to determine if the density of opiate receptors is reduced after chronic agonist exposure in the 7315c pituitary cell. The addition of naloxone (10 μM) after 72-hour morphine exposure resulted in very little, if any, increase in AC activity above control levels after removal of morphine. This is in contrast to the 80% increase above baseline AC activity first observed by Sharma et al. (1975) in the NG108-15 cells after removal of the treatment agonist. (This grant was supported by NIDA.)

64.9 DIFFERENTIATION OF CHRONIC μ OPIOID RECEPTOR BLOCKADE AND SUPERSENSITIVITY TO MORPHINE. B.C. Yoburn and C.E. Inturrisi. Dept. of Pharmacology, Cornell Univ. Med. Coll., NY, NY 10021.

Chronic exposure to opioid antagonists produces an increase in the number of brain opioid receptors (receptor upregulation) and increases the potency of opioid agonists (supersensitivity). However, it has not been determined if blockade of morphine's actions during chronic antagonist treatment, is a critical factor in the production of supersensitivity. In the present studies, mice were implanted subcutaneously with either a low (2.0mg) or high (7.5mg) dose naltrexone (NTX) pellet for 8 days. The high dose NTX pellet produces both upregulation and supersensitivity in the mouse (Yoburn et al., J.Pharmacol.Exp.Ther., 239:132,1986). Controls were implanted with placebo pellets. Morphine analgesia was tested using the tail flick test with analgesia defined as failure to respond by 10sec (baseline latency=2-3sec). In separate groups of mice tested at 4hrs and 8 days following pellet implantation, both NTX pellets blocked the analgesic effects of a dose of morphine (24mg/kg,s.c.) that produced analgesia in 95-100% of controls. At 8 days following implantation the high dose NTX produced nearly 2.5-fold greater plasma NTX (144.1ng/ml±18.7SEM) compared to low dose NTX (67.1±9.1) as determined by RIA. In order to examine supersensitivity, separate groups of mice were implanted with placebo, or, low or high dose NTX for 8 days. The pellets were then removed and 24hrs later mice were tested for morphine analgesia (8mg/kg, s.c.). Consistent with previous results, the high dose NTX produced significant supersensitivity (71% analgesic) compared to placebo-treated controls (28% analgesic). However, the low dose NTX failed to produce a significant increase in analgesia (45% analgesic) compared to controls and produced significantly less analgesia than the high dose NTX group.

The results of these experiments indicate that blockade of morphine analgesia over an 8 day interval is not sufficient to produce supersensitivity to morphine's analgesic actions. Both low and high dose NTX implants blocked the analgesic action of morphine. However, 8 day treatment with high dose NTX increased the percent analgesic by 43% compared to controls and by 26% compared to the low dose NTX. Low dose NTX did not significantly increase morphine's analgesic potency. These findings raise the possibility that opioid antagonist-induced supersensitivity may require antagonist action not only at μ (morphine) receptors, but also at receptors that do not mediate morphine analgesia.

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64.10 MECHANISMS UNDERLYING THE ACTION OF ENDOGENOUS OPIOID SYSTEMS IN NEURAL CANCER. I.S. Zagon and P.J. McLaughlin. Dept. of Anatomy, The M.S. Hershey Med. Ctr. of The Pennsylvania State University, Hershey, PA 17033.

Endogenous opioids are known to regulate oncogenic events (e.g., Science 221: 671-673, 1983), with opioids serving as trophic factors that exert an inhibitory influence on tumorigenicity. Opioid antagonist paradigms, which disturb opioid-receptor interactions, have proven particularly valuable in studies of opioids and cancer, with complete daily opioid receptor blockade (e.g., 10 mg/kg naltrexone) resulting in growth stimulation, and intermittent daily blockade (e.g., 0.1 mg/kg naltrexone) inhibiting growth. Using a murine S20Y neuroblastoma tumor model and naltrexone (NTX), we found that the mitotic index of tumors (12-15 mm diameter) from mice given a complete receptor blockade (10 mg/kg NTX) was 33% and 70% greater than control levels at 2 and 10 hr following NTX injection. Intermittent blockade (0.1 mg/kg NTX) resulted in an increase of 23% in mitotic index in contrast to controls at 2 hr, but a decrease of 25% at 10 hr; previous studies showed that receptor blockade was only effective for 4-6 hr/day in mice given 0.1 mg/kg NTX. Morphometric areal analysis of viable and necrotic tumor tissue showed no difference between control (26% viable area) and 10 mg/kg NTX mice, but a marked increase (45% viable area) was noted in mice given intermittent blockade. Radioimmunoassays revealed both β-endorphin and met-enkephalin were present in murine tumors, and were markedly elevated in tissues from both NTX groups. Met-enkephalin was detected in the cortical cytoplasm of neuroblastoma cells, but not in the nuclear region, using immunocytochemical techniques. Specific and saturable opioid receptor binding was detected in control tumor tissues for DADLE and EKC, but not for DAGO. Autoradiography experiments using ^{125}I-met-enkephalin showed a homogenous distribution in control tissues that was blocked by naloxone; densitometric measures revealed increases of 40-100% in NTX groups. Functional tests monitoring nociceptive response to a challenge with levorphanol showed a blockade during opioid antagonist presence (e.g., at 2 hr in the 0.1 NTX group) but a supersensitivity following receptor blockade (e.g., 8 hr after injection of 0.1 NTX). These results indicate neurotumors are dependent on opioids to control growth, and do so by actively regulating cell replication in an inhibitory fashion. Moreover, the mechanistic basis for antitumor effects by daily intermittent receptor blockade with opioid antagonists appears to be due to an exaggerated opioid action (e.g., mitotic inhibition), as a result of up-regulation of growth related opioids/binding sites and their interaction during the daily interval when the opioid antagonist is no longer present. Supported by NIH grants NS-20623 and NS-20500.

64.11 HUMAN NEUROBLASTOMA TRANSPLANTED INTO NUDE MICE IS REGULATED BY ENDOGENOUS OPIOID SYSTEMS. L. Nagy*, P.J. McLaughlin and I.S. Zagon (SPON: T. Lloyd). Dept. Anatomy, The M.S. Hershey Med. Ctr. of The Pennsylvania State University, Hershey, PA 17033.

Tumorigenic events associated with cancer in animals have been shown to be modulated by opioid agonists and antagonists, with the mechanism underlying this control involving endogenous opioid systems (i.e., endogenous opioids and opioid receptors). Involvement of endogenous opioid systems in human neoplasia is an important, but unanswered, question. In the present study, the role of endogenous opioid systems in human cancer was explored using an opioid antagonist paradigm and human neuroblastoma cells transplanted into nude mice. Mice were inoculated s.c. with 2.5 X 10^6 neuroblastoma (SK-N-MC) cells and separated into groups which received daily injections of either 0.1 or 10 mg/kg naltrexone (= 0.1 NTX or 10 NTX, respectively) or sterile water (= CO). The latency for appearance of a measurable tumor (5 mm or larger in diameter) in the 0.1 NTX group occurred 27% later than in control subjects (11 days), and the first death in this group occurred 33% later than in the control group (day 27). Mice inoculated with tumor cells in the 10 NTX group had an acceleration (18%) in latency time of tumor appearance. Moreover, 70% of the mice in the 10 NTX group had tumors on day 14, a time when only 10% of the CO group expressed a tumor. Forty-five days after tumor cell inoculation, only 33% of the mice in the 10 NTX group were alive, relative to 90% of the CO mice. Thus, complete blockade of endogenous opioid-receptor interaction resulted in enhanced oncogenic response, whereas intermittent blockade retarded human tumorigenesis. To determine the presence of opioid receptors in human neuroblastoma, saturation isotherms were generated utilizing 3 ligands (DAGO, DADLE, and EKC) which are prototypic for mu, delta, and kappa receptor types in neural tissues. Specific and saturable binding could only be demonstrated for DADLE and EKC. Measurements of opioid levels in tumor tissues revealed the presence of both β-endorphin and methionine-enkephalin. These studies indicate that components of endogenous opioid systems are present in a neural tumor, and are involved in the regulation of tumorigenesis. Endogenous opioids appear to function as trophic inhibitors of oncogenic events that actively control human, and animal, tumor development. The regulatory properties of opioids are mediated by opioid receptors that subserve growth-related functions.

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65.1 The Avian Suprachiasmatic Nucleus: Anatomical and Functional Aspects. R. B. Norgren, Jr.* and R. Silver. Dept. of Psychology, Columbia University, New York City 10027.

The suprachiasmatic nucleus is a circadian pacemaker in mammals. This has been demonstrated with a variety of methods, including lesion studies, recordings of electrical activity, transplants, glucose utilization. In birds, the function and location of the SCN is less well understood. Attempts to identify a nucleus homologous to the mammalian SCN in avian species have suggested two candidates in the anterior hypothalamus. One lies adjacent to the third ventricle. The other is in a more lateral position.

Several different methods were used to characterize these two nuclei in ring doves including retino-hypothalamic tract tracing, cytoarchitecture, immunocytochemistry, enzyme histochemistry, and deoxy-D-glucose-2-[1-^{14}C] (2-DG) labeling [in collaboration with V. Cassone]. **Retino-hypothalamic projections** were studied with intraocular injections of HRP and application of HRP to the cut end of the optic nerve. While both methods revealed a direct retinal input to the lateral hypothalamus, only the latter method suggested any retinal input to the medial hypothalamus, and this was at most very sparse. **Cytoarchitectural studies** revealed that the medial nucleus contained small, tightly packed neurons, while the lateral nucleus contained a mixed population of diffusely arranged cells. **Immunocytochemical analysis** failed to demonstrate vasoactive intestinal polypeptide or neurophysin in either nucleus. There was moderate **acetylcholinesterase staining** in the lateral nucleus and very little staining in the medial nucleus. Preliminary **^{14}C 2-DG experiments** suggested a circadian rhythm in metabolic activity in the medial nucleus.

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65.2 ANATOMIC AND FUNCTIONAL DEVELOPMENT OF THE SUPRACHIASMATIC NUCLEI IN A MARSUPIAL. S.A. Rivkees*, C.A. Fox*, S.M. Reppert, C.D. Jacobson. Children's Service, Massachusetts General Hospital and Harvard Medical School, Boston MA 02114, and Department of Veterinary Anatomy, Iowa State University. Ames, Iowa, 50011.

Due to profound immaturity at birth, marsupials provide a unique opportunity to study the development of the circadian timing system. In contrast to rats in which a functioning circadian clock in the suprachiasmatic nuclei (SCN) develops in utero, it is likely that the SCN of marsupials develop exclusively during the postnatal period. We thus characterized the anatomic and functional development of the SCN in a pouchless marsupial, Monodelphis domestica (the grey short-tailed opossum), during the postnatal period.

The time course of SCN neurogenesis was examined using [^{3}H]thymidine autoradiography. Dams were manually restrained, and each pup in a litter was injected sc with [^{3}H]thymidine along the dorsal midline. Each litter of pups received the isotope on a different postnatal day between days 4 and 12. Pups were killed on day 30, and 6 µm coronal brain sections were processed for autoradiography. The time of appearance of the SCN as distinct nuclei was determined by light microscopic examination of Nissl stained brain sections. To define the onset of SCN functional activity, the [^{14}C]2-deoxyglucose (DG) technique was employed. Animals of specified ages were placed in constant darkness for 24 hrs prior to injection. One-half of the offspring from each litter were then injected with DG (0.1 uCi/gm) during subjective day, and the remainder were injected during subjective night. At 45 min after injection, animals were killed, and 20 µm coronal brain sections were processed for autoadiography. The relative optical density (OD) of each animal's SCN was determined.

SCN neurogenesis was completed by postnatal day 8 (0=day of birth) and the SCN were recognizable as distinct nuclei beginning on day 16. There was no day-night rhythm in SCN metabolic activity on day 16: the nuclei were metabolically active during both day and night (n=10; day relative OD 1.28±.04 (M±SEM), night OD 1.26±.07). A clear day-night rhythm in SCN metabolic activity was first observed on day 20 (n=12; day OD 1.16±.02, night OD 0.99±.01, $p<.01$). The rhythm was also apparent on day 27 (n=6; day OD 1.24±.03, night OD 0.96±.04, $p<.01$). SCN metabolic activity was very similar among the pups of each litter. Pup circadian phase was synchronous with the phase of the dam; dam circadian phase was assessed by monitoring wheel-running activity.

We conclude that in Monodelphis domestica, (1) SCN neurogenesis occurs postnatally and is complete by day 8, (2) the SCN are morphologically apparent on day 16, (3) a daily rhythm in SCN metabolic activity is present on day 20, and (4) pup circadian phase is coordinated with the circadian phase of the dam and the prevailing light-dark cycle. Supported by NIH grants HD14427 and HD16148.

65.3 THE ORIGIN, ORGANIZATION AND PROJECTIONS OF VISUAL AFFERENTS TO THE RAT HYPOTHALAMUS: IMPLICATIONS FOR THE CONTROL OF CIRCADIAN RHYTHMICITY. J.P. Card, R.P. Meade and R.Y. Moore. Medical Products Department, E.I. du Pont de Nemours and Co., Wilmington, Delaware and Neurology Department, SUNY @ Stony Brook, Stony Brook, New York.

The suprachiasmatic nucleus (SCN) of the hypothalamus receives dense and overlapping projections from the retina and the intergeniculate leaflet (IGL) of the thalamus. Each of these projections upon the SCN has been implicated in the control of circadian rhythmicity and the projection from the IGL is also distinguished by neuropeptide Y (NPY) immunoreactivity. In the present investigation we have utilized retrograde transport of fluorescent tracers (fluorogold and rhodamine labeled latex beads) combined with immunohistochemistry to gain further insight into the organization of these projections. Four different injection regimes were combined with immunohistochemical localization of either NPY or vasoactive intestinal polypeptide (VIP). These included (1) bilateral tracer injection into the SCN, (2) bilateral tracer injection into the SCN with unilateral injection of another tracer into one IGL, (3) unilateral tracer injection into one IGL and (4) injection of a different tracer into each IGL. The findings of these studies support previous observations on the organization of NPY afferents terminating in the SCN and also reveal another order of complexity in the organization of neural circuits involved in the control of circadian rhythmicity. Our findings can be summarized as follows: First, projections to the SCN from the IGL are not restricted to neurons which contain NPY. An additional group of IGL neurons, of uncharacterized chemical content, is also retrogradely labeled following SCN injections and the number of these neurons is equivalent to the number of NPY neurons which project to the SCN. Second, although a substantial number of IGL neurons project to the contralateral IGL, these neurons do not contain NPY. In some instances, a small number of these neurons are also retrogradely labeled by injection of a second tracer into the SCN. Finally, tracer injections into the IGL reveal retrogradely labeled neurons in the SCN and anterior hypothalamus which do not contain VIP immunoreactivity. Most of these neurons project to the ipsilateral IGL, but a small percentage of these cells also project to the contralateral IGL. Taken together with the fact that both the SCN and IGL receive direct projections from the retina, these findings demonstrate that the neuronal circuitry which integrates visual information in the control of circadian rhythmicity is much more complex than previously appreciated.

65.4 EVIDENCE FOR DIRECT RETINAL INPUT TO THE PARAVENTRICULAR NUCLEUS IN A PHOTOPERIODIC RODENT (MESOCRICETUS AURATUS). T.G. Youngstrom, M.L. Weiss & A.A. Nunez. Psychology Dept. and Neuroscience Prog., Michigan State Univ., E. Lansing, MI. 48824.

A retino-hypothalamic tract (RHT) with projections primarily to the suprachiasmatic nuclei (SCN) has been reported in several species (JCN 146:1, '72; Brain Res. 49:403, '73; JCN 196:155, '81). The SCN are involved in the generation of circadian rhythms and photoperiod-dependent seasonal rhythms (Physiol. Reviews 59(3):449, '79; Sci. 227:714, '85). A few fibers of the RHT continue through the SCN into the region between the SCN and paraventricular nucleus (PVN; JCN 196:155, '81; Brain Res. Bull. 17:485, '86). Cholera toxin conjugated to horseradish peroxidase (CT-HRP) has recently been shown to be a sensitive anterograde tract tracer and has been used to reexamine the retinal efferents of the rat (Neurosci. Abstr. 12:549, '86). This method has revealed RHT projections to the SCN and anterior hypothalamic area. In addition, input to the lateral hypothalamus (dorso-lateral boundary of the supraoptic nucleus (SON)) via a second route was found. Outside the hypothalamus labelled processes were reported in anterodorsal (ADT) and anteroventral thalamus (AVT), lateral geniculate nucleus and superior colliculus. In the male Syrian hamster an earlier study using intraocular injections of free HRP reported evidence of retinal input to the piriform cortex and septal area but not to the SON, ADT or AVT (JCN 196:155, '81). In this study, retinal projections to the brain of the Syrian hamster (Mesocricetus auratus) were reexamined using CT-HRP. Following a 24 hour survival period male and female Syrian hamsters were perfused and the tissue processed using a previously published procedure (J. Histochem. Cytochem. 28(11):1255, '80). Areas of the visual system previously reported to receive direct retinal input were densely labelled as was the RHT projection to the SCN. A loose bundle of RHT fibers pass dorsal through the caudal SCN into the periventricular area to form a plexus in the sub-PVN area. Some fibers proceed laterally, beneath the PVN, while a small number of fibers and presumptive terminals were located within the PVN. Dorsal to the third ventricle in or near zona incerta and nucleus reuniens labelled fibers were observed. The supraoptic nucleus (SON) and adjacent hypothalamus also contained labelled fibers. Some fibers were traced into the superficial layer of the piriform cortex. A projection from the retina to dorsal thalamus was also identified. In addition to confirming the presence of a retinal projection to the SCN of the hamster these results provide evidence of RHT input to the AHA, periventricular hypothalamus, sub-PVN area, PVN and dorsal to the PVN. This direct retinal input to the PVN may mediate acute pineal responses to light with no involvement of the SCN. Supported in part by NIMH Grant MH 37877 to AAN and NRSA Postdoctoral Grant NS 08125 to MLW.

65.5 SEASONALITY IN THE SYRIAN HAMSTER MAY INVOLVE A TEMPORAL INTERACTION OF CIRCADIAN NEUROTRANSMITTER RHYTHMS IN THE RIGHT AND LEFT SUPRACHIASMATIC NUCLEI. J.M. Wilson and A.H. Meier*. Dept. Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803-1725.

Daily injections of 5-hydroxytryptophan (5-HTP; serotonin precursor) and L-dihydroxyphenylalanine (L-DOPA; catecholamine precursor) given at the same time of day for 9 days induced scotosensitive responses (reduced gonad weights and serum gonadotropin and thyroxine concentrations) in scotorefractory hamsters maintained on short daylengths (LD 10:14) for 5 weeks. On the other hand, daily injections of L-DOPA given 12 hours after daily injections of 5-HTP for 9 days induced scotorefractory responses (increased gonad weights and serum gonadotropin and thyroxine concentrations) in scotosensitive hamsters maintained on short daylengths (LD 10:14). Thus the endogenous mechanism controlling seasonality (scotosensitivity and scotorefractoriness) in the Syrian hamster may involve two circadian systems consisting of catecholaminergic and serotonergic components that vary seasonally in their phase relations.

Inasmuch as the suprachiasmatic nuclei (SCN) have been strongly implicated as a circadian pacemaker, they may also be a site for reentrainment of circadian oscillations by 5-HTP and DOPA. Accordingly, dopamine and serotonin concentrations were determined by high performance liquid chromatography with electrochemical detection in both SCN of scotosensitive and scotorefractory male hamsters maintained on short daylengths (LD 10:14). Marked circadian rhythms of dopamine and serotonin concentrations were observed in both SCN of scotosensitive and scotorefractory hamsters. In scotosensitive hamsters, highest concentrations of both neurotransmitters occurred near the onset of light in both left and right SCN. Similarly, highest conentrations of both neurotransmitters also occurred near the onset of light in the left SCN of scotorefractory hamsters. However, peak dopamine concentrations in the right SCN occurred 12-16 hours after the onset of light and the daily peak of serotonin concentration. Thus there is a 0-hr relation between the daily peaks of serotonin and dopamine concentrations in both SCN of scotosensitive hamsters and in the left SCN of scotorefractory hamsters. However, in the right SCN of scotorefractory hamsters there is approximately a 12-hr relation between the daily peaks of the two neurotransmitters. Thus the paired SCN may represent two circadian pacemakers and changes in the phase relations of their activities may produce alternations of seasonal conditions.

65.6 DISPERSED CELL SUSPENSIONS OF FETAL SUPRACHIASMATIC NUCLEUS (SCN) RESTORE CIRCADIAN LOCOMOTOR RHYTHMS TO SCN-LESIONED HAMSTERS. M.N. Lehman, R. Silver, M. Gibson, and E.L. Bittman. Dept. Anat. & Cell Biol., Univ. Cincinnati Coll Med.; Dept Psychol., Barnard Coll of Columbia Univ.; Dept. Medicine, Mt. Sinai Sch. Med.; and Dept. Zool., Univ. Mass. Amherst.

We recently demonstrated that whole tissue grafts of the fetal SCN . restore circadian locomotor rhythms to hamsters previously made arrhythmic by SCN lesions. To determine whether the intrinsic peptidergic organization of the SCN is a prerequisite for functional recovery, we attempted to restore rhythmicity to SCN-lesioned hamsters using dispersed cell suspensions. Adult male hamsters received bilateral electrolytic lesions of the SCN and their locomotor activity was monitored during 4 wks. of constant darkness (DD) and 2 wks. of light:dark (LD) 12:12 to verify the lack of rhythmicity. Twelve arrhythmic animals received bilateral stereotaxic injections, directed toward the medial hypothalamus, of dispersed cells prepared from fetal (E14) anteroventral hypothalamus (80,000 cells/5 ul injection). In order to mark injected cells, mothers received i.p. injections of ^{3}H-thymidine (7.5 uCi/gm body weight) on E11, when peak mitotic activity occurs in the SCN (Crossland & Uchwat, *Dev. Brain Res.* 5:99). Hamsters were housed in LD 12:12 for 1-3 wks. after surgery followed by DD for 10-12 wks. Hamsters with restored rhythms were transferred to LD 12:12 for an additional 10-12 wks. Animals were perfused and vibratome sections (50 um) were immunocytochemically stained for either vasoactive intestinal polypeptide (VIP) or vasopressin (VP), as previously described (Lehman *et al.*, *Neurosci. Abstr.*, 12:210). Alternate sections were processed for autoradiography to visualize the location of radiolabelled donor cells.

In 6 of 12 recipients, dispersed cells of fetal SCN re-established free-running rhythms in DD but not entrainment to LD. The period of these rhythms was 23.56 $\pm$ 0.02 hr (mean $\pm$ S.E.M.). In animals rhythms split into two components, which established a stable phase relationship. Measurement of testes width during 10 wks. of DD consistently revealed large gonads indicating the lack of appropriate reproductive response to photoperiod. In animals with restored rhythms, injected cells labelled with ^{3}H-thymidine were located within the medial hypothalamus, either adjacent to the third ventricle or between the fornix and paraventricular nuclei. Recovered hamsters possessed isolated or small groups of VIP cells in these areas, along with small plexuses of VIP and VP fibers. In animals that did not recover rhythms, radiolabelled cells were not found intracerebrally and were probably injected into the third ventricle. The results suggest that relatively few peptidergic cells and fibers, either isolated or in small clusters, need be present in order for circadian locomotor rhythms to reappear. The period of restored free-running rhythms following cell injections tends to be shorter than that of lesioned hamsters whose circadian rhythms recover after whole tissue grafts. [Supported by NIH NS24292 (R.S. & M.N.L.) NS 20335 (M.G.) and NSF BNS-8616935 (E.L.B.)]

65.7 SELECTIVE TRANSPLANTATION OF THE FETAL SUPRACHIASMATIC AND PARAVENTRICULAR NUCLEI: STABILITY AND SPECIFICITY IN CYTOLOGICAL AND IMMUNOHISTOCMEMICAL CHARACTERISTICS. S.J. Wiegand, D.J. Earnest and D.M. Gash. Dept. of Neurobiology and Anatomy, Univ. of Rochester Medical Ctr., Rochester, NY 14642.

Vasopressin-containing neurons which develop within transplanted hypothalami are not anatomically homogeneous, but exhibit a number of distinct morphological phenotypes. In addition to occasional large neurons which resemble those of the endogenous magnocellular neurosecretory system, several populations of parvicellular vasopressin-immunoreactive (VPir) neurons have been identified. Two types of parvicellular VPir neuron, in particular, are represented in significant numbers, and both types are often present within a single anterior hypothalamic graft. On the bases of their distinct cytological and connectional characteristics, and their discrete relationships to other chemically identified neuronal populations within the grafts, we have proposed that these two VPir cell types are derived from separate cell lines; one from the anlage of the suprachiasmatic nucleus (SCN) and the other from the developing the paraventricular nucleus (PVN). The present study was designed to test this hypothesis by determining if these two cytologically distinct types of VPir neuron could be harvested, independently, from the developing hypothalamus.

Donor hypothalami were obtained from normal Long Evans rat fetuses at 15-17 days post conception, and the rostral (AHr) and dorsal (AHd) divisions of the developing anterior hypothlamus were dissected free of the remainder of the hypothalamic primordium. The AHr and AHd contain the presumptive anlagen of the SCN and PVN, respectively. The AHv (n=9) or AHd (n=8) obtained from fetal donors were transplanted to the periventricular hypothalamus / third ventricle of adult, VP-deficient Brattleboro rats. Developing neocortex served as a tissue control (n=5). Host animals were sacrificed 6-12 weeks after transplantation, and alternate series of brain sections were processed for the immunohistochemical localization of VP, vasoactive intestinal polypeptide (VIP) and corticotropin-releasing factor (CRF). Viable grafts were recovered from all host animals. All AHv grafts contained VPir cells which resembled those of the endogenous SCN. These VPir neurons were organized into well defined clusters and, invariably, were associated with VIPir neurons of a similar small size (9-13μ). VPir fibers of very fine caliber ramified extensively around the cell bodies of origin and the associated population of VIPir cells, and projected to areas of the host brain that, ordinarily, are innervated by the VP neurons of the endogenous SCN. SCN-like neurons were the predominant VPir cell type in all AHv grafts, and 6 of 9 AHv grafts contained only SCN-like VPir cells. Conversely, SCN-like aggregations of VPir neurons were never identified in AHd transplants. Rather, in 7 of 8 cases, AHd transplants contained VPir neurons which resembled the parvicellular neurosecretory cells of the PVN. These neurons (or a morphologically similar companion population) also contained CRF, but were never associated with VIPir cells. PVN-like VPir neurons were slightly larger (12-18μ), less numerous and less densely packed than the SCN-type identified in AHv grafts, and they projected to vascular as well as neural targets within the graft and host brain.

These results demonstrate that discrete precursor populations of the vasopressinergic neurons of the SCN and PVN are established early in development, that these populations can be independently selected for transplantation using refined microdissection procedures, and that the principal cytological, neurochemical and connectional features of these distinct populations continue to develop in a histotypic fashhion following transplantation.

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65.8 TRANSPLANTATION OF SUBTYPES OF VASOPRESSINERGIC NEURONS INTO BRATTLEBORO RATS: SUPRACHIASMATIC GRAFTS GENERATE CIRCADIAN VASOPRESSIN RHYTHMS IN THE CEREBROSPINAL FLUID. D.J. Earnest, C.D. Sladek, D.M. Gash and S.J. Wiegand. Depts. of Neurobiology/Anatomy and Neurology, Univ. of Rochester Sch. of Med., Rochester, NY 14642

The suprachiasmatic nucleus (SCN) of the hypothalamus is an integral neural locus for the generation of circadian rhythms in mammals. Recent studies have utilized neural transplantation technology to underscore this function of the SCN. In view of the significant population of vasopressin (VP) neurons in the SCN and the role of the SCN in the generation of cerebrospinal fluid (CSF) concentrations of this peptide, the present study was conducted to determine whether transplanted SCN neurons release VP in a circadian fashion. Associated anatomical studies demonstrating that the anlagen of the SCN and the paraventricular nuclei (PVN) can be independently selected for transplantation provided a basis for examining the functional specificity of hypothalamic grafts containing these morphologically distinct types of VP neurons.

Developing dorsal and rostral hypothalamic fragments containing the anlage of the PVN or the SCN were obtained from normal Long-Evans (LE) fetuses at E15-17 and transplanted to a periventricular site in adult male VP-deficient Brattleboro hosts. Sham-operated LE rats and Brattleboro rats receiving transplants of developing neocortex were included as controls. Four-ten weeks post-transplantation, cannulae were chronically implanted into the cisternae magna. After a period of recovery, lighting conditions were changed from LD 12:12 to constant light and samples of CSF were collected from individual rats at six hour intervals for 2-5 days. VP concentration in aliquots of each CSF sample was measured by radioimmunoassay. Following CSF sampling, animals were sacrificed and the brains were processed using immunohistochemical procedures to confirm the presence of SCN- or PVN-like aggregations of VP neurons within the grafts.

The temporal profile of VP in CSF was clearly rhythmic in sham-operated LE rats, with peak levels observed during the sampling interval coinciding with the onset of the subjective day (0600h) and low values characterizing the remainder of the circadian cycle. In Brattleboro rats that received cortical grafts (n=4), VP was undetectable in all CSF samples. Measurable levels of VP were observed in all samples collected over a three-day period from a Brattleboro host transplanted with the anlage of the PVN, but no circadian variation was manifested in peptide levels. Similar to LE rats, VP concentrations in the CSF fluctuated on a circadian basis in all Brattleboro rats that received transplants of SCN anlage (n=4). However, the VP rhythms in three of these animals were not phase-coordinated with the rhythms observed in LE rats (i.e., the peaks of the individual rhythms were not coincident with the timing of the VP peak in LE rats), suggesting that the circadian function of these SCN grafts was not influenced by previous exposure to LD 12:12. In the remaining animal with an SCN graft, the peak of the VP rhythm coincided for the first 2 cycles with the onset of the subjective day and the rhythm was heavily damped thereafter.

These results demonstrate that SCN, but not PVN, grafts are capable of generating circadian VP rhythms in the CSF of Brattleboro hosts. In view of this data and the observation that the VP rhythms in 3 of the 4 animals that received SCN transplants were not influenced by lighting conditions before or during CSF sampling, it is unlikely that the host SCN plays a critical role in generating the circadian pattern expressed by grafted VP neurons. Supported by NIH Grant NS19900 (S.J.W.).

65.9 CIRCADIAN PACEMAKER (SCN) TRANSPLANTS INTO LATERAL VENTRICLES FAIL TO RESTORE LOCOMOTOR RHYTHMICITY IN ARRHYTHMIC HAMSTERS. M.E. Harrington, P.J. DeCoursey, D. Bruce, and J. Buggy. Dept. of Psychology, Dalhousie Univ., Halifax, NS, Canada, Dept. of Biology, Univ. of S.C., Columbia, SC 29208, and Dept. of Physiology, Univ. of S.C. Medical School, Columbia, SC 29208.

Ablation of the SCN in hamsters leads to locomotor arrhythmia, and our previous work demonstrated that transplantation of fetal SCN grafts to the 3rd ventricle restored circadian activity rhythms in about 50% of cases. The mechanism by which these grafts containing SCN-like neurons re-established rhythmicity is not known. For this reason, a series of SCN transplants were made in the lateral ventricles of SCN-lesioned hamsters distinctly separating the graft locus from the ablation site.

The protocol involved monitoring of locomotor activity as a behavioral indicator of circadian output. Wheel-running activity of male hamsters was recorded first in DD for 8 days; SCN were then lesioned and activity measured in DD to test for arrhythmia. Host hamsters received transplants into a lateral ventricle of a pair of SCN (1-4 µl) from 13-day old fetuses. Host activity was then recorded in DD for at least 8 weeks to detect recovery of rhythmic activity. Subsequently, animals were sacrificed and the brain prepared for immunocytochemical procedures. Using fifths of 40 µ thickness, frozen sections were stained sequentially for neuropeptide Y (NPY), neurophysin II (NP), vasoactive intestinal polypeptide (VIP), somatostatin (SS), and Nissl substance.

A compact globular graft, densely populated with neuronal cell bodies and processes, was consistently found in a lateral ventricle of each host, firmly attached to the fornix. In several instances, portions of the graft extended to the interventricular foramen as though possibly migrating towards the 3rd ventricle. Vascular connections between host and graft were apparent, but immunocytochemistry for the 4 neuropeptides provided little evidence of communicating nerve fibers between graft and brain. Clusters of cell bodies and processes containing SS, NP, and VIP, were often noted. Each graft contained at least one area of co-localized VIP and NP, indicating an SCN-like structure. In contrast to the dense NPY-staining in most of the graft, NPY fibers were rare within the VIP-NP plexus.

Lateral ventricle grafts containing SCN-like cells did not restore circadian rhythmicity. It appears likely, therefore, that the renewal of circadian rhythmicity by grafts of fetal SCN into the 3rd ventricle does not involve the release of hormone or neuromodulator into the cerebrospinal fluid or vascular circulatory systems.

Supported by NSF Grant DCB-8402104 to P.J. DeC.

65.10 A GENETIC MUTATION OF THE CIRCADIAN SYSTEM IN GOLDEN HAMSTERS. M.R. Ralph and M. Menaker, University of Virginia, Charlottesville, Virginia.

We have discovered a genetic mutation in golden hamsters which dramatically shortens the period of their circadian rhythms. The behavior of the mutation suggests that it has occurred at a single, autosomal locus, **tau**, and that the mutant allele, **tau$_s$**, is partially dominant. Animals that are heterozygous for the trait have freerunning periods (in the dark) of 22.3 ± 0.15 hours and homozygous animals have periods close to 20 hours, compared with wild-type siblings which exhibit normal periods of 24.1 ± 0.1 hr.

Entrainment is affected by the mutation. Heterozygous animals either cannot entrain to a 24 hour light/dark cycle (LD 14:10), or exhibit abnormal entrainment. For animals that are able to entrain, the onset of activity in LD 14:10 is 2-4 hours prior to the lights off transition compared with normal animals whose activity onsets normally occur within 1/2 hr. after lights off. The early onsets suggest that a large portion of the delaying region of the animals' phase response curve must be exposed to light to enable entrainment. Animals that cannot entrain to this light cycle show relative coordination. When the rhythm is not coordinated, the observed period is shorter than the freerunning period in constant dark, indicating that the system is being advanced by the light cycle. The latter observations suggest that although the freerunning period is shortened by this mutation, the basic response properties of the circadian system (the ability to delay and advance in response to light) remain intact.

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65.11 DROSOPHILA CIRCADIAN CLOCK PROBED WITH ANTIBODIES TO THE period GENE PRODUCT. Kathleen K. Siwicki*, Michael Rosbash*, and Jeffrey C. Hall, Biology Dept., Brandeis University, Waltham, MA 02254

The period (per) gene of Drosophila melanogaster influences behavioral rhythms. The normal 24 hour period of circadian activity rhythms is shortened to 18-20 hours in per^S flies and lengthened to 28-30 hours in per^L flies, whereas per^O flies are arrhythmic (Konopka & Benzer, 1971, PNAS 68:2112). Also, a rhythm in the male's courtship song, with a period of about 1 minute, is similarly disrupted by the per mutations (Kyriacou & Hall, 1980, PNAS 77:6929). This gene encodes a major 4.5 kb transcript, which is expressed in late embryos and adult flies. The sites of embryonic expression have been localized by in situ hybridization, revealing a metameric pattern of expression in the developing central nervous system (James et al., 1986, EMBO J 5:2313).

To investigate the biochemical properties of the per protein and the cellular sites where it acts to regulate behavioral rhythms, we have developed specific antibodies to the protein. From the amino acid sequence predicted by the genomic and cDNA sequences, we synthesized four peptides (12-14 amino acids each). Rabbits were immunized with the peptides crosslinked to thyroglobulin, then anti-peptide antibodies were affinity purified from immune sera.

We define specific binding to the endogenous per protein as immunoreactivity that is present in wild type and absent in per$^-$ flies, that are deleted of this genetic locus. By this criterion, antibodies to two of the peptides react with the per gene product. In sections of adult heads they label bilaterally about 12 neuronal somata between the protocerebral neuropil and the medulla of the optic lobes. These cells are reliably stained, during both the day and the night, in wild type flies entrained by light:dark cycles. Additional staining at the distal ends of the photoreceptors and in a band across the lamina ganglionaris is clear in the middle of the night but is undetectable in the middle of the day. Since the levels of the 4.5 kb transcript are relatively constant (Reddy et al., 1984, Cell 38:701), this difference in the per protein between day and night flies may reflect diurnal fluctuations in post-translational processes (e.g., covalent modifications or subcellular distribution) that affect the accessibility of the peptide epitope or the local concentration of the protein. Whether such changes or even these sites of per expression are involved in its regulation of rhythmic behaviors can now be addressed by using the anti-per antibodies to study the several kinds of genetic variants that lead to abnormal circadian rhythms. (Supported by NS-07873 to K.K.S. and GM-33205 to M.R. and J.C.H.)

65.12 EFFECTS OF DEUTERIUM OXIDE ON ACTIVITY RHYTHMS OF WILD TYPE AND PERIOD MUTANT DROSOPHILA. R.J. Konopka and D.J. Robinson*. Dept. of Biology, Clarkson Univ., Potsdam, NY 13676.

Mutations at the period locus of Drosophila melanogaster alter the period of the circadian locomotor activity rhythm, which is 24 hours in wild type flies. The per^{L1} and per^{L2} mutations increase period length to 29 hours at 24° C., while the per^S mutation shortens the period to 19 hours. The alterations in period induced by deuterium oxide in wild type and period mutant flies were measured at concentrations of 15%, 25%, 35%, and 50%. In all cases, deuterium oxide lengthened the periods of the activity rhythms. This result is in contrast to the effects of temperature on the per mutants; increasing temperature lengthens the period of per^{L1} and per^{L2} rhythms but decreases the period of per^S rhythms. The rhythm of per^{L1} flies was lengthened to a significantly greater extent than the rhythm of per^{L2} flies at concentrations of 15%, 25%, and 35%. The effects of deuterium oxide on the activity rhythms produced by two fragments of cloned wild type DNA from the per locus (8.0 kb and 12.8 kb) were also determined. For each of the two fragments, a lengthening of the period of the activity rhythm by deuterium oxide was observed. However, the characteristics of the lengthening response were different for the two cloned fragments.

66.1 INCREASE OF SUBSTANCE P, MET-ENKEPHALIN AND OTHER NEUROPEPTIDES IN NERVE CELL BODIES AND FIBERS IN A CASE OF DEMENTIA WITH INTENSE ATROPHY OF THE CAUDATO-PUTAMEN OF HUNTINGTON'S TYPE BUT WITHOUT CHOREA. S. Schiffmann* and J.-J. Vanderhaeghen (SPON: M. Meulders). Laboratories of Neuropathology and Neuropeptide Research and of Pathology and Electron Microscopy, Brugmann and Erasme Hospitals, Université Libre de Bruxelles, 808 route de Lennik, B-1070, Brussels, Belgium.

The brain of a 54-yr old man suffering from dementia, with rigidity but without choreoathetosis or familial history of Huntington's disease, was obtained at autopsy and compared to a control brain processed similarly. A severe bilateral atrophy of the caudate and the putamen as in Huntington's disease was present but no lesions characteristic of Pick's or Alzheimer's disease were observed in the sections taken from the cortex including the hippocampal formation, the basal ganglia and the brain stem.

CCK, SP, Met- Leu- and Syn-Enk, NPY, NT and glial fibrillary acidic protein were visualized by immunohistochemistry (PAP Sternberger technique) on thick 40 micrometer cryostat sections from 4% paraformaldehyde fixed tissue. In the caudate and the putamen, a heavy gliosis was present. SP, Met-, Leu-, Syn-Enk nerve cell bodies of large size (25-35 microns) and fibers were more numerous, more intensely stained, showing a dorso ventral gradient of staining, in some places distributed in a patchy pattern (striosomes). No CCK or NT nerve cell bodies, but a diffuse network of thin CCK and NT nerve fibers was easily detectable. A minimal augmentation of the number of the diffusely distributed NPY nerve cell bodies and a moderate augmentation of NPY nerve fibers was observed. There was no change in the pattern of SP wooly fibers in the internal globus pallidum and of Met-, Leu-, Syn-Enk wooly fibers in the external globus pallidus. Using radioimmunoassays with iodinated I125 substance P and CCK-8 (Amersham) an increase of SP but without change in CCK-8 was observed in the caudate and in the putamen. These results are in contrast with the diminution of immunoreactivity for SP and met-enkephalin reported in the striatum and globus pallidus of Huntington's disease both by radioimmunoassay (Emson P.C. et al. Brain Res., 199, 147, 1980) and immunohistochemistry (Ferrante R.J. et al., Neurosci. Lett. 71, 283, 1986). Shrinkage may partially have caused the elevation of neuropeptide levels observed by radioimmunoassay or some of the immunohistochemically observed augmentation of nerve cell bodies and fibers but cannot be responsible for the increase of staining. This increase of staining reveals higher concentration of positive neurons than in the normal brain. This may reflect an increase of synthesis for neuropeptides, especially substance P and Met-enkephalins, in some remaining neurons of the severely atrophied caudate putamen. Such increase may explain the surprising absence of choreoathetosis despite the severe caudate-putamen atrophy.

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66.2 CHOLECYSTOKININ AND VASOACTIVE INTESTINAL POLYPEPTIDE IN THE STRIATUM OF PATHOLOGICALLY GRADED CASES OF HUNTINGTON'S DISEASE. M.F. Mazurek, M.F. Beal, S.F. Knowlton*, D.W. Ellison*, E.D. Bird*, J.B. Martin, (SPON: G.M. Brown). McMaster Univ. Med. Ctr., Hamilton, ONT. and Mass. General Hosp., Boston, MA.

In Huntington's disease (HD) the striatum undergoes extensive atrophy. The brunt of this degeneration is borne by spiny projection neurons in the striatal matrix, aspiny neurons and afferent terminals being relatively spared. One might therefore expect transmitters localized to these latter elements to be elevated in concentration in HD, as the surviving neuronal elements increase in prominence in relation to the surrounding tissue matrix. Both cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) are found in striatum in subpopulations of aspiny intrinsic neurons and afferent terminals, the latter accounting for most of the immunoreactivity in each case. We studied levels of CCK and VIP in postmortem brain tissue dissected from 12 controls and 24 patients with HD, as defined by the family history, clinical presentation and neuropathological findings. The HD cases were subdivided into those with mild-to-moderate striatal atrophy (Grades I and II by the criteria of Von Sattel et al) and those with severe atrophy of the striatum (Grades III and IV). Concentrations of VIP were increased 3- to 4-fold over controls in both caudate and putamen in HD. These increases were completely unrelated to the degree of tissue atrophy, as values in the Grades I-II cases were no different from those observed in Grades III-IV. Levels of somatostatin and neuropeptide Y (NPY), which are colocalized in aspiny neurons and afferent terminals, were likewise elevated 2- to 4-fold in HD striatum, with no differences between mild-to-moderate and severe cases. CCK levels, by contrast, were normal in HD striatum, except for the caudate nucleus of severe cases, where a significant decrease was found.

These results indicate that the CCK- and VIP-containing neuronal systems are differentially affected in HD striatum. They further show that the increased striatal concentrations of VIP, somatostatin and NPY found in HD brain are not simply a reflection of tissue atrophy, since levels of all 3 peptides were increased as much in Grade I and II cases, many of which have relatively little striatal atrophy, as in the more severely affected Grade III and IV cases.

66.3 LOSS OF DOPAMINE D1 AND PRESERVATION OF D2 RECEPTORS IN HUNTINGTON'S CHOREA STRIATUM, RELATIONSHIP TO THE STRIOSOMAL ORGANIZATION OF THE STRIATUM. J.N. Joyce and A. Winokur. Dept. Pharmacology and Psychiatry, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

The hyperkinetic motor and cognitive disorders of the subcortical type associated with Huntington's disease (H.D.) has been thought to be caused by an overactivity of the dopamine system innervating the striatum. The difficulty with that hypothesis is that dopamine (DA) receptors are reduced in H.D. (Reisine et al. 1977; Bzowej and Seeman, 1986) suggesting that the effector system for H.D. is actually reduced. Recent studies have indicated that in H.D. the loss of neurons from the striatum is not homogeneous, a preservation of the aspiny interneurons occurs with a corresponding depletion of the spiny output neurons (Kowall et al, 1987). Moreover, the "striosomal" (patch)/matrix organization of the striatum is preserved, as evidenced by the sparing of the AChE and NADPH-diaphorase staining patterns (Kowall et al 1987; Feigenbaum et al, 1986). Since, the organization of the pre- and postsynpatic components of the DA system appears to obey the "striosomal" (patch) and matrix organization of striatum (Joyce et al, PNAS 83:8002, 1986; Joyce et al, Soc. Neurosci. Abstr., 1986), we decided to investigate whether in H.D. selective aspects of this system was changed.

Tissue from two H.D. patients (grade 3/4; 66 yr age, female) were obtained from the Brain Tissue Resource Center of McLean Hospital. Age-matched controls (CO) were obtained at autopsy (P.M.I < 20 hr) from the Hosptial of the University of Pennsylvania. The tissue was sectioned and processed for autoradiography as described previously. The CO striatum showed a correspondence betweeen D2 receptor density ([3H]spiroperidol), AChE patterning and [3H]mazindol binding to DA uptake sites in serial sections. The AChE-rich matrix being rich in D2 receptors and DA terminals The distribution of D1 receptors ([3H]SCH 23390) was also patchy and may be related to AChE-poor zones within the AChE-rich matrix. The H.D. striata showed a profound loss of D1 sites (90%) except in the nucleus accumbens (50% loss), where the patchy organization was still apparent. The D2 sites were reduced only 25 - 30%, although the area of the D2-rich matrix (and AChE-rich matrix) was reduced significantly more than the D2-poor regions. The density of DA terminals appeared to be unaffected in H.D. This suggests that DA input to the D2 receptors is intact in H.D and is unopposed by the D1 receptors because of their more selective loss. It is also consistent with the hypothesis that D2 receptors are located on the aspiny interneurons whereas D1 receptors are located on the spiny output neurons. This may provide a rationale for understanding the hyperkinetic syndrome of H.D.

(USPHS GM 34781)

66.4 CO-LOCALIZATION OF CHOLINE ACETYLTRANSFERASE-AND ACETYLCHOLINESTERASE-CONTAINING NEURONS IN HUNTINGTON'S DISEASE STRIATUM. R.J. Ferrante[1,1], N.W. Kowall[1], L.B. Hersh[2], G. Bruce[2], E.P. Richardson, Jr.[1], Mass. Gen. Hosp., Harvard Med. Sch., Boston, MA[1] and Department of Biochemistry, UTHSC, Dallas TX.[2]

We have previously reported that large aspiny acetylcholinesterase (AChE)-containing striatal neurons are relatively preserved in Huntington's disease (HD)(Ferrante, Brain Res., 1987;411:162-166). There is strong evidence that in experimental animals AChE and choline acetyltransferase (ChAT) co-localize within the same striatal neurons. In HD striatum, biochemical measurements of ChAT are decreased, despite preservation of AChE neurons. In order to resolve the differences between these findings, we examined 50 μm sections of striatal tissue from 10 HD patients and 6 normal controls; the sections were stained immunocytochemically with an antiserum against ChAT, and enzyme-histochemically for AChE, using the Hardy-Heimer method with which AChE is shown as black granular deposits (Hardy, Neurosci. Lett.,1976;3:1-5).

In both controls and HD, ChAT immunoreactivity was present in large multipolar neurons (controls: 37X27 μm, HD: 34X22 μm) uniformly distributed throughout the striatum. In all cases of HD, there was a striking persistence of ChAT neurons in striatal sections; ChAT cell densities were significantly increased in the caudate nucleus (667±32/cm2 vs. 215±13/cm2, $p<0.005$) and putamen (779±34/cm2 vs. 225±22/cm2, $p<0.001$) as compared to controls. In the neuropil, dense ChAT activity was confined to the striatal matrix zone with less activity in the patch compartments. Combined staining for ChAT and AChE within the same sections demonstrated nearly exact co-localization of each reaction product in large striatal neurons. The AChE reaction contrasted black granules with the brown colored end-product of the development of peroxidase with diaminobenzidine, delineating ChAT activity. In both HD and controls, less than 4% of the total population of large neurons in the head of the caudate nucleus contained AChE alone, and less than 2% of the cells were positive for ChAT activity alone.

Our findings confirm the persistence of large cholinergic neurons in HD striatum and extend the co-localization of AChE and ChAT to the human striatum. Reduced striatal ChAT activity in HD is not a consequence of loss of ChAT neurons, but perhaps the result of degeneration of highly collateralized axons and terminals of these cells. This is reflected in the differential reduction of the matrix compartment reported in HD (Ferrante, JNEN,1987,46:12-27).

66.5 THE QUINOLINIC ACID-SYNTHESIZING ENZYME IS INCREASED IN THE BRAINS OF HUNTINGTON'S DISEASE VICTIMS. R. Schwarcz[1], E.D. Bird[2] and W.O. Whetsell, Jr.[3]. [1]Md. Psych. Research Center, Baltimore, MD 21228; [2]McLean Hosp., Belmont, MA 02178 and [3]Dept. Pathology, Vanderbilt Univ., Nashville, TN 37232.

Intrastriatal injection of the endogenous excitotoxin quinolinic acid (QUIN) in the rat results in the degeneration of the majority of local neurons. The neurochemical and neuropathological features of the QUIN-lesioned striatum are virtually indistinguishable from those of the caudate nucleus/putamen complex of Huntington's disease (HD) patients. The enzymes responsible for the immediate biosynthesis (3-hydroxyanthranilic acid oxygenase; 3HAO) and degradation (quinolinic acid phosphoribosyltransferase; QPRT) of QUIN have been identified and partly characterized in human brain. K_m values for the substrates of 3HAO and QPRT are in the low micromolar range, compatible with the brain tissue concentration of QUIN. The V_{max} value for 3HAO, however, is far higher than that for QPRT, indicating stringent in vivo regulation of QUIN's biosynthetic enzyme.

As compared to controls (N=24), which were closely matched for age and post mortem interval (means: 5.5 hours for controls and 7.2 hours for HD patients), 3HAO activity in HD brains (N=25) was found to be elevated in ten of the eleven brain regions studied. No changes in QPRT activity were detected. Increases in 3HAO activity were particularly pronounced in the striatum (+ 370% in the caudate nucleus, $p<0.00001$; + 360% in the putamen, $p<0.0005$), which are known to exhibit the most prominent nerve cell loss in HD. Statistically significant (two tailed student's t-test) increments were also observed in the globus pallidus, substantia nigra, cerebellum, thalamus, hypothalamus, hippocampus and the frontal and parietal cortices but not in the medulla. Kinetic analyses of caudate tissues revealed a large increase in the V_{max} value for 3HAO (from 6.8 nmol/h/mg protein in controls to 31.3 nmol/h/mg protein in HD) and a smaller change in K_m-values (from 1.11 µM in controls to 2.86 µM in HD).

The results indicate that 3HAO in the human brain is predominantly localized in non-neuronal cells and that the increase in its activity in HD may at least in part be a reflection of the astrogliosis known to occur in diseased tissue. However, since significant increments were also noted in brain regions with little documented neuronal loss in HD, for example the hippocampus, yet unrecognized mechanisms of 3HAO regulation might also contribute to the enhanced ability of HD brains to produce QUIN. This finding may be of relevance for clinical, neuropathological and biochemical features associated with HD.

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66.6 ISOCORTICAL SUBSTANCE P NEURONS ARE DEPLETED IN ALZHEIMER'S DISEASE. B.J. Quigley Jr.* and N.W. Kowall (SPON:J. Nathanson) Neurology Service, Massachusetts General Hospital, Boston MA 02114.

Substance P-like immunoreactivity(SPLI) measured by radioimmunoassay is reduced in several cortical regions in Alzheimer's disease. (AD). We studied the morphology and distribution of SPLI elements in 6 cortical regions of 3 patients with confirmed AD and 3 normal controls using a monoclonal anti-substance P antibody (Sera Labs.)

In the dentate gyrus, dense terminal staining was seen in the molecular layer, supragranular plexus, and infragranular region. Granule neurons and a band deep in the molecular layer were devoid of staining. Multipolar SPLI neurons were observed throughout the CA fields of hippocampus, and were especially prominent in CA4. Bands of SPLI terminal staining were seen in stratum oriens, radiatum, and lacunosum-moleculare. SPLI fibers were seen in the most superficial and deepest layers of the subiculum that continued into the entorhinal area where SPLI terminals formed clusters in layer II and a continuous band in layer IV. Reactive neurons were not seen in the subiculum but were frequent in the deep layer of entorhinal cortex and infracortical white matter. SPLI perikarya and beaded fibers were found in all isocortical regions examined (Brodmann areas 39,40; 21,22; 10,11; 4,3,1,2; and 17). The greatest density of SPLI perikarya was in areas 3,1,2, and 39,40, while the lowest density was in area 17. In all regions most(78%) SPLI perikarya were located in deep cortical layers and infracortical white matter. Cortical SPLI perikarya were pleomorphic and multipolar with a spherical dendritic field. Infracortical neurons were more often bipolar with tangentially oriented dendritic fields. In all areas except 39,40 SPLI fiber density was greatest in layer I-II. Aggregates of fine, beaded, SPLI fibers were scattered in all cortical layers. The pattern of SPLI was unchanged in the hippocampal formation of AD patients. SPLI fibers were occasionally distorted in association with thioflavine S positive senile plaque cores in CA4. SPLI neurons in the CA fields were normal. Isocortical SPLI neurons, however, were drastically reduced in number in all regions and were often weakly stained, and disfigured with extensive dendritic pruning. SPLI fibers were irregular and formed coarse aggregates distributed in all layers. Intense SPLI globular deposits, at times associated with amyloid plaque cores, were seen in superficial cortical layers. To further assess the biochemical characteristics of SPLI neurons, sections were double stained for NADPH diaphorase and SPLI. All isocortical SPLI neurons contained NADPH diaphorase activity whereas the majority of SPLI hippocampal neurons were diaphorase negative. Previous studies have shown that, unlike SPLI neurons, the density of somatostatin neurons which also contain NADPH diaphorase, is unchanged in AD cortex. SPLI-NADPH diaphorase positive neurons may therefore represent a small subset of diaphorase neurons that degenerate in cerebral isocortex.

66.7 GLUTAMATERGIC NEURONS IN THE HIPPOCAMPUS ARE MORPHOLOGICALLY ABNORMAL AND DEVELOP NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE. N.W. Kowall K.S. Kosik*and M.F. Beal, Neurology Service Mass. General Hosp , and Center for Neurological Diseases, Brigham and Womens Hosp. , Boston MA 02114.

Although neurofibrillary tangle(NFT) formation is a major pathological feature of Alzheimer's disease(AD), the neurotransmitter content of NFT-bearing neurons has not been well defined. We studied the hippocampal formation of 4 patients with pathologically verified AD and 3 controls with a monoclonal antibody raised against glutamyl-glutamate(INCstar Inc.) and a polyclonal anti-glutaminase(GLN) antiserum. Preabsorption with the target antigen eliminated specific staining of both antisera. GLN specificity was further confirmed by solid phase immunoassay.

In the normal hippocampus, prominent glutamate(GLU) immunoreactive dendritic bundles arising from CA field and subicular pyramidal neurons were prominently stained. Dentate granule cells were moderately immunoreactive. Terminal staining was evident in the molecular layer of the dentate gyrus and stratum lacunosum-moleculare of CA3-1. Mossy fibers were moderately GLU reactive. Pyramidal neurons in the CA fields and subiculum, and dentate granule cells were GLN reactive. Fiber staining was not well seen with this antiserum.

In the AD material, the staining pattern of the dentate granule cells was unchanged. The terminal field of the GLU immunoreactive fibers in the molecular layer of the dentate gyrus was reduced while stratum lacunosum-moleculare staining was unchanged. GLU immunoreactive CA field pyramids were morphologically abnormal with irregular shortened and disorganized dendritic fields. Scattered abnormal GLU reactive neurites in the molecular layer of dentate gyrus and CA fields were associated with thioflavine S fluorescent amyloid cores.

GLU reactive neurons were examined for the presence of tau reactive NFT using double indirect immuno-fluorescence with either monoclonal or polyclonal tau antisera and either anti-GLN or anti-GLU respectively. Fluorescein and rhodamine conjugated second antibodies were used to label mouse monoclonal and rabbit polyclonal antibodies respectively. NFT were often located in GLU or GLN immunoreactive neurons. Occasional NFT were not associated with GLU reactive neurons and similarly many GLU neurons did not contain NFT.

These studies show that glutamatergic pyramidal neurons in allocortex frequently show morphological abnormalities and contain NFT's in AD. Not all glutamatergic neurons, however, are affected. The pyramidal neurons of the CA fields and entorhinal cortex are preferentially involved while dentate granule cells are spared.

66.8 FLUVOXAMINE IN OCD: ANTIOBSESSIONAL OR ANTIDEPRESSANT? W.K. Goodman, D.S. Charney, L.H. Price, P.L. Delgado, S.A. Rasmussen and G.R. Heninger. Clinical Neuroscience Research Unit, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508, and Brown University School of Medicine, Department of Psychiatry, Providence RI 02906.

The hypothesis that the pathophysiology of Obsessive Compulsive Disorder (OCD) is related to abnormal serotonin (5-HT) function is largely based on treatment response to the potent, but nonselective, 5-HT uptake inhibitor, clomipramine. To examine whether the serotonergic properties of a drug may confer antiobsessional efficacy, the potent and selective 5-HT reuptake inhibitor, fluvoxamine, was studied in depressed and nondepressed OCD patients. METHODS: 42 outpatients with a primary diagnosis of OCD gave informed consent and entered double-blind treatment with either fluvoxamine or placebo for 6 to 8 weeks. Approximately one-half the patients met criteria for major depression. Patients were assessed weekly for symptoms of anxiety, depression, and OCD using standard rating scales. OCD symptom severity was also rated using the Yale-Brown Obsessive Compulsive Scale (Y-BOCS). RESULTS: Fluvoxamine was significantly better than placebo on all measures of OCD and depression. There were 9/21 responders (= "much improved" on CGI) on fluvoxamine compared to 0/21 responders on placebo ($p<.001$, Fisher Exact Test). Y-BOCS scores decreased by 42% in responders but no patient was entirely free of symptoms. OCD response was not related to baseline depression and 6/9 responders were nondepressed. In some cases there was improvement in depression but no change in OCD. During subsequent open treatment with fluvoxamine in patients originally assigned placebo 12/20 were OCD responders. CONCLUSION: Fluvoxamine is effective in the treatment of OCD. The rate and degree of treatment response seems equivalent to published studies of clomipramine in OCD. Its antiobsessional effects do not seem to be dependent on the presence of depression. These data are consistent with the 5-HT hypothesis of OCD, however, since the magnitude of the response is modest, possible involvement of other neurochemical systems (e.g., dopaminergic) needs to be examined.

66.9 PLATELET ADENYLATE CYCLASE ACTIVITY IN PANIC DISORDER D.S. Charney, R.S. Duman, R.B. Innis, S.W. Woods, J.F. Tallman and G.R. Heninger. Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Several lines of evidence suggest that some Panic Disorder patients (PD) exhibit abnormal regulation of noradrenergic activity. These abnormalities include potentiated behavioral, biochemical, and cardiovascular responses to the alpha-2 receptor antagonist yohimbine and alpha-2 receptor agonist clonidine (Charney et al. Arch Gen Psych 1986;43:1042-1054). The molecular mechanism of the noradrenergic dysfunction is not known. It has been hypothesized that abnormalities beyond the alpha-2 receptor recognition site at the level of intracellular effector systems may be involved. In the present study, the platelet was used as a model to investigate the regulation of adenylate cyclase activity in PD patients. METHODS: 39 PD patients and 30 healthy subjects were studied. The effect of epinephrine (300 uM), PGE (30 uM), and NaF (10 mM) on adenylate cyclase activity was evaluated. Platelet adenylate cyclase was determined by method of Jacobs et al. (J Biol Chem 1982;257:2829-2833), and protein by Smith et al. (Anal Biochem 1985;150:76-85). Results are expressed as picomoles of ^{32}P-cyclic AMP formed per mg protein/minute. RESULTS: Basal adenylate cyclase activity was significantly reduced in PD patients (9.9 vs 11.4, $p<.05$). Epinephrine inhibition of adenylate cyclase activity was the same in both groups (68% PD, 69% healthy subjects). In PD, PGE_1 and NaF stimulated adenylate cyclase activity respectively, was decreased compared to healthy subjects (281 vs 314, $p<.02$; 61 vs 78, $p<.001$). IMPLICATION: The similar responses of adenylate cyclase activity to inhibition by epinephrine in PD and healthy subjects indicate that alpha-2 receptor regulation of adenylate cyclase activity may not be abnormal in PD. However, the decreased basal adenylate cyclase activity and blunted responses to PGE_1 and NaF in PD patients suggest possible abnormalities at the level of guanine nucleotide-binding regulatory protein or the catalytic subunit. Any relationship of these platelet findings to noradrenergic neuronal dysfunction in PD or brain intracellular effector function remains to be identified.

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66.10 QUANTITATIVE AUTORADIOGRAPHY DEMONSTRATES INCREASED 5-HT_2 RECEPTORS IN THE FRONTAL CORTEX OF SUICIDE VICTIMS. *V. Arango, P. Ernsberger, H. Tierney*, M. Stanley, D.J. Reis and J.J. Mann.* Div. of Neurobiology and Lab. of Psychopharmacology, Cornell Univ. Med. Coll., New York, NY 10021.

In homogenates of the frontal cortex of suicide victims there is a 28% increase in the number of serotonin$_2$ (5-HT_2), but not serotonin$_1$ (5-HT_1), binding sites (Mann et al., *Arch. Gen. Psych. 43*:954-9, 1986). Since there are reduced levels of the 5-HT metabolite 5-HIAA, the results may represent postsynaptic receptor up-regulation secondary to reduced presynaptic serotonergic activity. Using quantitative autoradiography we sought to determine whether the increased receptor number in frontal cortex was localized to specific cortical layers.

Studies were carried out on Brodmann area 9 of the prefrontal cortex (PFC) in brains of suicide victims (N=4) and controls (N=4) matched for postmortem delay and age. Brains were obtained from the NYC ME's office and had a postmortem delay < 18h. Each PFC sample was divided in two: one sample was homogenized to study membrane binding by conventional methods and the other was sectioned for autoradiography. Membranes (P_2 fraction, N=8 subjects) were used to generate ^{125}I-LSD saturation curves (see Mann et al., 1986). For autoradiographic studies (N=8) 15 µm sections were mounted on gelatin-subbed glass slides. The slides were incubated in Tris-maleic acid buffer (pH 7.4) containing 2nM ^{125}I-LSD for 90 min. Nonspecific binding was determined in parallel incubations of adjacent sections in the presence of 5µM ketanserin (to block 5-HT_2 sites) or 10µM 5-HT (to block 5-HT_1 sites). After exposure to Ultrofilm (9-15h) with ^{3}H standards calibrated for ^{125}I, autoradiograms were quantified using computer-assisted image analysis. Specific binding was obtained by subtracting the image of a section displaying non-specific binding from the image of an adjacent section displaying total binding.

In membranes, 5-HT_2 binding sites were increased 39% in the suicide group, while 5-HT_1 sites were unchanged. Integrated measurements of the cortical layers in autoradiograms indicated that mean 5-HT_2, but not 5-HT_1 binding was also increased over controls by 48% (2-way ANOVA by layer and group; $p<0.05$). ^{125}I-LSD binding measured in autoradiograms and in membrane preparations from the same region were highly correlated ($r=0.92$, $p<0.01$). In cortex of both groups 5-HT_1 and 5-HT_2 receptor binding differed between cortical layers: intermediate>outer>inner (2-way ANOVA: $p<0.01$). The increase of 5-HT_2 receptors in the brains of suicides differed between cortical layers (ANOVA, $p<0.01$). The greatest increase was in the outer layers (I & II) which had an increase of 53 ± 19%. The smallest increase was found in the inner layers (V & VI, 25 ± 18%). The intermediate layers, where most of the receptors were found in both groups, showed an increase of 46 ± 25%. There was no difference in the binding to white matter between the suicide (17 ± 6 fmol/mg) and the control (16 ± 6 fmol/mg) groups.

We conclude that a) 5-HT_1 and 5-HT_2 receptors in the PFC are differentially distributed across all cortical layers, b) 5-HT_2 but not 5-HT_1 receptors are increased in gray matter of area 9 of suicide victims, and c) while this increase occurs across the cortex it is greatest in the outer layers.

66.11 IN VIVO MEASUREMENT OF D2 DOPAMINE RECEPTOR ABNORMALITIES IN DRUG NAIVE AND DRUG FREE MANIC-DEPRESSIVE PATIENTS. D.F. Wong*, G. Pearlson, C. Ross, L. Tune*, V. Villemagne*, R.F. Dannals*, J. Links*, H. Ravert*, A. Wilson*, M. Kuhar, H.N. Wagner, Jr.*, A. Gjedde*+ (SPON: W. Jankel). Johns Hopkins Med. Inst., Baltimore, MD 21205; + Montreal Neuro. Inst., Montreal, Canada H3A 2B4.

Increases in caudate D2 dopamine receptor numbers (Bmax) have been found in schizophrenics, but it is unknown if these are specific to schizophrenia or would be seen in any psychotic major mental illness (Wong, D.F., et al. Science, 234:1558-1563, 1986). Furthermore there is evidence that dopamine may be involved in affective illness, since dopamine blocking agents are effective anti-manics, and acute administration of dopamine agonists can cause a hypomanic like state. We therefore measured D2 dopamine receptors in symptomatic patients with major affective disorders (diagnosed according to DSM III) and age matched controls, using ([11C]-N-methyl)spiperone (NMSP), and 2 PET scans, with and without a blocking dose of haloperidol. Six were drug naive and three were drug free for more than one year. We calculated Bmax using a four compartment model (Wong, D.F., et al. J. Cereb. Blood Flow and Metab., 6:137-146 and 6:147-153, 1986). Clinically, the hallucinations, delusions and thought disorder ("psychosis") were quantified using a modified version of the Present State Examination ("mini-PSE"), which yielded an overall psychopathology score.

Bmax values for patients were as follows: (initials, age, sex, diagnosis, mini-PSE score, Bmax in pmoles/g): R.M. 20 yr M, psychotic manic, 37, 45; C.W. 25 yr F, psychotic manic 41, 38; E.W. 40 yr F, psychotic mixed, 47, 28; F.A. 52 yr M non-psychotic hypomanic, 12, 11; L.A 68 yr M, psychotic depressed, 17, 30; J.C. 54 yr M, psychotic manic, --, 12; F.K. 39 yr M, non-psychotic depressed, 9, 11; L.P. 43 yr M, psychotic manic, 13, 27; MD 48 yr F, non psychotic depressed vs. personality disorder, --, 13. Values for controls showed an age related decrease from approximately 20 pmole/g at age 20 yrs to less than 10 pmole/g at age 60 yrs.

Our preliminary conclusions are: 1. Patients with affective disorders overall had higher Bmax values than controls. 2. Patients with psychosis, regardless of whether they were depressed or manic, had higher Bmax values than those who were not psychotic. 3. There was a trend for higher values on the mini-PSE to be associated with higher values of Bmax. These preliminary data suggest that Bmax elevation may not be limited to schizophrenia, but may be present in psychosis of affective disorder as well.

66.12 REDUCED CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTOR NUMBER IN FRONTAL CORTEX OF SUICIDE VICTIMS. C.B. Nemeroff, M.J. Owens, M. Stanley, A. Andorn* and G. Bissette. Depts. Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710; Dept. Psychiat., Columbia Univ., New York, NY 10032 and Clev. Metro. Gen. Hosp., Cleveland, OH 44109.

Corticotropin-releasing factor (CRF), the hypothalamic releasing hormone which regulates the synthesis and secretion of ACTH and other POMC products from the anterior pituitary, also apparently acts as a neurotransmitter in higher CNS centers. There is evidence to show that CRF neuronal activity or integrity is altered in major depression, anorexia nervosa, and Alzheimer's disease. Our two studies (Nemeroff et al., Science 226:1342, 1984 and Banki et al., Amer. J. Psychiat., in press) demonstrating increased CSF CRF concentrations in depressed patients, taken together with a plethora of behavioral data, have led our group to hypothesize that depressed patients hypersecrete CRF. If this is the case, then one would expect to find a decrease (down-regulation) in the density of CRF binding sites in the brains of depressed individuals. In the present study, we measured the number (Bmax) and affinity (Kd) of CRF receptors in the frontal cortex of suicide victims (n=26) and age- and sex-matched controls (n=29).

Approximately 150 mg of tissue was homogenized in cold isotonic sucrose. After low speed and high speed centrifugations, the resulting pellet was resuspended in a Tris-$MgCl_2$-EDTA buffer. Of this suspension, 100 µl was incubated in triplicate with 100 µl of buffer containing 30,000-35,000 cpm of [^{125}I]-Tyr^0-ovine CRF and 100 µl of buffer containing varying amounts of unlabeled r/hCRF in a microcentrifuge tube. After a two hour incubation at 22°C, the tubes were centrifuged. The resulting supernatant was aspirated and the pellet washed with phosphate-buffered saline. The tubes were centrifuged again and aspirated. The remaining pellets were counted for two minutes in an LKB Rackgamma counter. Scatchard analysis revealed no difference between the affinity (Kd) of the radioligand in the two groups. However, a significant decrease (23%, $p=0.020$) in CRF receptor density (Bmax) was found in the suicide group. No significant correlations were found between the Bmax for CRF and sex, age or post-mortem delay.

These findings are concordant with the hypothesis that CRF is hypersecreted in suicide victims as evidenced by a decrease (down-regulation) in the number of CRF binding sites. These findings, taken together with the CSF studies cited above, the studies of Gold and his colleagues demonstrating a blunted ACTH response to CRF, and the preclinical reports of behavioral effects of centrally administered CRF in laboratory animals, are all concordant with the hypothesis that CRF is hypersecreted in endogenous depression. (Supported by NIMH MH-42088, MH-39415, MH-40524, MH-40159, MH-41847 and NIA AG-05128.)

67.1 Behavioural Tolerance Following Continuous Infusions of a Dopamine D-2 Agonist is Reversed by Either Stress or a D-1 Agonist. M.T. Martin-Iverson, S.M. Stahl and S.D. Iversen* Merck Sharp and Dohme Neuroscience Research Centre, Harlow, Essex, UK CM20 2QR

We have previously observed that rats given continuous infusions via Alzet Osmotic Minipumps of a selective dopamine (DA) D-2 agonist, [+]-4-propyl-9-hydroxynaphthoxazine (PHNO) developed complete tolerance to the motor stimulation effects of this drug, but only during the light cycle (Martin-Iverson et al., submitted). Activity induced by PHNO does not develop tolerance during dark periods of a 12 h light-dark cycle. In the present experiments, we investigated possible mechanisms underlying the differential development of tolerance, depending upon the light-dark cycle.

Male Sprague-Dawley rats (300-400g, 6 per group) were implanted subcutaneously with minipumps infusing either PHNO (5µg/h) or vehicle (0.5µl/h) while under ether anaesthesia. Locomotor activity was assessed hourly throughout the duration of the infusions (12-14 days), by recording interruptions of photobeam assemblies in the rats' home cages. Room lights were on each day from 09.00-21.00, and the rats had continuous access to food and water. Additional treatments, consisting of either continuous infusions of the DA D-1 antagonist (20µg/h, sc) or single daytime injections of the D-1 agonist SKF 38393 (6mg/kg i.p.) for 4 successive days beginning on Day 7 of PHNO infusion, were given to some groups. All groups were subjected to a mild stress on some days, which consisted of disturbance associated with changing of food, water and cage litter, lasting about 20 minutes. Activity for the 2h during and after this disturbance was compared to the same time period on days without disturbance.

Vehicle-infused rats exhibited higher levels of activity during the night and after disturbance than during the non-disturbed daytime. Infusions of the D-1 antagonist attenuated nocturnal activity, but had no effect on daytime or daytime stress-induced activity. Daytime injections of the D-1 agonist had no significant effect on activity. Rats receiving infusions of PHNO initially displayed high levels of activity, which exhibited complete tolerance by day 4, but only during the day. Daytime tolerance was reversed for the 2h during and following disturbance. The D-1 antagonist blocked initial daytime activity, stress-induced reversal of tolerance, and attenuated nocturnal motor stimulation by PHNO. The D-1 agonist reversed daytime tolerance to PHNO. These data suggest that tolerance to a selective DA D-2 agonist is mediated by changes in the activation of the D-1 receptor, likely by the endogenous ligand.

67.2 LESIONS OF MEDIAL PREFRONTAL CORTEX DOPAMINE NEURONS: EFFECTS ON AMPHETAMINE SELF-ADMINISTRATION AND DOPAMINE SYNTHESIS IN THE RAT BRAIN. A.P. Leccese and W.H. Lyness. Psychology Dept., Kenyon College, Gambier, OH 43022 and Dept. of Pharmacology, Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

It has been suggested that dopamine neurons within the medial prefrontal cortex subserved some role in the positive reinforcing effects of the psychomotor stimulants. Microinjections of 6-hydroxydopamine (6-OHDA) into this region, which destroyed a major portion of the dopamine innervation but maintained the integrity of noradrenergic and serotonergic neurons, failed to alter either the acquisition or maintenance of i.v. d-amphetamine self-administration in rats. Compared to vehicle injected controls (sham lesions) the 6-OHDA treated animals acquired the drug abuse behavior and maintained comparable, stable rates of self-injection. The lesions did increase the concentrations of dihydroxyphenylacetic acid and homovanillic acid in nucleus accumbens septi but not the striatum. The increased synthesis of dopamine in the nucleus accumbens septi (demonstrated by increased dihydroxyphenylalanine accumulation) was abolished by the administration of i.v. d-amphetamine in patterns mimicking those of trained self-administration animals.

67.3 REDUCED GABA & GLUTAMATE AND INCREASED SEROTONIN ACTIVITY IN MIDBRAIN-THALAMUS OF RATS FOLLOWING RECOVERY FROM ACUTE THIAMINE DEFICIENCY. P.J. Langlais, R.G. Mair, C.D. Anderson and W.J. McEntee. Research Svc. (151C), VA Medical Center, Brockton, MA 02401.

The anatomic substrates and neurotransmitter substances involved in the cognitive and memory deficits of thiamine deficiency induced Korsakoff's disease remain unknown. In acutely thiamine deficient rats, regional brain monoamine and amino acid neurotransmitter disturbances have been reported but their impact on cognition and memory could not be assessed. In two previous studies we observed significant learning and performance deficits in rats following recovery from a two week bout of PTD-induced thiamine deficiency (pyrithiamine [0.5 mg/kg/day] + thiamine deficient diet) (Mair et al., Brain Res., 360:273-284, 1985; Mair et al., Neurosci. Abstr., 12:745, 1986). Cortical norepinephrine (NE) levels were significantly reduced (by 35%) in the first study, but were reduced by only 6% in the second study (Langlais et al., Brain Res., in press). These differences may have been due to the use of a more severe, prolonged bout of thiamine deficiency in the first study (35% reduction).

In this study, rats were made thiamine deficient and the brains dissected as in the original study and the concentrations of monoamines, metabolites and amino acids measured. Equal size groups of PTD treated and pairfed control rats were sacrificed 2 weeks (N=10) and 9 weeks (N=7) following nutritional restitution. Consistent with our previous results, significant elevations were observed at both recovery periods in NE content of cerebellum and 5-HT & 5-HIAA of midbrain-thalamus and striatum. In cortex, NE was reduced by 23% at 2 weeks and unchanged at 9 weeks, thus failing to demonstrate a persistent diminution of cortical NE. Significant reductions in GABA, glutamate and aspartate levels in midbrain-thalamus but not any other brain areas were observed at 2 and 9 weeks. These findings agree with an earlier report of reduced GAD activity within the thalamus of recovered PTD rats (Thompson & McGeer, Neurochem. Res., 10:1653-1660, 1985) and suggest a permanent loss of thalamic GABAergic activity in this model of Korsakoff's disease. This possibility is supported by our observation of consistent thalamic lesions in recovered PTD rats (Langlais et al., Neurosci. Abstr. 12:751, 1986). The findings of reduced GABA and increased serotonin activity may be related to the purported GABA mediated-inhibition of serotonin activity within midbrain-thalamic nuclei. Supported by Veterans Administration research grant.

67.4 ASYMMETRY IN STRIATAL DOPAMINE RELEASE AND METABOLISM: BILATERAL IN VIVO DIALYSIS IN NORMAL RATS. S.D. Glick, J.N. Carlson, J.L. Baird* and K.L. Drew. Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208.

Asymmetry in the dopaminergic nigrostriatal system of the rat brain has been well documented in this laboratory and others (cf. Glick, Cerebral Lateralization in Nonhuman Species, Academic Press, 1985). Normal hemispheric differences in striatal dopamine levels, metabolites, release, uptake, and receptors have been reported. Studies of such differences have previously been conducted only with postmortem brain tissue (either homogenates, slices or synaptosomes). Using the technique of in vivo brain dialysis, we have monitored release of dopamine and its metabolite DOPAC in both striata of awake, freely moving female rats. Under baseline conditions, an asymmetry in dopamine release was reciprocally related to an asymmetry in DOPAC; that is, DOPAC was higher on the side with lower dopamine and vice-versa. Administration of d-amphetamine sulfate (1.25 mg/kg) enhanced dopamine release and decreased DOPAC-- these changes were frequently much greater in one striatum than the other and appeared to be related to the direction and intensity of d-amphetamine-induced rotation (circling). In accordance with other findings (e.g., Zetterstrom et al., Europ. J. Pharmacol., 132: 1, 1986), the reciprocal relationship between dopamine and DOPAC suggests that when more dopamine is released, there is less intraneuronal substrate for MAO and hence lower DOPAC. Further studies are in progress to determine the relationship of these asymmetric changes to regional variations in dialysis sites within the striata. (Supported by NIDA grant DA03817 to S.D.G.)

67.5 STRIATAL DOPAMINE RELEASE ASSESSED WITH MICRODIALYSIS FOLLOWING UNILATERAL NIGROSTRIATAL DAMAGE. Terry E. Robinson and Ian Q. Whishaw. Departments of Psychology, University of Michigan, Ann Arbor and University of Lethbridge, Lethbridge, Canada.

Most studies on the presynaptic compensatory changes in dopamine (DA) release that are thought to occur following partial damage to nigrostriatal DA neurons have involved indirect measures of DA utilization or in vitro methods for assessing release. The present study was designed to determine the effects of nigrostriatal damage on striatal DA release in vivo, measured with microdialysis in freely moving rats.

Rats received unilateral 6-OHDA lesions of varying severity in the substantia nigra. At least 1 month later concentric (250 µm O.D.) dialysis probes were placed bilaterally in the striatum. The next day extracellular concentrations of DA, DOPAC, HVA and 5-HIAA in dialysate were measured in both the denervated and intact striatum, first during the resting state and then following an injection of 1.5 mg/kg d-amphetamine (AMPH). Striatal tissue was removed and assayed at the end of the experiment.

The most striking finding was that striatal tissue DA concentrations did not predict DA concentrations in dialysate over a wide range of lesion sizes. Massive tissue DA depletion (90-99%; denervated relative to intact side) was accompanied by only relatively small changes in DA levels measured in dialysate during the resting state. In contrast, the depletion of DA metabolites in tissue was accompanied by a comparable depletion in dialysate, and one was highly predictive of the other. In rats with a <95% DA depletion (tissue) AMPH produced a large increase in DA release (dialysate) from both the intact and lesion sides; although the intact side showed greater release than the denervated side. Rats with a >95% DA depletion showed a large increase in DA release from the intact side, but a progressive reduction in DA levels in dialysate obtained from the lesion side (often to non-detectable levels). All rats turned away from the side showing the greatest AMPH-stimulated DA release.

In summary, after even a >90% loss of DA from one striatum the extracellular concentrations of DA are maintained at close to normal levels (in absolute terms), presumably due to compensatory changes in the remaining neurons. If the tissue DA depletion is <95% the denervated side can even release considerable quantities of DA in response to AMPH. However, if the tissue depletion is >95% there appears to be no reserve capacity, and thus no increase in release in response to AMPH. The data may help explain why relatively normal function is maintained after even extensive damage to the nigrostriatal DA system, and why deficits often often appear only when the system is 'challenged'.

67.6 ADRENERGIC RESPONSE TO COGNITIVE ACTIVITY IN A COLD ENVIRONMENT. S.T. Ahlers, J.R. Thomas, J. Schrot, J.F. House, K.F. VanOrden, and R.L. Hesslink. Environmental Stress Department, Naval Medical Research Institute, Bethesda, MD 20814-5055.

Plasma norepinephrine (NE) and epinephrine (E) were sampled in six male subjects before, during, and after a 90 minute exposure in a controlled climate chamber at 4°C and during exposure in the chamber at ambient temperature (22°C). After 45 minutes of exposure subjects performed a cognitive test battery lasting a total of 40 minutes.

NE increased markedly within 2 minutes after exposure to the cold environment and remained asymptotic for 30 minutes during which subjects engaged in no programmed activity. Just prior to, and during the test battery however, NE increased to nearly three times basal levels. A significant but smaller increase in plasma NE was observed when subjects were administered the cognitive test in the ambient environment.

In contrast to NE, E did not increase above baseline in the first 30 minutes of cold exposure but did increase substantially just prior to and during the administration of the cognitive test. A slight increase in E was observed when the test battery was given at normal temperature.

These data indicate that while NE and E responded differentially to cold with NE increasing and E levels unchanged, both catecholamines increased when subjects were engaged in focused mental activity. Moreover, the combined effects of cold stress and mental activity appeared to synergistically modify the release of catecholamines, especially in the case of E, where the release during the cognitive test was augmented during exposure to the cold environment. This work was supported by the Naval Medical Research and Development Command, Research Task No. M0095.04.1054.

67.7 **STRESS- AND COCAINE- LIKE EFFECTS ON AMPHETAMINE- INDUCED ROTATIONAL BEHAVIOR CAUSED BY THE ANXIOGENIC BENZODIAZEPINE INVERSE AGONIST METHYL β-CARBOLINE -3-CARBOXYLATE.** J.N. Carlson and S.D. Glick. Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208

This laboratory has been investigating lateralized changes in dopamine (DA) activation which take place following exposure to mild stress and various stimulant drugs. One technique for assessing these alterations has been to evaluate differences in unlesioned rats' rotational behavior, presumed to result from lateralized nigrostriatal DA activation. We have previously shown that, in the Sprague-Dawley rat, mild footshock stress causes a change in the direction and intensity of d-amphetamine (d-A) induced rotational behavior resembling that induced by cocaine alone (Carlson, J.N., et. al. Pharm. Biochem. Behav. 26:17,1987). We have hypothesized that mild stress and cocaine both selectively activate mesoprefrontocortical DA systems while d-A preferentially activates nigrostriatal and limbic DA; and that stress- induced changes in rotation are subserved by a lateralized cortical modulation of striatal DA function.

It has recently been shown that certain beta-carboline benzodiazepine receptor inverse agonists selectively activate mesoprefrontocortical DA systems (Tam, S.-Y. and Roth, R.H., Biochem. Pharmacol 34:1595,1985; Claustre, Y. et.al., J.P.E.T. 238:693,1986). Previous findings have indicated that cortical DA activating stressors such as uncontrollable footshock and 48 hours of food deprivation (Carlson, J.N. and Glick, S.D., Neurosci. Abstr. 12:1535,1986) cause an increase in the intensity of d-A induced left rotation in the Long-Evans rat while leaving right rotation unaffected. It was thus of interest to compare the effects on d-A rotation of methyl β-carboline-3- carboxylate (β-CCM) to those of mild stress and cocaine in this strain of rat. Rats were tested for d-A rotation (1.56 mg/kg males, 1.25 mg/kg females, i.p.) and were again tested 1 week later 45 min. following a 5.0 mg/kg s.c. dose of β-CCM. In another experiment male and female rats were tested for rotation following 20.0 mg/kg i.p. cocaine HCl. β-CCM caused a selective increase in the intensity of left rotation while lovering or leaving unchanged the intensity of right rotation. Similarly, cocaine caused a strong left rotational bias. These findings indicate that stress, cocaine and β-CCM share a common lateralized action on DA systems. Using these manipulations, neurochemical studies are now in progress to explore lateralized relationships among cortical, limbic and striatal DA systems. **(Supported by NIDA grant DA03817 to S.D.G.)**

67.8 IS THERE A RELATIONSHIP BETWEEN DOPAC RELEASE FROM THE CAUDATE NUCLEUS AND YAWNING BEHAVIOR IN THE FREELY MOVING MALE RAT? N. J. Laping and V. D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

It has been shown previously that the nigro-striatal dopamine system is involved in prolactin- and apomorphine- induced yawning. Herein we describe the in vivo dopamine activity of the caudate nucleus via DOPAC measurement from push-pull-perfusates of the caudate nucleus sampled at two minute intervals. This technique allows us to more closely examine any correlation between dopaminergic activity and yawning behavior.

Male Holtzman albino rats were implanted with a 22g stainless steel syringe into the caudate nucleus (2.3mm anterior to the bregma; 2.5mm lateral from the superior sagittal sinus; 4.5mm ventral from the dura mater). Seven to ten days after the surgery the caudate nuclei of these freely moving animals were perfused with a modified KRP medium (16ul/min.), and sampled every two minutes starting between 12:00 and 14:00h. The subjects were monitored simultaneously for motor activity by an animal activity monitor and yawns were noted visually by the investigator.

The two minute sampling rate of DOPAC concentrations of the caudate nucleus revealed an episodic pattern of release with a period of about 8-10 minutes with a mean amplitude of 11.56±2.1pg/min. Interestingly, in an untreated animal that yawned spontaneously, there was a sharp drop in DOPAC and dopamine release just prior to or during the two samples at which yawning occurred (>20pg/min.).

In order to experimentally manipulate the dopaminergic release, subjects received a systemic injection of apomorphine (50ug/kg) at a dose known to induce yawning. Perfused animals in those experiments yawned 15.5±6.0 times in 30 minutes following a systemic APO injection. A pairwise comparison was then performed between DOPAC release over a 10 minute period prior to apomorphine injection and DOPAC release during the sample at which the first yawn occurred. The results show that DOPAC release rates dropped significantly during the first yawn ($p<0.05$) (delta = 22.5±5.9 pg/min., $n=4$). These findings suggest that yawning behavior is displayed with a concomitant drop in dopaminergic activity and support the hypothesis that cholinergic activation of yawning is under dopaminergic inhibitory control.

67.9 CHARACTERIZATION OF HIPPOCAMPAL NOREPINEPHRINE EFFLUX USING IN VIVO DIALYSIS: PHARMACOLOGICAL AND BEHAVIORAL STUDIES. E.D. Abercrombie, R.W. Keller, E.M. Stricker and M.J. Zigmond. Dept. of Behavioral Neuroscience, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

We have examined norepinephrine (NE) efflux in the dentate gyrus of hippocampus of freely moving animals in response to pharmacological and environmental stimuli. A probe was constructed from hollow dialysis fibers (15,000 mw cut-off) so as to provide an active region of dialysis fiber 4.5 mm in length, calibrated with standards, and then stereotaxically implanted under chloral hydrate anesthesia into the dorsal hippocampus. Most of the loop was located in the hilus of the dentate gyrus, the area known to receive the densest NE innervation within the hippocampus. Perfusion (2 ul/min) using artificial CSF was carried out using a Harvard syringe pump via a fluid swivel that permitted relatively unrestricted movement. Animals were allowed to recover from surgery overnight and efflux was monitored for a minimum of two hours prior to each manipulation in order to ensure stable baseline values. Experiments were performed 1-5 days post-operatively. Perfusate was collected every 15 min and 20 ul of each sample was assayed for catecholamines using HPLC with electrochemical detection.

The concentration of extracellular NE in this region was 50-75 nM. After systemic administration of the α_2-adrenergic receptor antagonist, yohimbine (2.0 and 5.0 mg/kg, ip), NE was increased to 125% and 230% of baseline, respectively. Addition to the perfusate of desipramine (0.1 mM), an inhibitor of high affinity NE uptake, increased NE more than 3-fold. Intermittent electric shock to the tail for 30 min provoked a short-lived increase in extracellular NE levels to approximately twice that of baseline. Similarly, a brief 3-fold increase in extracellular NE levels was observed in response to restraint stress of 30 min duration. These stress-induced increases in hippocampal NE efflux were limited to the two 15 min dialysate samples which corresponded to the application of the stressor.

These results demonstrate that changes in hippocampal NE efflux can be measured in response to both pharmacological and behavioral manipulations using in vivo dialysis. In addition, these data lend further support to the hypotheses that the level of activity in central NE systems is partly under the control of α_2-adrenoceptors, that reuptake processes are important in regulating extracellular NE levels, and that the noradrenergic projections of the locus coeruleus are involved in the response of the central nervous system to stress. (Supported in part by USPHS grants MH29670, NS19608, and MH18273.)

67.10 SEQUENTIAL INCREASE IN TYROSINE HYDROXYLASE ACTIVITY IN NORADRENERGIC CELL BODIES AND TERMINAL AREAS AFTER EXPOSURE TO CHRONIC COLD. L.K. Gladstein, E.D. Abercrombie, E.P. Weisberg, B. Kaplan, M.J. Zigmond. Depts. of Behavioral Neuroscience and Psychiatry, Cntr. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

The norepinephrine (NE)-containing neurons originating in the locus coeruleus (LC) have been implicated in the response of the central nervous system to stress. One mechanism by which this system adapts to conditions of increased demand is via enhanced biosynthetic capacity. This is accomplished by an elevation in the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in NE biosynthesis. Previous studies have shown that cold stress results in an elevation of TH activity in the LC. We have further examined the stress-induced alterations in TH.

Adult, male rats were shaved and placed in a cold room (5°C) for 3-7 days, while control animals remained in a temperature-controlled environment at 22°C. Animals were killed by decapitation and a region of brain stem containing the LC cell group dissected out. The cerebellum, which contains axon terminals from LC, also was removed. Tissues were immediately placed on dry ice and then stored at -70°C until assayed. TH activity was measured by a coupled decarboxylase assay. TH protein was quantified using immunoblot analysis.

After three days of cold stress, there was a 29% increase in TH activity in LC which was accompanied by an increase in TH protein. After seven days of cold exposure, TH activity was further elevated to 61% of control activity. These results are consistent with our previous finding that TH mRNA is elevated in the LC after chronic cold stress (Stachowiak et al., 1986) and suggest that cold stress increases the synthesis of TH protein. In contrast to our observations in LC, no change in TH activity was found in cerebellum after three days of cold exposure. However, after seven days of cold, a 33% increase in enzyme activity was observed. This delayed rise in TH activity seen in the cerebellum parallels reports of sequential increases in TH activity in the LC, cerebellum, and hippocampus following partial injury to LC neurons with 6-hydroxydopamine (Acheson and Zigmond, 1981) and treatment with reserpine (R. Zigmond, 1979). It suggests that once synthesized, the new TH protein is transported to axon terminals.

These results provide further support that during conditions of increased demand, TH is synthesized in the cell body regions and is transported down the axon to the terminal fields. Additional experiments are currently underway to determine whether the arrival of new TH protein in terminal regions is associated with an increase in the capacity for NE synthesis and release. (Supported in part by USPHS grant NS19608.)

67.11 D-AMPHETAMINE (D-AMPH) DISCRIMINATION BEHAVIOR IS NOT ALTERED BY L-TRYPTOPHAN (L-TRY) OR FLUOXETINE PRETREATMENT. F.L. Smith* and W.H. Lyness. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

In experiments where rats self-administer i.v. d-AMPH for its reinforcing properties, injections of 100 mg/kg L-TRY or 5 mg/kg fluoxetine significantly reduces drug intake (A. Leccese and W. Lyness, Brain Res., 303: 153, 1984; F. Smith, et al., Pharm. Biochem. Behav. 25: 849, 1986). A concern was that drugs which increase 5-HT receptor activity might alter dopamine mediated reinforcement, causing subsequent reductions in self-injected d-AMPH. In one study where zimelidine reduced conditioned place-preference in rats trained on d-AMPH, it was proposed that increased 5-HT transmission reduced the reinforcing properties of d-AMPH (A. Kruszewska, et al., Eur. J. Pharmac., 125: 283, 1986). To examine this concern, drug discrimination experiments were conducted to determine whether 5-HT agents alter the degree of d-AMPH mediated discrimination behavior. Male Sprague-Dawley rats were trained to discriminate 1.0 mg/kg d-AMPH from saline in a dual-lever food operant paradigm, utilizing a fixed ratio-10 (FR-10) schedule. Using doses between 0.1 and 0.7 mg/kg d-AMPH, a dose-response curve was generated which had an effective dose-50 (ED-50) of 0.34 mg/kg. Serotonergic drugs were then combined with both low and high doses of d-AMPH (0.2 and 0.7 mg/kg) to determine whether increased 5-HT receptor activity alters the degree of d-AMPH mediated discrimination. Activation of 5-HT neurons with 100 mg/kg L-TRY or blockade of 5-HT reuptake with 5 and 10 mg/kg fluoxetine, when combined with d-AMPH, did not significantly enhance or reduce drug-lever responding.

Verifying that dopamine neurons mediate the discriminative properties of d-AMPH was accomplished by combining dopamine receptor agents with d-AMPH. Increasing doses of apomorphine (APO), when combined with 0.2 mg/kg d-AMPH, produced significant increases in drug-lever responding at 1.0 mg/kg. Subsequent combinations of 1.0 mg/kg APO with various doses of d-AMPH produced a significant shift-to-the-left in the d-AMPH dose-response curve. On the other hand, when increasing doses of haloperidol were combined with 0.7 mg/kg d-AMPH, significant reductions in discrimination occurred at 0.2 mg/kg haloperidol.

Thus, d-AMPH discrimination behavior is not enhanced or reduced by manipulations which increase 5-HT receptor activity. This appears to answer two questions: first, d-AMPH reinforcement is not reduced with increased 5-HT transmission as proposed in the place-preference experiments, and second, the reductions seen with self-administered d-AMPH is not the result of 5-HT enhancing dopamine mediated reinforcement. Possibly, by enhancing 5-HT receptor activity, aversive qualities are elicited which produce subsequent reductions in self-injected d-AMPH.

67.12 HALOPERIDOL BLOCKS THE INCENTIVE MOTIVATIONAL PROPERTIES OF FOOD REINFORCEMENT IN RATS. A. Ettenberg and J.C. Horvitz. Department of Psychology, University of California, Santa Barbara, CA 93106

A primary assumption of incentive motivational theories of behavior (e.g. Bindra, 1974) is that the presentation of incentive stimuli can serve to activate a "central motive state" underlying the initiation and maintenance of animal behavior. The "motivating" action of rewarding stimuli has been demonstrated in studies where previously extinguished operant responses are reinstated following a single "prime" with the original reinforcer. If central dopamine substrates play an important role in the neural mediation of food reward, then one might predict that the incentive properties of food (in such a "priming" task) would be prevented by disruption of dopamine (DA) neurotranmission.

In the present study, hungry rats (n=7 per group) were trained to traverse a straight runway for a reward of five 45mg food pellets. Training consisted of a single trial per day over seven consecutive days. On Day 8, the animals were tested with no food reward available in the goal box. Such non-rewarded extinction trials continued until the animals' running times slowed to an arbitrary "extinction criterion" (i.e. 3 times slower than the mean running speed over the final 3 reinforced trials). Once operant running had extinguished, animals were given one final extinction trial (which served as a baseline) and then were tested in one of the following ways: 1) one group (HAL.15/FOOD) was pretreated with 0.15 mg/kg IP haloperidol (a DA antagonist prepared in warm vehicle solution of 0.002M lactic acid and injected in a volume of 1.0 ml/kg) 45 mins prior to a single food rewarded trial; 2) a second group was similarly treated with a higher, 0.3mg/kg, dose of haloperidol (HAL.3/FOOD); 3) group 3 (VEH/0) pretreated with the vehicle solution alone and then experienced a non-rewarded trial; 4) group 4 (VEH/FOOD) was similarly pretreated with the lactic acid vehicle solution but later experienced a food-rewarded trial; and 5) the final group (FOOD/HAL.3) served as a "motor impairment" control group in that these animals were not injected with haloperidol until 1 hr after a rewarded runway trial. The next day the effectiveness of the reward presentation on the previous day's trial was assessed by examining the running speeds of each of these five groups of rats.

The results clearly demonstrated that a) a single food-prime was sufficient to reinstate operant runway responding; b) this "priming" effect was blocked by haloperidol pretreatment; and c) the drug effects could not be accounted for by motor impairment since the FOOD/HAL.3 group behaved indistinguishably from nondrugged food-rewarded animals (i.e. there was no residual motor deficit one day after haloperidol treatments). Together these results suggest that DA systems are involved in mediating the incentive motivational properties of food reward.

67.13 **DIABETES-INDUCED POLYDIPSIA IN RATS: DEPENDENCE ON INTACT DOPAMINE FUNCTION AND MEDIATION BY CENTRAL INSULIN** **M.H. Lewis, M.F. Keresztury*, Q.D. Walker*, L.S. Cook*, B.E. Mileson*, and R.B. Mailman. Departments of Psychiatry and Pharmacology and Biological Sciences Research Center, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514**

When administered to rats, the pancreatic beta cell toxicant, streptozotocin (55 mg/kg iv) induces a well-characterized model of insulin dependent diabetes mellitus (IDDM). Rats so treated become hypoinsulemic, hyperglycemic, polydipsic and polyuric, hyperphagic (despite weight loss), and, later, develop secondary complications such as retinopathies and peripheral neuropathies. We have selected diabetes-induced polydipsia (DIP) as a behavioral/physiological endpoint with which to examine some of the effects of diabetes on central catecholamine function. Diabetic rats (blood glucose > 400 mg/dL) increased their daily water consumption from ca. 45 mL to ca. 300 mL. In the first study, the effect of selective D_1 and D_2 dopamine antagonists on daily water consumption was assessed. Administration of haloperidol (1 mg/kg ip) significantly suppressed DIP, while not affecting water consumption in control rats. Conversely, SCH23390 treatment did not suppress DIP at any dose tested (0.05 to 0.5 mg/kg ip). Thus, DIP is attenuated by D_2 but not D_1 receptor blockade. In contrast either angiotensin II or lithium-induced polydipsia can be attenuated by both D_1 and D_2 antagonists; effects known to involve the nigrostriatal dopamine system. These data may indicate a differential effect on the sensitivity of dopamine receptor sub-types as a consequence of diabetes.

In a second study, rats were chemically lesioned with the catecholamine neurotoxicant, 6-OHDA, following pretreatment with desipramine (25 mg/kg, i.p.). Water consumption in dopamine depleted animals was found to be markedly reduced relative to controls following induction of diabetes. These data further support the dependence of DIP on intact dopamine pathways. Data from experiments using site-specific injections of 6-OHDA point to the importance of the nigrostriatal dopamine in mediating DIP. We have also examined the role that insulin may play centrally in mediating DIP, independent of its peripheral effects. Preliminary data have demonstrated that icv infusions of insulin suppress DIP. The mechanisms by which insulin may suppress DIP are currently being examined.

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67.14 BEHAVIORAL SUPPRESSION IN RATS FOLLOWING BILATERAL MICROINJECTION OF 5-HYDROXYTRYPTOPHAN (5-HTP) IN LATERAL HYPOTHALAMUS SUPPORTS ROLE OF CENTRAL SEROTONERGIC MECHANISMS IN 5-HTP ANIMAL MODEL OF DEPRESSION. J.N. Hingtgen, A. Shekhar*, J.A. DiMicco and M.H. Aprison. Depts. of Psychiatry and Pharmacology and Toxicology and Biochemistry and Program in Medical Neurobiology and Section of Applied and Theoretical Neurobiology, Institute of Psychiatric Research, Indiana Univ. School of Med., Indianapolis, IN 46223.

In a series of studies over a 27 year period 5-HTP has been shown to produce operant response suppression in pigeons and rats working on food reinforcement schedules; these changes in behavior have been correlated with serotonergic increases in specific areas of the brain and have led to the development of the hypersensitive postsynaptic serotonin receptor theory of depression (Aprison et al. In: Neuropharmacol. and Behav., Plenum, p. 23,1978; Aprison and Hingtgen, In: Serotonin, Plenum, p. 267, 1981). Although many studies from our and other laboratories have proposed central mechanisms in explaining this animal model of depression, there remains a possibility that peripheral effects may play a role, since 5-HTP typically has been administered by the systemic route. To demonstrate more clearly that the 5-HTP induced suppression is a centrally mediated phenomenon, we injected rats directly into the hypothalamus, an area previously shown to reflect changes in 5-HT associated with behavioral changes (Loullis et al., Pharm.Biochem. Behav., 959, 1980). Male Wistar rats were trained on a VI schedule for milk reinforcement. After response baselines were established, guide cannulae were stereotaxically placed bilaterally in the lateral hypothalamus. After a recovery period of 4-5 days, 100-500 ng D,L-5-HTP was microinjected bilaterally 15 min after the start of the VI session. Subsequent changes in response rates were monitored over a 2-3 hr session. Significant decreases in responding were observed (50-100% below baseline levels) for as long as 200 min following injection, a behavioral effect comparable to that obtained after a systemic injection of 50 mg/kg D,L-5-HTP. Rats receiving a microinjection of 5-HTP in the posterior hypothalamus did not exhibit a behavioral effect. Rats working on avoidance schedules, when injected systemically with 5-HTP, do not exhibit response suppression (Aprison and Hingtgen, Rec. Adv. Biol. Psychiat. 8, 87, 1966). Similarly rats in the present study working on Sidman shock avoidance schedules failed to exhibit response suppression when microinjected with 5-HTP in the lateral hypothalamus. These data support the important role previously assigned to central mechanisms in the 5-HTP animal model of depression. (Supported in part by training grant PHS MH 17107-03 and USPHS NS 19883).

67.15 EFFECTS OF AMPHETAMINE AND HALOPERIDOL ON CORE TEMPERATURE IN RAT. W. F. Caul, S. D. Comer*, J. D. Nussdorf*, and R. J. Barrett*. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Pharmacodynamic adaptive processes are induced by administration of many centrally and peripherally acting drugs (Lupolover, R., et al., Int. Pharmacopsychiat., 17:194-237, 1982). These adaptive processes are thought to be the basis for biphasic responses to drug (Barrett, R. J., and Rock, R. S., Psychopharm. In press) as well as rebound effects observed following termination of chronic drug treatment (Haefely, W., Pharmacopsychiat. 19:353-361, 1986). One would expect, therefore, that a biphasic temporal pattern of drug response would be present in every homeostatic system affected by the drug. Since the stimulus properties of amphetamine follow a biphasic temporal pattern as revealed using the drug-discrimination procedure (Barrett, R. J., and White, D. K., In prep.), and since amphetamine-induced anorexia is followed by hyperphagia (Caul, W. F., et al., Beh. Neurosci., In press), the purpose of this experiment was to determine the temporal pattern of amphetamine's effect on temperature. The effects of haloperidol were assessed also because it has been reported that while this drug potentiates the effects of dopamine agonists, alone it produces no change in temperature (Lai, H., and Horita, A., 82:335-337, 1984).

Lightly restrained rats received injections (s.c.) of either distilled water, amphetamine (1, 2, or 4 mg/kg), or haloperidol (1, 2, or 4 mg/kg). Oiled number 402 thermistor probes were inserted 8 cm into the rectum and taped to the base of the tail. Air and core temperature were then continuously monitored immediately after drug injection for 8 hours 10 minutes by a Grass Model 7 polygraph. Each rat received the same injection three times with two days intervening between each injection day. Animals were deprived of food and water for 16 hours prior to each injection.

Over the 8-hour recording period, the temperature for control animals increased during the initial 30 minutes and then declined over the next 120 minutes toward an asymptote of 36.7° C. Relative to this control curve, amphetamine elicited an initial hypothermic response that lasted from 30 to 60 minutes. This hypothermia was always followed by hyperthermia. The hyperthermia persisted from 180 to 280 minutes. The data suggest that at higher doses of amphetamine, rebound hyperthermia occurs later in time, is of greater magnitude, and lasts longer. In contrast to these cubic components that describe the effects of amphetamine, the orthogonal polynomial analysis of the effects of Haloperidol reveals that the major component was quadratic. Haloperidol elicited long lasting hypothermia at all doses. No rebound was apparent within the measurement period. Thus, while the explanation for haloperidol's effect is not apparent, amphetamine's pronounced biphasic effect on temperature conforms to expectations derived from knowledge of the biphasic nature of the drug's stimulus properties and it effects on food consumption.

67.16 PANIC DISORDER: TREATMENT, SYMPTOMS, AND PLASMA MHPG. Matthew J. Edlund* and Alan C. Swann. Department of Psychiatry, University of Texas Medical School and Mental Sciences Institute, Houston, TX., 77225, USA.

Norepinephrine may be involved in the physiology of anxiety and the pathophysiology of anxiety disorders. Panic episodes are associated with increased norepinephrine turnover, and drugs or other manipulations that increase norepinephrine release can precipitate panic in susceptible individuals. Yet, there is little information about noradrenergic function in patients with panic disorder under naturalistic conditions, or about relationships between norepinephrine and clinical variables such as symptoms or treatment response. We examined plasma MHPG and clinical measures including Beck Depression Index (BDI), Hamilton Depression Score (HDS), Zung Anxiety Score (ZAS) and frequency of panic attacks in 28 otherwise healthy patients with panic disorder. The patients were compared to 21 healthy controls matched for age, gender, and time of day. The patients had received no psychotropic medicine for at least one month (26 for at least six months). After evaluation, patients were offered outpatient pharmacologic treatment. An average of eight months later, the evaluations were repeated in 24 patients, of whom 12 had remained in treatment. Initial plasma MHPG was lower in patients than in controls (3.31 ± 1.07 (SD) vs 4.41 ± 1.23 ng/ml; $P < 0.002$). Patients were mildly depressed, with mean HDS of 13.4 ± 5.8, BDI 13.4 ± 8.2, and ZAS 42.2 ± 9.3. Plasma MHPG correlated negatively with BDI ($r = -0.49$; $P < 0.01$). Patients who subsequently remained in treatment had significantly lower initial plasma MHPG than those who did not, but did not differ significantly in clinical measures. Clinical measures improved between initial and follow-up evaluations in treated patients. Plasma MHPG increased in the overall group but not in treated or untreated patients alone. Change in plasma MHPG correlated negatively with symptom change in untreated patients but positively in treated patients. Previous reports have found no difference between plasma MHPG between panic patients and controls; in those studies lower plasma MHPG in panic patients could have been obscured by anticipatory anxiety (in double blind studies of manipulations that provoke panic episodes) or rebound effects from recent drug treatment. If low plasma MHPG in panic disorder represents low norepinephrine turnover under resting conditions, this could lead to compensatory hypersensitivity of postsynaptic noradrenergic receptors and subsensitivity of inhibitory autoreceptors, as has been reported (Albus et al., Clin Neuropharmacol 9 (Suppl 4):359). These changes would predispose patients to labile noradrenergic responses, and are compatible with reports that panic patients have increased behavioral sensitivity to norepinephrine (Charney et al., Arch Gen Psychiat 41:751).

67.17 **ALTERATIONS IN DOPAMINE AND SEROTONIN MEDIATED BEHAVIORS PRODUCED BY LESIONS TO THE LATERAL HABENULA.** Paul M. Carvey, LiChiung Kao, Racheal Amdur and Harold L. Klawans. Dept. of Neurological Science, Rush Presbyterian St. Lukes Medical Center. Chicago, IL 60612.

We have previously presented evidence that bilateral lateral habenula (LHb) lesions induced by kainic acid potentiated stereotypic behavior (SB) induced by dopamine (DA) agonists (Soc. Neurosci. Abstr. 12:2 p.920, 1986). We have extended these preliminary findings in relationship to other DA and serotonin (5HT) mediated behaviors.

Rats and/or guinea pigs with verified LHb lesions exhibit the following behavioral profiles relative to sham-operated controls:

1- Increased SB in response to moderate doses (0.2 to 0.75 mg/kg) of apomorphine [both the guinea pig and rat].
2- Decreased cataleptic response to haloperidol (0.3 mg/kg; hanging grid test) [rats].
3- Trials necessary to learn a conditioned avoidance response were the same as sham-operated controls although the escape latency was reduced [rats].
4- Reduced myoclonic jumping response to LSD (300 mmg/kg) [guinea pigs].
5- Prevention of hypersensitive SB response to apomorphine following chronic haloperidol treatment (0.75 mg/kg/3 weeks [rats] 9 weeks [guinea pigs]). Sham-operated, haloperidol treated animals exhibited the usual hypersensitive response in both species.

In addition to these behavioral effects rats exhibited increased striatal DA and DOPAC content in response to 0.75 mg/kg haloperidol administered acutely. Sham-operated, haloperidol treated animals exhibited decreased DA and increased DOPAC levels in their striata relative to saline treated, unoperated controls.

These results suggest that the LHb participates in the expression of both DA and 5HT mediated behaviors. Because of the unique anatomical connections of the LHb it is possible that this structure plays a central role in regulating, and integrating, the activity of the dopaminergic and serotonergic projection nuclei.

67.18 EARLY PREWEANING MATERNAL BEHAVIOR INFLUENCES THE SENSITIZATION OF AMPHETAMINE-INDUCED STEREOTYPY FOLLOWING HALOPERIDOL IN THE ADULT OFFSPRING. H. L. Schreiber, M. Ortiz*, W. Marquez*, M. Garcia*, M. Gonzales, C. Maez, R. Klein, M. Bibb, and L. Calhoon. Dept. Psychology, New Mexico Highlands University, Las Vegas, NM 87701.

In previous studies (see Abst. Soc. Neurosci., 12:1120,1986), we showed that brief relocation of the dam-plus-litter but not mild direct stress of the litter during the early preweaning period diminished the intensity of haloperidol-induced catalepsy but not morphine-induced catatonia in adulthood. The present study investigated the effect of these early manipulations on various drug-induced behaviors, especially the sensitization of amphetamine-induced stereotypy seen after repeated haloperidol or morphine administration.

After litter reduction on the day of birth, dams and litters received the following treatments for the next 10 days: the CONTROL group received ordinary care; the DISTURB group had their nestcages placed in a room adjacent to the vivarium for 30 min/day; the EHANDL group received standard early handling, i.e., the pups were removed from their nestcages for 3 min/day; the EH/DISTURB group received early handling first, then the nestcage relocation manipulation. The offspring were weaned and housed in ordinary fashion until, in adulthood, they were assigned in split-litter fashion to receive water, haloperidol (.33 mg/kg), or morphine (3.33 mg/kg) and testing for activity wheel locomotion and open-field/holeboard exploration in counterbalanced fashion (4 days/week; 2 weeks). Then (week 3), rats received water or progressively higher doses of their assigned drug (haloperidol, 0.5, 1.0, and 1.5 mg/kg; morphine, 5, 10, and 15 mg/kg) and forepaw-on-dowel catalepsy testing for 3 days. Finally (48 hrs later), all rats received d-amphetamine (4 mg/kg), were placed in activity wheels for 30 min and then were moved to a clear plexiglass chamber and videotaped for 4 min. Blind observers rated stereotypy.

A significant interaction of early handling by nestcage disturbance by drug history in stereotypy was found, $F(2,72) = 4.27$, $p < .02$. Further analysis indicated that, while a history of haloperidol treatment increased amphetamine-induced stereotypy in all groups, only the DISTURB group showed significantly less stereotypy than the CONTROL group, indicating that this particular early manipulation had diminished sensitization in adulthood. Other results as well as the rationale for considering nestcage relocation a manipulation of maternal behavior will be presented.

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67.19 THE EFFECTS OF LOCUS COERULEUS LESIONS ON MONKEY P300-LIKE POTENTIALS. J. A. Pineda, S. L. Foote, and H. J. Neville. Departments of Neuroscience and Psychiatry, University of California, San Diego, La Jolla, California 92093, The Salk Institute, and Scripps Clinic and Research Foundation, La Jolla, CA. 92037

The hypothesis that the noradrenergic locus coeruleus (LC) system participates in the generation and/or modulation of electrocortical activity associated with specific cognitive processes (i.e., P300) was tested by recording event-related potentials (ERPs) in monkeys before and after bilateral LC lesions. Untrained squirrel monkeys (Saimiri sciureus) were presented with auditory stimuli (2 KHz and 6 KHz tones, 40 ms duration, 60 dB above nHL) occurring once a second in a random Bernoulli sequence. One tone constituted 90% of the trials and the other 10%. ERPs were recorded once a week for four weeks pre- and post-lesion from a montage of chronically implanted epidural electrodes. Pre-lesion ERPs included a long-latency potential recorded in response to the infrequent tones and shown by Pineda et al. to be analogous to the human P300 (Electroenceph. clin. Neurophysiol., in press). Lesions were then made by first localizing the LC with glass micropipettes or metal micro- electrodes to record unit activity. Once localized, cathodal current (0.5 mA, 15 sec) was passed through the metal electrode to make an electrolytic lesion. A knife cut was also made by placing the electrode at the anterior pole of the nucleus and moving it 750 um in the medio-lateral dimension. The extent of LC damage was assessed through reconstructions of the lesions from Nissl-stained sagittal sections through the brainstem. Immunohistochemical verification using dopamine-B-hydroxylase and tyrosine hydroxylase antisera as specific markers of noradrenergic neurons and axons and dopaminergic axons, respectively, was also obtained.

The results indicated that damage to LC cell bodies and to the ascending noradrenergic dorsal bundle (DB) fibers resulted in decreased amplitudes of the monkey P300. In contrast, interruption of DB fibers alone did not significantly affect these potentials. Lesions did not affect the amplitude, latency, or surface distribution of shorter latency potentials such as P52, N106, and P172 or the temporally overlapping N250-900. The specificity of this effect supports the hypothesis that LC activity in response to surprising and attention-eliciting events produces a behavioral state that is a necessary precondition for the generation or modulation of P300-like activity. Supported by AFOSR-F49620-87-C-0038

67.20 A PARTIAL REVERSAL OF AMPHETAMINE ANOREXIA AFTER INJECTION OF DOPAMINE ANTAGONISTS INTO THE NUCLEUS ACCUMBENS. G. J. Mogenson and M. Wu.* Dept. of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

Food intake is reduced substantially by amphetamine, a classic anorectic compound. Since amphetamine releases dopamine from axon terminals and blocks the reuptake of this neurotransmitter, it appears that amphetamine anorexia is dopamine-mediated. Consistent with this suggestion is the observation that amphetamine anorexia is reduced or blocked by the systemic administration of dopamine antagonists (Burridge and Blundell, Neuropharmacology, 18:453-457, 1979). The present study was undertaken to investigate whether or not the administration of dopamine antagonists (haloperidol and sulpiride) into the nucleus accumbens, which receives dense dopaminergic projections, also reduced amphetamine anorexia.

Rats were prepared with chronic bilateral cannulae into the accumbens and adapted to a 3 hr feeding schedule which followed 21 hr of food deprivation. Intraperitoneal administration of amphetamine sulphate (2 mg/kg) 10 to 30 min before the feeding period reduced food intake by 70-80% (from 14-18 g to 3-6 g). The effects of bilateral injections of haloperidol (2.5 nmoles in 0.5 ul) into the accumbens on this amphetamine anorexia were compared to bilateral injections of the lactic acid vehicle. Food intake during the first 60 min was 8.58±1.58 g after the administration of haloperidol compared to 3.63±0.66 g after the administration of lactic acid ($t=3.21$, $p<0.02$). However, injections of haloperidol into caudate nucleus did not influence the amphetamine anorexia. The effects of bilateral injections of sulpiride (0.2 ug in 0.5 ul) into the accumbens were also compared to bilateral injections of saline vehicle in a second series of rats. Food intake during the first 60 min was 9.55±1.07 g after the administration of sulpiride compared to 5.45±0.82 g after the administration of saline ($t=3.73$, $p<0.02$).

These observations implicate the dopamine projections to the nucleus accumbens in amphetamine anorexia. Since these dopamine projections have also been shown to contribute to the hoarding of food (Kelley and Stinus, Behav. Neurosci., 99:531-545, 1985) it is of interest to investigate the mechanisms further.

(Supported by NSERC of Canada)

68.1 PROGESTERONE RECEPTOR INDUCTION AND SEXUAL BEHAVIOR BY DIFFERENT ESTROGEN TREATMENT IN MALE AND FEMALE RATS.

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Sex differences in behavioral response to estrogen (E) and progesterone (P) in rat have been correlated with different levels of estrogen-induced progesterone receptors (CPR) in hypothalamic (HYP) nuclei such as the periventricular preoptic area (PVPOA) and the ventromedial nuclei (VMN) where large sex differences were found. In this study we investigated the possibility that this dimorphism may depend on the E dose used for CPR induction. Repeated daily administration of 10 µg of estradiol benzoate (EB)/day for 3 days produced higher levels of CPR in female than in male hypothalamus-preoptic area (HPOA), while single injections of 2 or 8 µg of EB which are sufficient to facilitate estrous behavior in females do not produce significant sex differences in HPOA. Other authors using a treatment of 3 days of Silastic capsules filled with 10% or 100% estradiol (E2) were able to find significant sex differences in CPR in VMN and arcuate nucleus (ARC) with the lower dose. We measured the levels of CPR in discrete HYP nuclei in adult male and female rats following of 6, 24 and 48h of E2 in Silastic capsules and also measured the feminine sexual behavior caused by these treatments 2h after removal of the implants and 2h following an injection of 0.5 mg P.

CPR levels increased with the time of E2 exposure. Sex differences appear in the same areas at defined times of E2 treatment; e.g. PVPOA showed a significantly higher CPR levels in females (33.5 ± 1.8 fmol/mg prot.) than in males (23.9 ± 3.1 fmol/mg prot.) at 48h E2 ($p < 0.05$) while in VMN these differences appear at 24h E2 (F = 24.2 ± 1.1 ;M = 16.4 ± 1.0 fmol/mg prot.) ($p < 0.05$) and 48h E2 (F = 35.3 ± 2.7 ;M = 21.4 ± 1.1 fmol/mg prot.) ($p < 0.01$). The ARC nucleus showed significantly higher CPR levels in males than in females at 24h E2 (M = 29.8 ± 2.1 ;F = 11.3 ± 0.6 fmol/mg prot.) ($p < 0.01$) and 48h E2 (M = 28.5 ± 1.4 ;F = 25 ± 1.5 fmol/mg prot.) ($p < 0.05$). Lordosis behavior also increased monotonically with the E2 dose. Females showed lower lordosis quotient (LQ) with E2 alone than E2 + P; a similar pattern appears for the lordosis quality score (QS). After 48h of E2 treatment, both LQ and QS were maximal. In males, after 48h E2 treatment and after P administration a few showed poor lordosis.

These results suggest that using large doses of E2 for 48h we are able to consistently show a large sex difference of CPR levels in VMN. On the other hand the induction of CPR in ARC nuclei had a slower response in females than in males with a reduction of differences with longer exposure to E2. Sex differences in display of lordosis behavior, which involves VMN as tirsty, may depend in part on sex differences in VMN CPR levels after E2 priming. Supported by NIH TW03617 (HC) and NSO7080 (BMc).

68.2 LACK OF PROGESTERONE-FACILITATED LORDOSIS IN IMMATURE FEMALE GUINEA PIGS MAY BE REFERABLE TO A DEFICIENCY IN HYPOTHALAMIC NUCLEAR PROGESTIN RECEPTOR (NPR) ACCUMULATION.

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Ovariectomized (OVX) neonatal guinea pigs do not exhibit lordosis in response to estradiol benzoate (EB) or EB plus progesterone (P) treatments which are behaviorally effective in OVX adults. This is thought to be due, in part, to a deficiency in the induction of cytosol progestin receptors (CPR) in the mediobasal hypothalamus (MBH) by EB in young females; paradoxically, however, a corresponding decrease in NPR accumulation after P injection in neonates is not observed (Ryer, H.I. and Feder, H.H., Dev. Brain Res. 13:15, 13:23, 1984). The present study was designed to reevaluate neural NPR accumulation in immature and adult guinea pigs and to determine whether inadequate nuclear estrogen receptor (NER) accumulation may underlie the deficiency in CPR induction by EB in the MBH of immature animals, thus contributing to the inability of these animals to display P-facilitated lordosis.

OVX immature and adult (16 days and 9 wks old, respectively, n=7-8/group) guinea pigs were given EB (10 ug) followed by P (0.5 mg) or oil 40 h later, and tested for lordosis by manual stimulation. Confirming the previous report, 85.7% of EB + P treated adults displayed lordosis, whereas none of the immature females responded. Cytosol estrogen receptor levels in the MBH or POA did not differ in the 2 age groups, but CPR concentrations (fmol specifically bound ^{3}H-R5020/mg protein) in the MBH after EB injection (10 ug 40 h earlier) in adults (22 ± 2, n=8) exceeded those observed in immature animals (18 ± 1, n=8). In contrast to the previous report, a significant difference in NPR accumulation in the MBH 2 h after injection of P (0.5 mg) in EB-primed guinea pigs was also observed: 118 ± 13 fmol/mg DNA in adults vs. 68 ± 10 fmol/mg DNA in immature females (n=12/group). Finally, whereas NER accumulation in adult and immature OVX guinea pigs did not differ in the MBH or POA 2 h after a saturating dose (100 ug) of estradiol was administered, it appears that NER saturation is achieved at a lower absolute dose of estradiol in adults (10 ug) than in immature females (50-100 ug).

These data suggest that the absence of P-facilitated lordosis in immature guinea pigs is due to a deficiency in NPR accumulation in the MBH; this, in turn, may be secondary to a deficient induction of CPR by estradiol. Furthermore, the results suggest that poor NER accumulation may contribute to the smaller induction of CPR by estradiol in immature, as compared to adult, guinea pigs. (Supported by NS 1927, RCDA NS 00970 and BRSG RR07048, all from the NIH.)

68.3 DILUTE ESTRADIOL IMPLANTS, RECEPTIVITY AND PROGESTIN RECEPTOR INDUCTION IN THE VENTROMEDIAL HYPOTHALAMUS OF THE FEMALE RAT.

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The hypothesis that cell bodies of the ventromedial nucleus of the hypothalamus (VMN) constitute an essential neural substrate for the hormonal induction of female sexual behavior is supported by results from both behavioral and biochemical studies. Dilute estradiol (E_2) implants very near or in the VMN have been reported to prime female sexual behavior in rats. Induction of cytosolic progestin receptors (PRc) in hypothalamic blocks have been correlated with facilitation of receptive behavior. These studies have been hampered by the need to flood the system with supraphysiological levels of E_2 and/or to pool animals to allow measurement of PRc. The Palkovits punch microassay allows measurement of PRc in discrete CNS nuclei; induction in VMN (and other areas) is now detectable with E_2 doses in the physiological range. We combined dilute E_2 implants with the punch assay to examine VMN-PRc and receptive behavior in individual animals.

OVX female Long-Evans rats were given bilateral guide cannulae aimed at the area of the VMN, and primed for 3 days with either: 1) blank implants + oil, 2) blank implants + 0.5 ug estradiol benzoate (s.c.), 3) 0.4% E_2 implants, or 4) 2.0% E_2 implants. On day 4 all rats were injected s.c. with progesterone (P) and tested for receptive behavior. The following week they received the same priming treatments and were sacrificed on day 4 without P injection. Frozen brains were sectioned (300 um), cannulae locations were determined, and the VMN punch-dissected using a 1000 um needle and assayed for PRc.

High average LQs were seen in all groups but group 1. VMN PRc induction was higher in groups 2 and 4 than in group 3. PRc levels in both E_2 implanted groups were higher than in the oil treated controls. LQ scores were higher in the animals implanted with E_2 in the rostral VMN than was the case with more caudally placed implants. With the 0.4% E_2 implants (group 3) LQ was not positively correlated with VMN PRc induction, indicating that PRc levels in the entire VMN are not a good predictor of female sexual behavior in response to local E_2/ systemic P treatment. These results suggest that E_2 exposure of only a subset of neurons in the area of the VMN may be sufficient for activation of lordosis behavior. The positive correlation between rostro-caudal implant placement and LQ suggests that a particularly sensitive site for the activation of this behavior may lie within or near the rostral VMN. (Supported by NIH grants NS 07807 to TJB, MH 36041 to AME, and HD 04484 to RJB).

68.4 AN ULTRASTRUCTURAL AND MORPHOMETRIC STUDY OF THE ARCUATE NUCLEUS IN FEMALE RAT BRAIN FOLLOWING ESTRADIOL TREATMENT.

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We have demonstrated that a short (2 h) or a discontinuous (2h on/ 7hr off/ 2 hr on) schedule of estradiol (E2) administered to ovariectomized (OVX) rats results in dramatic changes in nuclear ultrastructure in neurons within a brain region critical for controlling reproductive behavior, the ventromedial hypothalamic (VMN) nucleus (Jones et al., J. Comp. Neurol., 1985). These changes are consistent with alterations in RNA synthesis and with the hypothesis that E2 acts in a cascade manner to affect reproductive behavior. More recent molecular data (Jones et al., Molec. Cell Endocrinol., 1986; Molec. Brain Res., 1987) suggest that E2 may act in the VMN and arcuate nucleus (ARC) through mechanisms partly unique to each area. To examine this hypothesis, we ultrastructurally and morphometrically analyzed the early and discontinuous effects of E2 action on neurons within the ARC, a brain region involved in the endocrine components of reproduction. E2 capsules were implanted in OVX rats under the time courses outlined above, with OVX rats serving as controls. The rats were perfused, ARC groups dissected with the aid of a vibratome, and the tissue blocks processed for routine TEM. For EM work, 25 micrographs per rat were collected, for a total of 300 neurons. For morphometry, computerized image analysis was used to collect nucleolar, nuclear, and somal area, and nuclear perimeter and shape from randomly selected 1 um sections through the ARC. The data were analyzed with 2-way ANOVA and the Student-Newman-Keuls test at $p < 0.05$. The results were quite different from the effects of early and discontinuous E2 on VMN neurons. No ultrastructural changes in the nucleolus, nucleus, or RER were noted with either hormone treatment in the ARC. No alterations in nucleolar, nuclear, or somal size, or nuclear shape or envelope length were found. These results suggest that the VMN is more sensitive and exhibits a faster response to E2 than the ARC. The regional differences provide further evidence in support of the hypothesis that estradiol-concentrating brain regions are not homogeneous in their response to steroid hormone administration, and may reflect the functional differences the VMN and ARC subserve in the neural control of reproduction. Supported by grants MH15125 (KJJ), HD05791 (DWP), NSO7080 (BSM).

68.5 REGIONAL SEX DIFFERENCES IN NUCLEAR ESTROGEN BINDING IN THE RAT HYPOTHALAMUS AND PREOPTIC AREA. T.J. Brown and N.J. MacLusky. Dept. of OBS/GYN, Yale University School of Medicine, New Haven, CT 06510.

Numerous studies have demonstrated sex differences in neuroendocrine and behavioral responsiveness to estrogen and progesterone exposure in the adult rat. These sex differences have been correlated with a lower level of estrogen-induced progestin receptors in the periventricular region of the preoptic area (PV-POA) and the ventromedial nucleus of the hypothalamus (VMN) of the male, as compared to the female. To determine if sex differences in estrogen action within these two regions of the brain could be due to a sex difference in nuclear estrogen receptor concentrations, we measured nuclear estrogen binding in discrete microdissected regions of the hypothalamus and preoptic area from gonadectomized/adrenalectomized (GDX/ADX) male and female rats. Rats were treated with a single i.v. injection of 3.6 μg estradiol (E_2)/Kg b. wt. or 36 μg E_2/Kg b. wt. 1 h prior to sacrifice. Animals were perfused with 10% DMSO and their brains were removed and frozen onto cryostat chucks. Serial sections (300 μm thick) were prepared, and the PV-POA, medial preoptic nucleus (mPON), arcuate-median eminence region (ARC-ME) and VMN were removed from the sections using a 500 or 1,000 μm diameter steel punch. Cell nuclei were isolated, extracted with 0.4M KCl and estrogen binding assayed in the nuclear KCl extracts by exchange with 2 nM [^{3}H]E_2. Similar results were obtained with the two dose levels of E_2. Higher levels of estrogen binding were observed in the PV-POA and VMN of the female than the male (PV-POA 3093 ± 154 vs. 1919 ± 95 fmol/mg DNA; VMN 1594 ± 58 vs. 1062 ± 32 fmol/mg DNA, respectively), with a more modest sex difference in estrogen binding in the mPON (1730 ± 113 vs. 1364 ± 80 fmol/mg DNA) (Means ± SEM; N = 9-10). No significant sex difference in nuclear E_2 binding was observed in the ARC-ME. The sex differences in the PV-POA, mPON and VMN do not appear to result from a difference in the retention of estrogen receptors in the cell nuclear fraction: the magnitude of the sex differences in estrogen binding was similar at both 1 and 2 h after E_2 injection. Scatchard analysis of saturation binding data revealed that these sex differences in nuclear estrogen binding reflect a difference in the number of binding sites rather than in binding affinity for [^{3}H]E_2. Parallel studies examined the distribution of estrogen binding sites in the brains of male and female rats using quantitative autoradiography. GDX/ADX rats were administered a saturating dose of 11β-methoxy-16α-[^{125}I]iodoestradiol (sp. act. 40-50 Ci/mmol) and killed 1 h later. Thin cryostat sections (10 μm) were cut through the preoptic region and hypothalamus, exposed against LKB ultrafilm for 3-4 weeks and the resultant autoradiograms quantified densitometrically. Results were consistent with the biochemical studies, indicating a sex difference in estrogen binding in the ventrolateral portion of the VMN and in the medial and periventricular regions of the preoptic area.

These results demonstrate regional sex differences in nuclear estrogen binding which may, at least in part, underly sex differences in responsiveness to estrogen. Supported by NIH grants HD13587 and NS07807.

68.6 UNILATERAL LESIONS OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS DISRUPT LORDOSIS IN FEMALE RATS. Susan B. Greene* and Pauline Yahr. Department of Psychobiology, University of California, Irvine CA 92717.

The ventromedial nucleus (VMN) of the hypothalamus plays a critical role in the hormonal induction of lordosis in female rats. The present study tested the hypothesis that female sexual behavior requires bilateral hypothalamic control.

Ovariectomized Sprague-Dawley and Long-Evans female rats were tested six times for female sexual behavior. At 48 and 24 hr before each test, each female was injected with estradiol benzoate (EB). Six hours before each test, the females were injected with 500 ug progesterone. Progressively decreasing doses of EB were administered until the mean lordosis quotient (LQ) fell below the threshold of 70. For the first test, the first injection was 2 ug EB and the second was 1 ug EB (2/1 ug). By the sixth test, the females received either .5/.5 ug or .2/.2 ug EB. Within each strain, the females were randomly divided into three groups. One group received a left VMN lesion. The second group received a right VMN lesion. The third group was the sham-operated controls. The animals were retested for female sexual behavior 2, 6, 10, and 14 days after the lesions at their assigned threshold dose. On day 18 after the lesions, all females were tested with 2/2 ug EB.

At both the threshold and 2-ug doses, unilateral lesions of the VMN resulted in severe deficits in lordosis compared to the effects of sham operations. At the threshold dose, mean LQ scores after left, right or sham lesions were: 17.9 ± 6.4, 32.1 ± 8.0 and 65.9 ± 8.7, respectively [$F(1, 34) = 18.26$, $p < .001$]. With 2 ug EB, the means were: 56.2 ± 11.9, 60.0 ± 11.9 and 91.4 ± 3.8, respectively [$F(1, 34) = 9.27$, $p < .005$]. The right and left lesions were both effective and there were no differences between strains. At the threshold dose, the deficits produced by the unilateral lesions were as severe as those reported after bilateral VMN lesions. At the 2 ug dose, the unilateral lesion deficits were bimodally distributed with 36% of the females having an LQ ≤ 20 and 54% having an LQ ≥ 80. Only 2 of the 26 lesioned females scored in the midrange. Our data indicate that when the estrogen dose is close to the threshold for eliciting lordosis, the VMN must be intact on both sides of the brain, in most female rats, for the behavior to occur; i.e., bilateral hypothalamic control appears necessary. When the estrogen dose is increased, about half of the females with unilateral VMN lesions respond as well as normal females. Thus under these conditions, unilateral hypothalamic control is often sufficient. Yet a third of the females with unilateral VMN lesions can not respond even to a large dose of estrogen. In these females, hypothalamic control of lordosis may be unilaterally organized.

68.7 SMALL NEOCORTICAL LESIONS IMPAIR THE PERFORMANCE OF LORDOSIS BY GOLDEN HAMSTERS. A. C. Thomas* and J. D. Rose. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Substantial differences have been identified in the subcortical structures controlling lordosis in rats and golden hamsters. These differences in neurobehavioral control appear to extend to the role of the neocortex as well. Cortical ablation or spreading depression facilitates the performance of lordosis in rats, but in hamsters, spreading depression impairs lordosis responding. The present study utilized small, localized neocortical ablations to examine the possiblity that certain cortical regions play a relatively greater role in the control of hamster lordosis than other regions. Ovariectomized golden hamsters in which lordodis responding was induced by sequential injection of estradiol benzoate and progesterone, were tested twice on a test battery evaluating lordosis in response to a sexually active male, and three tests with manually-applied somatosensory stimulation (lordosis maintenance, lordosis elictation latency and lordosis interruption latency). The animals then received, under barbiturate anesthesia, small aspiration lesions of one of the following types: 1) the lumbosacral projection zone, bilaterally; 2) the lumbosacral projection zone, unilaterally; 3) the central face projection zone, bilaterally; 4) the occipital pole, bilaterally; or 5) sham lesions. After a 4-7 day recovery period, the animals were given the battery of lordosis tests three times, at weekly intervals, to assess the effects of the cortical lesions. The results showed that these lesions impaired lordosis responding, but largely without respect to the cortical locus of the lesions. At least 2 hamsters from each of the 4 lesion conditions exhibited complete failure of lordosis in one of the three postlesion tests. Analysis of variance revealed the following lesion effects on the separate response measures: 1) maintenance of lordosis in response to flank stimulation was reduced by all cortical lesions; 2) elicitation of lordosis by stimulation of the lumbar midline region was impaired by all cortical lesions; and 3) interruption of an ongoing lordosis response by face tactile stimulation was impaired by lesions of the face projection region. These lesion effects demonstrated an important degree of neocortical control over a hormone-dependent, species-typical reproductive behavior. The high degree of sensitivity of golden hamster lordosis to localized cortical damage may result from the more sustained nature of the response and broader, more sensitive tactile control of the response than that seen in rats. In addition, these results raise the possiblity that the neocortex may be an important substrate for the action of ovarian hormones in the induction of hamster lordosis. Supported by NIH Grant NS13748.

68.8 **DEFICITS IN FEMALE SEXUAL BEHAVIOR IN THE GOLDEN HAMSTER FOLLOWING KAINIC ACID LESIONS IN THE LATERAL SEPTAL AREA.** G.A. Myatt* AND D.M. Nance. (SPON: D.G. Gwyn). Department of Anatomy, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada

Patterns of female sexual behavior in the hamster are qualitatively different from those observed in the rat. Female hamsters demonstrate tonic immobility and a sustained lordotic response that can last for several minutes following minimal stimulation from the male while in rats, the lordotic response is highly dependent upon continued stimulation from the male and lasts for only a few seconds following a mount or intromission. Also, the hamster requires both estrogen and progesterone priming in order to display female sexual behavior but the rat can show lordotic behavior following treatment with estrogen alone. Electrolytic lesions in the lateral septal area of female rats produce a facilitation in female sexual behavior and increased behavioral sensitivity to estrogen. However, lesions in the lateral septal area produced by the neurotoxin kainic acid (KA) result in deficits in female sexual behavior following estrogen and progesterone treatment (P.B.&B. 18; 605. 1983). Given these major differences in female sexual behavior between the rat and hamster, we have examined the effects of KA lesions in the lateral septal area of hamsters on patterns of female sexual behavior. Adult female golden hamsters (120-160 g) were ovariectomized and subsequently tested twice for female sexual behavior at two week intervals prior to receiving brain surgery. For all behavior tests, animals were injected sc with 60 μg of estradiol benzoate/kg per day for two days and 0.5 mg of progesterone three hours prior to the behavior test on day three. Behavior tests consisted of placing the test animal in a male's home cage for 10 minutes and recording the latency to the first lordosis (maintaining a lordotic posture for atleast 5 sec), length of the longest single bout of lordosis (300 sec maximum) and total lordosis duration for the entire test. All animals were then randomly assigned to either lesion or sham operated control groups. Animals were deeply anesthesized with Somnotol and either KA (0.375 μg in 0.5 μl) or saline (control) were infused bilaterally into the lateral septal area at the rate of 0.1 μl/minute. The microsyringe was left in place an additional 10 minutes before removal. All animals were subsequently tested again for female sexual behavior as described above at one and three weeks following brain surgery. At the end of the test, the animals were perfused and the brain lesions verified histologically. There were no differences between the lesion and control groups for the two pre-lesion behavior tests. Similarly, performance of the sham operated animals were comparable between the post- vs pre-lesion tests. However, following brain surgery, the KA lesioned animals were significantly different from the sham operated controls on all behavioral measures and for both tests. Relative to the saline group, the KA lesioned animals showed significantly longer latencies to the first lordosis, significantly shorter single bouts of lordosis and significantly shorter total duration of lordosis for both behavior tests. Thus, despite the large species differences in the display of female sexual behavior between rats and hamsters, KA lesions of the lateral septal area produce similar deficits in female lordotic behavior. Therefore, in addition to the known inhibitory role of the septal region, this brain area also exerts facilitory control on female sexual behavior in both rats and hamsters. Supported by MRC of Canada.

68.9 ALPHA-1 AND ALPHA-2 NORADRENERGIC RECEPTORS ARE INVOLVED IN REGULATION OF LORDOSIS BEHAVIOR IN THE GUINEA PIG. P.A. VINCENT AND H. H. FEDER. INSTITUTE OF ANIMAL BEHAVIOR AND DEPT. OF BIOLOGICAL SCIENCES, RUTGERS UNIVERSITY, NEWARK, NJ 07102.

Modulation of lordosis behavior by stimulation of noradrenergic receptor subtypes was examined in ovariectomized, estradiol benzoate (10 ug) primed guinea pigs. In the first experiment, systemic administration of the alpha-2 agonist, UK14304-18 (0.01, 0.5, or 0.1 mg/kg, Pfizer Central Research), produced a significant increase in lordosis behavior when these responses were compared with responses seen in control animals injected with saline. In a second experiment, animals were injected systemically with the alpha-1 agonist methoxamine (0.5 mg/kg), UK14304-18 (0.5 mg/kg), or both drugs given together. Methoxamine or UK14304-18 administered alone facilitated lordosis in only a moderate percentage of animals (17% and 39%, respectively). However, when both drugs were given together, 76% of the animals became sexually receptive. A third experiment showed that lordosis behavior facilitated by UK14304-18 could be attenuated by the administration of the alpha-2 antagonist, idazoxan (2.5 mg/kg). Only 29% of sexually receptive animals continued to show lordosis after they received idazoxan. The results obtained from these and previously reported experiments suggest that both alpha-1 and alpha-2 noradrenergic receptors are involved in regulation of lordosis behavior in the guinea pig.

68.10 NORADRENERGIC FACILITATION OF FEMALE SEXUAL RECEPTIVITY IS INDEPENDENT OF PROGESTIN RECEPTOR STIMULATION. J.E. Thornton, P.A. Vincent and H.H. Feder. Institute of Animal Behavior and Department of Biological Sciences, Rutgers University, Newark, NJ 07102.

The noradrenergic (NE) system plays a role in the modulation of lordosis behavior in the female guinea pig. The alpha NE agonist clonidine facilitates lordosis whereas NE synthesis blockers and alpha NE antagonists decrease lordosis behavior. Alpha NE antagonists also decrease hypothalamic progestin receptor levels. Since progestin receptors play a strong role in the lordosis behavior shown by female guinea pigs, the present study determined whether the alpha NE agonist clonidine facilitates lordosis by stimulating progestin receptors.

Ovariectomized adult Hartley strain female guinea pigs were given a behaviorally subthreshold dose of estradiol benzoate (EB: 10 ug). In Experiment 1, females were then given either the progestin receptor antagonist RU486 (10 mg/kg, Roussel-Uclaf, N=11) or vehicle (N=13) at hr 39 and then all were given the NE agonist clonidine (1mg/kg) at hr 40. In Experiment 2, females were given RU486 (10 mg/kg, N=10) or vehicle (N=10) at hr 39 and then progesterone (P; 0.1 mg) at hr 40. All drugs were injected subcutaneously. Females were checked for lordosis just before clonidine or P and then for 14 hours afterwards.

The progestin receptor antagonist RU486 did not decrease the lordosis induced by EB + the alpha NE agonist clonidine. There was no effect on the number of animals responding, the duration of the response or the mean or maximum response. In contrast, RU486 did significantly decrease all of these measures in animals treated with EB + P. This indicates that although P acts through progestin receptors to facilitate lordosis, the alpha NE agonist clonidine does not.

68.11 REGULATION OF MONOAMINE OXIDASE ACTIVITY (MAO) IN THE HYPOTHALAMUS V.N.Luine and J.C. Rhodes*Dept. Psychology, Hunter College and Rockefeller University, New York, N.Y. 10021.

Monoaminergic endings within preoptic-hypothalamic nuclei participate in gonadal hormone regulation of sexual behavior and gonadotropin secretion. In this study the possible involvement in neuroendocrine regulation of the enzyme responsible for catabolism of serotonin and norepinephrine, MAO, was investigated. MAO activity was measured in gonadectomized (GDX) male or female rats and GDX rats receiving estradiol benzoate (EB) at a dose (5 ug for 42 hr.) which was insufficient to induce lordosis. Activity was also measured in males and females when the same EB dose was followed by 500 ug of progesterone (P). In the EB + P groups, maximal lordosis responding was measured in females, but not males, 3 hrs after P. All rats were sacrificed approximately 15 min following behavioral testing, and MAO activity was measured in micropunched samples of brain. EB increased MAO activity of females in the ventromedial nucleus (VMN) and midbrain central gray (MCG) but not in the arcuate-median eminence (Ar-ME), medial preoptic nucleus (mPOA), or dorsal raphe (DR) cell body region. Responses to EB in males showed a totally different pattern since EB decreased activity in the VMN and Ar-ME and did not affect activity in the other areas. Activity in EB + P treated females was decreased in the VMN and mPOA as compared to EB treatment alone. In males, P administration after EB priming did not affect activity in preoptic - hypothalamic areas, but activity in the DR decreased. These sexually dimorphic responses of MAO to gonadal hormones may be related to serotonergic regulation of lordosis.

The sensitivity of MAO to ovarian hormones suggested that MAO activity, like monoaminergic activities, may show flucuations over the estrous cycle. Thus, activity was measured in females who showed repeated 4-day estrous cycles. Consistent with the pattern of changes found after P administration to EB primed females, activity of MAO in the VMN and POA, but not the Ar-ME, was decreased on estrus. In conclusion, the location of changes in activity of MAO over the estrus cycle and the sexually dimorphic response of MAO to hormones strongly support a role for Type A MAO in monoaminergic regulation of hormone dependent sexual behavior and gonadotropin secretion. (Supported by NIH Grant HD12011).

68.12 ESTRADIOL IMPLANTS IN THE RAT STRIATUM STIMULATE LOCOMOTOR ACTIVITY IN RUNNING WHEELS. Edward J. Roy. Psychology Department, University of Illinois, 603 E. Daniel St., Champaign, IL 61820.

Estradiol (E2) affects several aspects of dopaminergic function in the striatum, including sensorimotor performance (Joyce et al., 1982; Becker et al., 1986). Estradiol also has pronounced effects on the spontaneous activity of rats in running wheels, causing a sharp increase in activity on the night of proestrus. This latter response has been thought to be mediated by the preoptic area (POA), because implants of E2 in the POA of ovariectomized (OVX) animals increase activity, whereas implants in the basal hypothalamus do not (Wade and Zucker, 1970). We report here that implants of estradiol into the striatum also increase activity, apparently without spreading to the POA. Adult Long-Evans female rats were housed in cages with activity wheels attached, beginning at 6 weeks of age. Ten rats that displayed regular 4-day cycles of activity for 5 weeks were ovariectomized and implanted with a 23 g guide cannula (coordinates: AP 0.8 mm from bregma, L + 2.2 mm, DV - 2.5 mm from dura). Five days later hormonal treatments were begun. Intracerebral treatment for 6 rats was a 28 g inner cannula extending 2 mm below the end of the guide cannula, filled with either estradiol (3:7 E2:cholesterol) or cholesterol. Implants were left in place for 5 days and then removed. After 3 days animals were given the opposite treatment. Four animals were given injections of either estradiol benzoate (EB, 2.5 ug s.c.) or sesame oil. Implants of estradiol in the striatum caused increases in running wheel activity with a similar delay and to a similar extent as injections of systemic EB. To determine the spread of estradiol from the site of implantation, animals were treated with the same application of E2. After 3 days, animals were sacrificed, the brains were sectioned coronally, and cuts made along the cannula track and below to the ventral surface. Estradiol was extracted and assayed by RIA. E2 was present at the tip of the cannula and along the track of the cannula dorsally. No estradiol was detectible in the POA (the assay sensitivity was 1-2 nM E2 in tissue). These results raise the possibility that the effect of estradiol on locomotor activity in running wheels is a consequence of actions in the striatum rather than the POA, since implants of E2 into the POA might provide E2 to the striatum via diffusion up the cannula track. If estradiol does affect activity by actions in the striatum, it would be an example of hormonal effects on behavior by non-genomic actions of estradiol, since classical estrogen receptors are not present in the striatum.

68.13 EFFECTS OF PROGESTERONE AND NALTREXONE ON SEROTONIN TURNOVER. D.L. Allen and V.N. Luine.
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Gonadal steroids are important regulators of gonadotropin secretion and sexual behavior. Some of the actions of progesterone on LH secretion and sexual behavior are thought to be mediated by serotonin (5-HT). By measuring the increase in levels of 5-hydroxytryptophan (5-HTP) after inhibition of 5-HT synthesis by NSD-1015, King et al. (Neuroendo. 42:344, 1986) reported that progesterone (P) increased the synthesis of 5-HT in preoptic area-anterior hypothalamus, but had no effect in the medial basal hypothalamus. An increase in 5-HT turnover in the medial preoptic area (POA) was also reported after measuring the increase in levels of 5-HT after inhibition of degradation by pargyline. However, in the ventromedial nucleus of the hypothalamus (VMN), P decreased 5-HT turnover. The present study measured 5-HT turnover after P in discrete brain nuclei using the NSD-1015 method in order to localize the effect seen by King et al., and to compare results with studies using the pargyline method to determine turnover. In addition, we studied the effect of naltrexone (NTX), which like P affects gonadotropin secretion and sexual behavior, on 5-HT turnover.

Female rats were ovariectomized and five days later injected with 5 ug estradiol benzoate. After forty-two hours, saline, NTX (3mg/kg) or P (500 ug) was injected, and rats were sacrificed three hours later. NSD-1015 (50 mg/kg) was injected 30 minutes before decapitation. Discrete brain nuclei were removed by the Palkovits "punch" technique and levels of 5-HTP in hypothalamic and preoptic nuclei or the midbrain central gray were analyzed by HPLC. In the medial preoptic area (POA), both NTX and P increased the accumulation of 5-HTP. Neither treatment altered 5-HTP accumulation in the VMN, dorsomedial nucleus, arcuate-median eminence, midbrain central gray, or anterior hypothalamus.

The action of naltrexone on sexual behavior and gonadotropin secretion may be mediated in part by changes in 5-HT turnover in the POA. The effect of progesterone on 5-HTP accumulation in the preoptic area-anterior hypothalamus reported by King et al. can be localized to the POA. Our data agree with King on the lack of an effect in the basal medial hypothalamus, which contrasts with the effect in the VMN seen using the pargyline method. Therefore, progesterone may be altering serotonin turnover in the VMN without affecting its synthesis. (Supported by NIH Grant HD12011 and NIH Training Grant).

68.14 THE EFFECTS ON AGGRESSION AND SEXUAL BEHAVIOR OF BILATERAL ESTROGEN IMPLANTS IN THE FOREBRAIN OF FEMALE SYRIAN HAMSTERS. M.R. Sterner and R.L. Meisel. Purdue University, W. Lafayette, IN 47907.

Earlier studies have implicated several forebrain sites as potential areas for estrogen action on sociosexual behavior in female Syrian hamsters (e.g. Takahashi and Lisk, Physiol & Behav, 34:233). It is not clear whether the decline in aggression reported in these studies is the direct result of hormonal treatment or whether it is a by-product of the testing situation; a receptive female being unlikely to attack a potential mating partner. To avoid this problem, we tested implanted animals with a female stimulus partner (aggression test) as well as an experienced male stimulus partner (sexual receptivity test) to maximize the expression of both aggression and sexual behavior.

In this study, female Syrian hamsters were singly housed for 3 weeks before ovariectomy and guide cannulae implant. Bilateral cannulae were aimed at 1)the ventromedial hypothalamus (VM, n=17), 2) the anterior hypothalamus (AH, n=10), or 3) the medial amygdala (AMY, n=7). After recovering, implanted animals received a baseline behavioral test with no hormone present, followed by a test 3-4 days after intracranial application with 100% powdered estradiol(E_2). Following this test, implant animals received a systemic injection of progesterone (P; 500 ug in 0.1cc oil) followed by the final behavioral test 5-6hrs later.

Animals with VM implants showed a significant decline in the number of attacks on the E_2 + P aggression test as compared to the E_2 alone condition ($p<0.01$). There was no significant difference between the baseline and E_2 only test. The proportion of VM animals displaying lordosis on the receptivity test after E_2 + P was 70.6% (12/17). Animals in the AH group showed a decline in aggression following P which approached significance ($p<.06$). For the AH group, 30% (3/10) of the animals responded with lordosis; however the cannulae of these three receptive animals were located right at the border between the AH and the VM. Finally, animals in the AMY group showed no significant hormone effects on aggression, while 28.6% (2/7) responded with lordosis. These results suggest that the VM is the site most sensitive to E_2 action on sociosexual behavior in the Syrian hamster.

[This study was supported by N.I.H. grant HD21478.]

68.15 PRIMING EFFECTS OF MEDIAL PREOPTIC AREA IMPLANTS OF ESTRADIOL ON MATERNAL BEHAVIOR. H.B. Ahdieh, A.D. Mayer* and J.S. Rosenblatt*. Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102.

Previous studies have shown that female rats exposed to low circulating levels of estradiol (E2) during pregnancy, or ovariectomized and treated with estrogen and progesterone for 16 days, exhibit maternal behavior in response to systemic low doses (5 ug/kg) of estradiol benzoate (EB). Nonpregnant ovariectomized-hysterectomized (HO) females require large (100-200 ug/kg) doses of EB to exhibit maternal behavior. The present study investigated whether dilute bilateral implants (1%) of E2 in the medial preoptic area (MPOA) of nonpregnant HO females for 48 hr would enable them to exhibit maternal behavior in response to the low systemic dose of EB. A 1% E2 implant which elevated MPOA nuclear estrogen receptor concentrations to day 16 pregnancy levels resulted in the onset of short-latency maternal behavior 48 hr after systemic treatment with 5 ug/kg EB. The E2 implants in the absence of EB, or cholesterol implants with EB, were ineffective in enhancing maternal behavior. The results support both a priming and triggering role for estrogen in the stimulation of maternal behavior.

68.16 COMPLEX OPERANT BEHAVIOR ACROSS THE MENSTRUAL CYCLE IN THE BABOON: A MODEL FOR PERIMENSTRUAL STRESS. M.G. Paule, M.J. Hulstyn* and E.K. Killam*. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079 and Department of Pharmacology, UCD School of Medicine, Davis, California 95616.

Performance under Incremental Repeated Acquisition (IRA) schedules was monitored in 3 young adult female baboons (Papio papio) across 3 to 8 regular menstrual cycles (26-35 day mean cycle lengths). Menstrual cycle status was scored by days since the beginning of last observed menses and by visual inspection of perineal tumescence (ischial swelling) and correlated with operant performance. The IRA tasks consisted of the acquisition (learning) of a new sequence of lever presses each test day for food reinforcers. Percent task completed (number of correct lever sequences performed/number correct lever sequences possible in a 30-min session) peaked during the peri-ovulatory periods (individual means = 54, 77 and 89, respectively) and declined to significantly lower values perimenstrually in all 3 animals (individual means = 22, 52 and 54, respectively). Efficiencies (correct responses/total responses) were generally unaffected by menstrual cycle status whereas, response rates (lever presses/min) decreased significantly during perimenstrual periods in most cases. These data suggest that complex operant performance in the nonhuman primate may serve as an indicator of hormonally-related changes in CNS function that may model changes in the human CNS now associated with the pre- and/or perimenstrual period. Supported in part by a grant-in-aid of research from The Society of the Sigma Xi and a UCD President's Undergraduate Fellowship.

68.17 COMPARATIVE BEHAVIORAL EFFECTS OF PROGESTERONE AND ALPHAXALONE.
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Studies on social behaviors in syrian hamsters have shown a decrease in aggressiveness following treatment with progesterone. The mechanisms by which progesterone may inhibit aggression in hamsters are not clear, but it has been suggested that this effect could be related to the anesthetic properties of progestins. We have addressed this hypothesis by comparing the effects of progesterone and alphaxalone, one of the most potent anesthetic agents in the progestin family, on aggressive and sexual behaviors and on locomotor activity.

Adult gonadectomized male and female hamsters housed singly were used. One week after arrival the animals were tested for their basal aggressive and sexual behaviors. A week later the individuals assigned to the aggression study were injected s.c. with progesterone or alphaxalone at the same dose (1mg/0.2ml oil) during four consecutive days and they were tested on the last two days, 4-5 hours after injection, in a home-away rotation. Pairs tested were of the same sex, receiving the same treatment.

A week after the preliminary test, the individuals in the sexual behavior study were injected s.c. with 10ug of estradiol benzoate, followed 48 h later by s.c. injection of progesterone or alphaxalone (1mg/0.2ml oil). All animals were tested 4-5 h later for their feminine sexual behavior with a control, group-housed, intact male hamster.

In the locomotor activity study the animals were housed individually in a Wahmann activity wheel. After an habituation period the individuals were injected s.c. 4 days with oil and the following 4 days with progesterone or alphaxalone (1mg/0.2ml oil). The activity was quantified 5 h after the injection.

Alphaxalone treatment did not affect the aggressive display of either sex, whereas progesterone decreased the frequency of aggressive postures and increased the latency to attack, as has been reported previously (Physiol. Behav. 39: 225-229, 1987). Alphaxalone induced precopulatory responses (tail-up) in females but failed to promote receptive behavior, while progesterone induced sexual responses in both sexes. Alphaxalone, but not progesterone, decreased the locomotor activity in both sexes 5 h after the injection, although it was statistically significant only in males on days 1 and 2.

Our results show that a potent anesthetic progestin, alphaxalone, s.c.-injected, may decrease locomotor activity slightly, but it does not affect aggressive performance in hamsters. Therefore, the reduced aggressiveness in the progesterone-treated animals is not likely to be related to the hormone's anesthetic or sedative properties.

68.18 THE EFFECTS OF PROGESTERONE ON PREGNANCY-INDUCED AGGRESSIVE BEHAVIOR IN THREE INBRED STRAINS OF MICE. S. Ogawa, Lab. of Behavior Genetics, Univ. of Connecticut, Storrs, CT 06268

Previously, we reported (Soc. Neurosci. Abs., 12, 841, 1986) the variation in plasma levels of progesterone in three inbred strains of mice which differ in the levels of pregnancy-induced aggressive behavior (PIA). In the present study, the effects of supplemental progesterone on PIA were examined.

Nulliparous females from AKR/J, BALB/cN, and DBA/2J (10-11 wks of age) were mated with a CFW male during the night of estrus and placed in an individual cage on the next morning if a vaginal plug was found (Gestation Day 0). On Day 1, they were treated with subcutaneous silastic implants that contained crystalline progesterone. Females from AKR/J received one (1P) or two (2P) 10mm silastic capsules. Females from BALB/cN and DBA/2J received two (2P) or three (3P) capsules. Control mice from each strain received an empty capsule. They were either tested once on Day 5 or tested repeatedly on Days 5, 9, 13 and 17 (N=6-8/group/hormone treatment/strain). Female aggressive behavior (defined as eight different behavioral acts) toward an unfamiliar, olfactory bulbectomized, group-housed CFW male was recorded during a 10-min observation period. Blood samples were taken immediately after the end of behavioral tests on Day 5 or Day 17 for the determination of plasma levels of progesterone by radioimmunoassay.

On Day 5, 2P-treated and 3P-treated BALB/cN and DBA/2J had similar levels of circulating progesterone as 1P-treated (64-74 ng/ml) and 2P-treated (88-98 ng/ml) AKR/J, respectively. Supplemental progesterone treatment failed to elevate the incidence of PIA further in the highly aggressive strain, AKR/J. Levels of PIA in 1P and 2P-treated AKR/J mice did not significantly differ from those in control mice on either days tested. In the moderately aggressive DBA/2J, progesterone increased the levels of PIA across the gestation period and advanced the onset of PIA. A higher percent of animals showed PIA on Days 5 and 9 in progesterone-treated groups than in the control group. Progesterone-treated DBA/2J mice showed the highest levels of PIA on Gestation Day 9, whereas control mice showed very little aggressive behavior on Day 9 and the maximum levels of PIA on Day 13. Progesterone treatment induced aggressive behavior in the nonaggressive BALB/cN strain on Days 9, 13, and 17 but not on Day 5. PIA levels of progesterone-treated DBA/2J and BALB/cN did not reach the levels of AKR/J.

These results suggest that progesterone might have facilitatory probably modulatory, effects on PIA. Progesterone and estradiol sysnergism in the onset of PIA should be investigated in further studies.

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68.19 PRENATAL STRESS AFFECTS STRESS-INDUCED AND MORPHINE ANALGESIA IN RATS. C.H. Kinsley, P.E. Mann and R.S. Bridges, Department of Anatomy and Cellular Biology, Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, MA 02115.

Prenatal stress (PS) affects many of the same behaviors which in adulthood are under the influence of opiates and endogenous opioids. For example, aggressive, maternal, regulatory, and sexual behaviors have all been tied to opioid function in the adult. Recently, Hammer (Brain Res. 360: 65, 1985) reported that opiate receptors develop in a sexually dimorphic pattern, and that androgens normally suppress their formation. Prenatal stress results in severe perturbation of the developmental actions of androgens, and therefore, may disrupt both the formation of opiate receptors and accompanying analgesia. We were interested in determining if two forms of analgesia, morphine-induced (opioid receptor mediated), and stress-induced (cold-water swim, CWS, non-opioid) would altered in prenatally-stressed (P-S) male and female rats. Timed-mated Sprague-Dawley females were exposed to heat and restraint stress (three daily half-hour sessions, 0830, 1230, and 1630 h) from days 14-21 of pregnancy. Control animals remained undisturbed throughout pregnancy. All offspring were cross-fostered to untreated lactating dams at birth. Between 100-120 days of age, baseline tailflick latencies were recorded for all animals. P-S and Control animals were exposed to 3.5 min CWS (2^{o}C) and pain thresholds were determined at 30, 60, 90, and 120 min post-swim. P-S females exhibited significantly lower pain thresholds than Control females, whereas P-S and Control males did not differ. Five to six days later, these same animals were assessed for morphine (5.0 mg/kg) analgesia in a three-hour test, tailflick latencies recorded every 30 min. P-S females exhibited significantly greater analgesia following morphine treatment than Control females, whereas P-S males were significantly less analgesic than Control males. These data suggest that one manner in which prenatal stress may be acting to influence adult behavioral potentials is through alterations of endogenous opioid systems. Supported by NIH 5T32-HD-07130, C.H.K. trainee, NIH 5T32-AM-07337, P.E.M. trainee, and by NIMH (RSDA K02-MH00536) and NIH (HD-19789) R.S.B., awardee.

68.20 FEMINIZATION OF MALE SLEEP CYCLE RHYTHMICITY BY PRENATAL STRESS. W. Fishbein and P.F. Bright*. Psychobiology Lab. CUNY, The City College and Graduate School, New York, 10031.

It is generally recognized that differentiation of sexual behaviors depends on the secretion of testosterone by the fetal testes and prenatal stress alters the perinatal release of circulating testosterone with concomitant changes in adult male sexual behaviors (Ward, I.L., Psychoneuroendocrin., 9:13. 1984). Since sleep-cycle rhythmicity is gender specific (accompanying paper at this meeting), we considered that sleep rhythms might also be altered in males born of stressed mothers.

The procedures are identical to those in our accompanying paper except the pregnant dams received 90 min (0730-0900) per day of heat-restraint stress beginning on day 7 of gestation and lasting 14 days. Within 12 hrs postpartum, 6 male and 6 female CF-1 mice were randomly cross-fostered to a nonstressed lactating dam and reared with five other litter mates until weaning. None of the subjects is a sibling or was reared with any other subject. On day 50 EEG and EMG leads were implanted. The animals were placed in a recording chamber and on day 57 connected to a commutator for 72 hrs adaptation; on day 61 sleep-wakefulness cycles were recorded for the next 48 hours.

The prenatally stressed subjects time asleep (or awake) was indistinguishable in the two sexes. Males averaged 11.07 hrs/24 hrs asleep, females 10.22 hrs, ($F(1,10)=2.04$). Despite this similarity, the stressed males slept significantly more than the control males (9.71 hrs), ($F(1,10)=6.25$ $p<.03$), whereas the stressed females were indistinguishable from the control females (9.63 hrs).

Stressed males and females were indistinguishable on all measures of REMS and SWS, as were stressed and control females.

However, the comparisons of the stressed males with normal males revealed that the stressed males spend significantly more time in REMS than control males ($F(1,10)=9.87$ $p<.01$). Moreover, the difference was totally accounted for by a greater number REMS episodes in the stressed males; 2.33/hr, control males 1.60/hr ($F(1,10)=37.14$ $p<.001$), whereas mean duration of REMS episodes was identical. The stressed males were indistinguishable from the control females.

The results imply that sleep-cycle rhythmicity follows the same general rule as reproductive behaviors: the basic plan is inherently female. In the absence of male hormones the genetic male develops a female brain and female sleep-cycle rhythms. Apparently prenatal stress acts to change the organization of rhythmicity as well as reproductive behavior per se. We think it likely that the critical brain region affected is the sexually dimorphic medial preoptic nucleus of the forebrain by gonadotrophic influences.

68.21 DIETARY TRYPTOPHAN RESTRICTION PRODUCES AN UPREGULATION OF THE NEUROENDOCRINE RESPONSE TO INFUSED TRYPTOPHAN IN HEALTHY HUMAN SUBJECTS. P.L. Delgado*, D.S. Charney, L.H. Price, G. Anderson, H. Landis* and G.R. Heninger, (SPON: J. Cooper) Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Both increases and decreases in tryptophan (TRP) intake can alter brain serotonin (5-HT) levels in laboratory animals. In humans, dietary TRP restriction has been shown to produce mild impairment of attentive performance and to increase subjective reports of negative moods, although effects on brain 5-HT levels or turnover or neuroendocrine effects have not been demonstrated. There is considerable data indicating that prolactin (PRL) release is mediated, via the 5-HT system, and large doses of intravenously infused TRP have been shown to increase plasma PRL in humans. In order to assess the neuroendocrine effects of dietary TRP restriction, a TRP infusion was administered before and during 2 different magnitudes of TRP restriction diets in healthy human subjects. METHODS: The average USA diet contains 1,200 mg TRP per day. Subjects participated in a 7 day TRP restriction diet composed of normal food designed to be nutritionally balanced which contained either 700 mg TRP per day (11 subjects) or 200 mg per day (6 subjects). Within 7 days prior to beginning the diet and on the morning of the 8th day of the diet each subject received an infusion of TRP (100 mg/kg) at 9 a.m. and plasma was collected at regular intervals for assessment of PRL levels using standard RIA methods. Subjects were fasting the 12 hrs. before the 9 a.m. TRP infusion. Total plasma TRP levels were measured by HPLC prior to each TRP infusion. RESULTS: The peak prolactin response (peak minus base $\pm$ SD) before and on the 8th day of the 700 mg/per day diet was respectively, 11.4 $\pm$ 7.9 and 12.2 $\pm$ 8.1 ng/ml (mean, on diet minus prediet = .9 $\pm$ 4.1 ng/ml, NS). On the 200 mg/per day diet all six subjects demonstrated an increased response and the corresponding values were 16.3 $\pm$ 6.3 and 22.0 $\pm$ 5.5 ng/ml respectively (mean, on diet minus prediet = 5.8 $\pm$ 4.1 ng/ml, $p<.03$). The increase of 5.8 ng/ml in the prolactin response during the 200 mg diet was significantly larger than the increase of .9 ng/ml on the 700 mg diet ($p<.03$). Only the 200 mg diet produced a drop (13%) in the 9 a.m. fasting total TRP levels on the day of the infusion. DISCUSSION: The 200 mg per day TRP diet reduced plasma TRP levels and produced an apparent supersensitivity of the PRL response to the TRP infusion. Since the 700 mg per day TRP diet did not reduce plasma TRP levels or produce a change in the PRL response, this is evidence that the observed effect is specific to dietary TRP levels. The 200 mg TRP diet was well tolerated by the healthy subjects. It may be a useful method for studying the role of dietary TRP and modifying the functional capacity of the 5-HT systems in investigations of human medical and neuropsychiatric disorders.

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68.22 LEARNING IMPAIRMENT AFTER REVERSAL OF SLIGHT PTU-INDUCED POSTNATAL HYPOTHYROIDISM IN RATS. M. Akaike*, N. Kato, H. Ohno*, T. Kobayashi* and T. Sakaguchi*. Pharma R&D Division, Hoechst Japan Ltd., Kawagoe, Saitama 350. Dept. of Psychiatry, Shiga Univ. Med. Sci., Otsu, Shiga 520-21, Japan.

Thyroid hormones are essential for the growth and development of the brain, and congenital lack of thyroid secretion induces cretinism marked by mental retardation. The screening of the newborn for such lack has recently started, making it possible to prevent the onset of cretinism. However, hyperactivity and learning deficits have frequently been reported to occur even in children treated within 3 months of age. Rats with severe induced hypothyroidism have been used extensively as a model of cretinism but there has been no good model system for the above hyperactivity and learning impairment. This investigation was undertaken to produce such model in the rat by giving newborn animals via milk the antithyroid drug, n-propylthiouracil (PTU). Methods: Eight females of Sprague-Dawley rats were allowed to litter normally. Four animals were given drinking water containing PTU at 0.02% ad libitum on days 0-19 of lactation and other 4 animals served as controls. Four male and 4 female offspring were selected per litter at delivery and were weighed weekly. The serum level of thyroxine (T_4) was determined at 4 and 10 weeks of age. At 6 weeks, the animals were given 3-min open field trials 3 times at about 15-min intervals and observed for ambulation and rearing. Fifteen males and 15 females were selected per group and given, at and after 13 weeks of age, a trial on the radial eight-arm maze (RAM) per day for 20 days. The 20 trials begun with the first well-performed trial (WT). The brain weight was measured at the end of the maze test. Results: PTU rats showed depressed body weight gains from 2 weeks onward but no change in either serum T_4 level or brain weight. In hypothyroid animals ambulation increased in both sexes during the study while rearing was comparable to that of controls. The RAM test revealed that (a) more trials were required for PTU rats to establish WT, (b) only 3 males and one female reached criterion in the treated group while 13 male and 10 female controls did, and (c) PTU animals made more errors in total and less correct responses in the first 8 choices than controls. Conclusion: In PTU rats, T_4 levels were already normal at 4 weeks of age but ambulation and the number of trials required to establish WT were increased, indicating hyperactivity and lowered adaptability to the environment which are signs of cerebral disturbances. These results suggest that the abnormalities may be related to hippocampal damage known to cause severe impairment for RAM.

68.23 ALTERATION OF THE GABA RECEPTOR-COUPLED Cl^- CHANNEL AND IN VIVO BENZODIAZEPINE RECEPTOR BINDING IN LEARNED HELPLESSNESS. A.L. Morrow, R. Weizman*, A. Weizman*, S.I. Deutsch*, J.N. Crawley, S.M. Paul* and R.C. Drugan, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892

Recent behavioral evidence suggests that inescapable tail shock may produce alterations in the GABA/benzodiazepine receptor Cl^- channel complex. A putative animal model of depression, learned helplessness involves the failure to learn a shuttle-escape task 24 hours after a session of inescapable tailshock. Thus we have investigated whether the development of learned helplessness alters the function of the GABA-gated Cl^- channel, measuring muscimol-stimulated $^{36}Cl^-$ uptake in vitro. Further, we have labelled central benzodiazepine receptor binding sites in vivo with [^{3}H]Ro15-1788 in rats subjected to this paradigm.

$^{36}Cl^-$ uptake was measured in synaptoneurosomes from cortex (Schwartz et al, 1986) immediately following the shuttlebox learning task. Stimulation of $^{36}Cl^-$ by the GABA agonist, muscimol (50 µM) was reduced approximately 22% in the rats which failed the task ("helpless") compared to naive control rats (ANOVA $p<.05$, Newman Keuls $p<.05$). Rats which learned the task did not show a significant reduction in $^{36}Cl^-$ uptake, compared to naive rats. A similar reduction in $^{36}Cl^-$ uptake in the "helpless" rats (25%) was detected using 5µM muscimol.

In vivo benzodiazepine receptor occupancy was measured by injecting [^{3}H]Ro15-1788 (2.5 µCi) via the lateral tail vein immediately following the final learning trial and sacrificing the rat 20 min. later. Nonspecific binding was assessed by administering clonazepam (5 mg/kg i.p.) immediately prior to the radioligand. Failure to learn ("helpless" condition) was associated with a decrease in specific [^{3}H]Ro15-1788 binding in cerebral cortex (43%), hippocampus (35%), and striatum (33%) compared to naive rats (ANOVA $p<.05$, Newman-Keuls $p<.05$). In hypothalamus and cerebellum, there was a decrease in specific [^{3}H]Ro1501788 binding in both animals that failed and animals that learned compared to naive controls. We are presently investigating whether these alterations in in vivo [^{3}H]Ro15-1788 binding and $^{36}Cl^-$ uptake are due to the stress of inescapable shock or are specific to learned helplessness. These data add support to previous observations that suggest that the GABA/benzodiazepine receptor complex may be functionally altered following exposure to stress.

69.1 SPIDER VENOM BLOCKADE OF DENDRITIC CALCIUM SPIKING IN PURKINJE CELLS STUDIED *IN VITRO*. M. Sugimori and R. Llinás. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Purkinje cells are known to generate Na and Ca-dependent spikes upon direct stimulation and following activation of the climbing fiber afferents. *In vitro* recordings in guinea pig Purkinje cells have demonstrated that the AG1 toxin from Agelenopsis aperta venom (BioActives, Inc.) is capable of blocking dendritic Ca spikes quite specifically at a concentration of 20 nM, without affecting the rapid Na conductances or the low-threshold non-inactivating Na conductances of the same cells. Thus, direct somatic depolarization of Purkinje cells produced rapid, Na-dependent, repetitive firing as well as oscillatory Ca-dependent spikes. After bath application of AG1 toxin the TTX-sensitive, fast action potentials were modified in the sense that the large afterhyperpolarizations (in part due to activation of a Ca-dependent K conductance) were reduced in amplitude. In addition, the presence of somatic plateau potentials, due to the activation of the low-threshold non-inactivating Na conductance, was unaffected by the toxin. The action of this toxin on dendritic spiking was quite dramatic. Ca-dependent all-or-none responses were clearly blocked as recorded from the somatic level and from direct dendritic impalement. A similar set of experiments were implemented in which the Purkinje cells were activated synaptically via the climbing fiber afferents. Initially, in the presence of AG1 a blockage of dendritic Ca spikes and a simplification of the complex spike in the cells was observed. However, as the blockage continued, the synaptic potential produced by the climbing fiber activation was not blocked. This indicates that the toxin affects Ca-dependent conductance in the dendrites but does not seem to modify greatly the Ca current which generates transmitter release from the climbing fiber. These results suggest that the Ca channels reponsible for mediating dendritic Ca spikes may be different, in some measure, from those responsible for synaptic transmitter release. [Supported by NS-13742 from NINCDS.]

69.2 **BLOCKADE OF HARMALINE-INDUCED TREMORS BY ALCOHOLS MAY BE MEDIATED BY THE LOW THRESHOLD CALCIUM CHANNEL.** C.M.Sinton[1], B.Krosser*, K.D.Walton and R.R.Llinas, Dept. Physiol. Biophys., New York Univ. Med. Ctr., NY 10016 and [1]Pharmaceuticals Divn., Ciba-Geigy Corp., Summit, NJ 07901.

Intracellular recording of inferior olive cells in the guinea-pig brainstem slice (Llinas & Yarom: Neurosci. Abstr. 12, p. 174, 1986) has demonstrated that higher molecular weight alcohols act as blockers of the low threshold calcium channel (LTCC). These blockers thus provide a possible tool for understanding the function of the pacemaking cellular networks of the olivo-cerebellar system, since the LTCC has been implicated in neuronal oscillatory behavior in this system (Llinas & Yarom: J. Physiol. 315, p. 569, 1981). These experiments were designed to address this question by examining the effects in vivo of some alcohols on harmaline-induced tremor. Male rats (Mbf:[SD]) were chronically implanted, under pentobarbital anesthesia, with EMG electrodes inserted between the splenius and acromiotrapezius muscles. After recovery from surgery, the electrodes were connected to a polygraph recorder (Grass Model 78D). Baseline EMG signals were recorded for 15 min, before harmaline was administered (9 mg/kg i.p.), and EMG recordings were then continued for a further 60 min. Harmaline produced marked alterations in the EMG, with behaviorally evident tremors of the head, neck and body corresponding to high amplitude rhythmic EMG oscillations. The animals also tended to crouch to minimize movement, so that the tremors were most evident when the rats were moving. EMG signals were recorded on FM tape for subsequent off-line spectral analysis: the EMG power frequency spectrum after harmaline treatment showed a marked peak between 6 and 9 Hz, the tremor frequency. In subsequent experiments, various doses of 1-octanol, mixed in a Tween-80 vehicle, were administered 15 min after the harmaline treatment, and the EMG power spectrum compared to that obtained with harmaline alone. The vehicle itself had no effect on the tremors. At a dose of 0.25 mg/kg i.p., 1-octanol blocked the harmaline-induced tremor for about 30 min, an effect which was evident both behaviorally and on the EMG power spectrum. Lower doses had little effect, and higher doses caused behavioral sedation that lasted for about 60 min. To examine the specificity of this action of 1-octanol, other isomers of octanol were tested in this protocol, including 2-octanol, 3-octanol and 4-octanol. Results with these isomers were similar although they were not equally effective in blocking the tremors. These data imply that the reported action of higher alcohols on the LTCC may be observable in vivo, and that this model can provide information about the relative potency of different compounds in blocking this channel. Novel therapies for treating tremor may eventually be developed from compounds which selectively block the LTCC.

69.3 USE OF VOLTAGE SENSITIVE DYES TO IMAGE SPATIAL PATTERNS OF CEREBELLAR NEURONAL ACTIVITY. J.H. Kim, M.B. Dunn* and T.J. Ebner. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455.

Voltage sensitive dyes have been shown to be useful for studying the spatial and temporal properties of neuronal ensembles. In this study we used voltage sensitive dyes and image processing technology to evaluate spatial features of the neuronal circuitry of the cerebellar cortex. The cerebellar cortex is an excellent candidate for voltage sensitive dyes with its shallow laminar architecture and known circuitry and physiology. In the anesthetized, ventilated and paralyzed rat, voltage sensitive dyes were used to stain the exposed cerebellar cortex. Although different stryl dyes were tried RH 237 or RH 414 were used in most experiments. The animals were mounted stereotaxically in a modified Zeiss Universal Microscope equipped for epi-fluorescence and electronically shuttered. Parallel fiber stimulation was achieved with a microelectrode inserted just into the molecular layer. Dyes were excited at the appropriate wave lengths from a stabilized DC mercury lamp. After filtering the emitted light the images were recorded using a low light, charge coupled device video camera (384 X 419 pixels) equipped with a second generation microchannel plate intensifier. Removal of DC components, filtering, and windowing of the video signal was performed prior to storage and processing. Image capture and surface stimulation were coupled to the cardiac cycle to reduce movement artifacts. Images of cerebellar activity evoked by surface stimulation were averaged (up to 1200) and appropriate control images subtracted. Image processing was done using the Biomedical Image Processing Laboratory at the University of Minnesota. Electrophysiological field maps evoked by the surface stimulation were also constructed. Resultant images to surface stimulation revealed a "parallel fiber beam" arrangement running longitudinally which was restricted in width but 1-2 mm in length. More complex patterns were also observed. The spatial patterns were graded and modifiable with the intensity of surface stimulation and could be eliminated along with the evoked field potentials by topically applied lidocaine. These preliminary results suggest that voltage sensitive dyes will be an important tool for studying spatial patterns of activity in the cerebellar cortex. Supported in part by NIH grant NS-18338, Minnesota Medical Foundation, EMPI, Inc., and the Graduate School of the University of Minnesota.

69.4 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF RAT CEREBELLAR NEURONS IN MATURE AND DEVELOPING CULTURES. D.L. Gruol and C.P. Crimi*, (SPON: M. Howard), Div. Preclin. Neurosci. and Endocrin., Scripps Clinic and Res. Foundation, La Jolla, CA 92037.

We have used immunohistochemical techniques and antibodies to gamma-aminobutyric acid (GABA), parvalbumin (PAV) and cyclic GMP dependent protein kinase (cGPK) to identify populations of cerebellar neurons in culture that exhibit morphological features similar to each of the six neuronal types in the cortical region of the cerebellum in vivo: the Purkinje neuron (PN), granule cell, basket cell, stellate cell, Golgi cell and Lugaro cell. The cultures were prepared from the cortical region of immature cerebella obtained from 20 day rat embryos as previously described (Gruol, Brain Res. 263, 1983). Immunohistochemical procedures were as previously described (Gruol and Franklin, J. Neurosci., 1987). These studies revealed that neurons identified as PNs and cerebellar interneurons (granule, stellate, basket, Golgi, Lugaro) were present at all culture ages. The morphological and physiological properties of these neuronal types were compared at three culture ages: 7-9 DIV, 12-16 DIV and >21 DIV (DIV = days in vitro) reflecting early, intermediate and late periods in cerebellar development. The morphological features of the interneurons were similar at all culture ages, in contrast to the PNs which did not exhibit prominent dendritic structure until 12-16 DIV. Extracellular recordings from the neurons at the different culture ages revealed that all classes of neurons exhibited spontaneous activity but that only a portion of the interneurons were spontaneously active. Spontaneously active interneurons exhibited similar patterns of activity at all culture ages. The patterns were characterized by low firing rates (~2-6 Hz) and irregular spiking. At young culture ages, the PNs exhibited patterns of activity similar to the interneurons; at older culture ages the patterns were significantly different and were characterized by high frequency (>10 Hz) spike activity usually in regular patterns. All cerebellar neurons were excited by the transmitter glutamate (Glu). The response of the interneurons to Glu consisted of a brief burst of spikes at all culture ages. In young PNs, the response to Glu was similar to that observed in the interneurons; at older culture ages the response to Glu was prolonged and multiphasic. These data indicate that the cerebellar interneurons in culture express morphological and physiological properties that are significantly different from the PN. These differences may relate to the different functional roles played by the neuronal types in cerebellar circuitry. (We thank Dr. Paul Greengard for the cGPK antibody and Dr. Marco Celio for the PAV antibody; Supported by NINCDS grant NS271777 and NIAAA grant AA06665).

69.5 MODULATION EFFECT OF CEREBELLAR STIMULATION ON MOTOR SYSTEM EVOKED POTENTIALS IN THE CAT. S.S.Haghighi and W.J. Levy. Dept. of Surgery, Division of Neurosurgery, Sch. of Med. Univ. of Missouri, Columbia, MO 65212.

Excitation of the synaptic membranes in the pyramidal cells of the motor cortex as a result of cerebellar surface stimulation has been reported. Furthermore, similar excitatory postsynaptic potentials (EPSPs) have been documented in the neurons within brainstem and the motor neurons of the spinal cord in decorticated cats.It has been reported that cerebellar stimulation, either directly on the cortex or transcranially through the occipital bone, produces evoked responses in the spinal cord, the peripheral nerves and the muscles. These cerebellar evoked potentials (CEPs) are predominately ipsilateral, are unaffected by pyramidotomy, and may differentially activate extrapyramidal pathways. An interaction between the MEP from motor cortex stimulation and the CEP has also been reported.

To explore this, 7 cats, anesthetized by ketamine, xylozine anesthesia and paralyzed with Pavulon, had MEPs from anodal surface stimulation of the somatomotor cortex. CEPs were elicited through bipolar surface stimulation of the paravermal areas of the cerebellum. The simultaneous stimulation of MEP and CEP produced a 250% increase in the spinal cord response amplitude. Cerebellar stimulation prior to motor cortex stimulation was examined at several interstimulus delays (ISDs). This had a modulation effect with a 450% increased in MEP amplitude. The maximum modulation effect recorded at spinal cord was at 2 msec ISDs (p .02). This modulation effect of the MEP by the CEP may be a useful indicator of motor system status and pyramidal- extrapyramidal interactions

69.6 CEREBELLAR STABILIZATION OF THE VESTIBULOSPINAL REFLEX IN SHARKS - A MODEL SYSTEM. Richard S. Babb. Rockefeller University, York Ave., N.Y., N.Y., 10021.

The classical three-neuron pathway which provides part of a negative feedback loop for stabilizing the retinal image using vestibular information, has its counterpart for stabilizing the body. This latter feedback loop uses the descending branch of the medial longitudinal fascicle (MLF), instead of the ascending branch (Smeets and Timerick, 1981) which is used by the oculomotor system. However, the vestibular organs cannot analyse the angular acceleration into components produced by either passive external torques or torques actively produced by the shark's own trunk musculature. The MLF descending vestibulospinal system would thus have the undesirable characteristic of "correcting" for active movements produced by the shark itself. Such feedback by the vestibulospinal system acting alone could result in instability such as uncontrolled oscillations of the shark's body.

According to the proposed model, active shark movements would be compensated for by activity of the cerebellum and its pathways. A substantial primary vestibular input to the cerebellum is known to be received by the ipsilateral vestibulolateral lobe of the cerebellum (Boord and Roberts, 1980), and some Purkinje cells of the cerebellum are thought to project back to and terminate directly on the vestibular nucleus. Further, the spinocerebellar tracts could carry information about intended movements of the shark's body to the cerebellum. The vestibulocerebellar system is thus a viable candidate for the neural substrate which could act to subtract the active movement component from the vestibular information, leaving the passive component only for the error term used to correct body movement for external perturbations. Such a model entails that the cerebellar cortex would function to compute the amplitude and phase of the active movement compensation. One might speculate that the uniform arrays of Purkinje cells and parallel fibers, common to the cerebella of all extant vertebrates, are necessary for these computations. Such structural commonality suggests that the vestibulocerebellar system might well have originated in our common vertebrate ancestor.

69.7 ZONAL ORGANIZATION OF OLIVO-NODULUS PROJECTIONS IN RABBITS. Raymond T. Henry, John D. Connor and Carey D. Balaban*. Departments of Pharmacology, Anatomy and Surgery. College of Medicine, The Pennsylvania State University, Hershey PA 17033.

Anatomic and physiologic studies indicate that sagittal groups of Purkinje cells form basic functional units of cerebellar cortex. These groups, termed *zones*, are defined by climbing fiber inputs and output projections to distinct pools of cerebellar and vestibular nuclear neurons. The organization of olivo-nodulus projections was assessed with anterograde autoradiographic, anterograde degeneration and retrograde tracing techniques. For anterograde tracing, adult New Zealand white rabbits received injections of either [2,3,4,5-^{3}H]-L-leucine (20 μCi/50 nl) or 3-acetylpyridine (200-250 nl/injection, 27.5 μg/μl) in the dorsal cap (DC), ventrolateral outgrowth (VLO) and beta nucleus (β). After a 1-3 day survival period, rabbits were perfused transcardially under deep pentobarbital anesthesia. Brains were processed histologically with standard autoradiographic or cupric-silver degeneration methods. The retrograde injections were into the nodulus and posterior lobe lobules VII-IX. Four rabbits received iontophoretic injections (2 μA, 1-2 min) of horseradish peroxidase (HRP). Eleven rabbits were given pressure injections (100-150 nl) of both HRP-wheat germ agglutinin complex (10% solution in saline) and rhodamine-labeled latex microspheres (Tracer Technologies, Bardonia, NY) in distinct sites. After a 1-2 day survival, they were perfused transcardially and sections were stained for HRP with a tetramethylbenzidine substrate. Rhodamine microspheres were identified on a Zeiss fluorescence microscope. The data indicate that the caudal aspect of DC projects to a sagittal strip in the medial 0.5-1 mm of the nodulus. The rostral aspect of DC and VLO projects to an adjacent 0.5-1 mm band in the nodulus. The intermedio-caudal DC and adjacent regions of dorsal β project to the lateral margin of the ventral surface of the nodulus. The lateral margin of the dorsal surface of the nodulus and the ventral surface of lobule IXd, though, appears to be innervated by the rostral aspect of the dorsomedial cell column and the rostral medial accessory olive. Finally, regions of the nodulus and IXd on the bank of the postero-lateral fissure represent a transition between DC-VLO and β projection fields to the nodulus and more rostral lobule IX, respectively. These data provide a basis for investigating the significance of nodulus climbing fiber zones for visual-vestibular integration and alterations in cardiovascular tone. (Supported by USPHS NS 19850 (C.D.B.), DA 03454 (J.D.C.) and RCDA NS 00891)

69.8 VESTIBULAR MODULATION OF THE ACTIVITY OF INFERIOR OLIVARY NEURONS IN THE BETA NUCLEUS OF THE RABBIT. N.H. Barmack, E. Mugnaini and B. Nelson*. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209; and Lab of Neuromorphology, Univ. of Connecticut, Storrs, CT 06268.

Previously we demonstrated that the beta nucleus of the inferior olive contains a dense cluster of GABAergic terminals. Using a double label technique we traced the origin of these GABAergic terminals to the ipsilateral descending vestibular nucleus (DVN). In the present experiment we have recorded the activity of beta nucleus neurons in rabbits during natural vestibular, visual and neck proprioceptive stimulation. Rabbits were anesthetized and positioned in a triaxial servo-controlled rate table with the head fixed at the center of rotation. Horizontal neck proprioceptive stimulation was provided by rotating the body independently of the head. Contrast-rich visual stimuli were rear-projected onto a 70 deg tangent screen. Neurons in the beta nucleus were identified by antidromic stimulation of the nodulus-uvula, the site of termination of the climbing fibers which originate from the caudal beta nucleus. Alternatively, recording sites in the beta nucleus was verified by subsequent histological analysis of marking microlesions. More than 70% of the identified beta nucleus neurons responded to roll vestibular stimulation about the longitudinal axis. Each of these neurons was excited when the rabbit was rolled onto the side which was contralateral to the recording site, and inhibited when the rabbit was rolled ipsilaterally. More than 65% of the cells which were responsive to roll vestibular stimulation evinced a response to static roll positions, indicating an otolithic origin of the vestibular input conveyed from the DVN. These roll-sensitive neurons were not responsive to horizontal vestibular stimulation, horizontal neck proprioceptivestimulation or visual stimulation in either the horizontal or vertical axes. Although most of the roll-sensitive neurons were more sensitive to lower stimulus frequencies (.01-.05 Hz), the range of sensitivity extended to higher frequencies (.1-.5 Hz). Two neurons were sensitive to vestibular stimulation about the vertical axis, but not to vestibular stimulation about the longitudinal axis, nor were they sensitive to visual stimulation in either the horizontal or vertical axes. Only one neuron was sensitive to stimulation of neck proprioceptors.

These data are consistent with our immunocytochemical demonstration of a presumed inhibitory GABAergic DVN projection to the beta nucleus, and further substantiate the general proposition that the characteristic low discharge frequency (1-4 imp/sec) of inferior olivary neurons is entirely adequate to encode sensory events of low temporal frequency. (Supported by EY04778, the Oregon Lions' Sight and Hearing Foundation, and NS09904).

69.9 RELATION OF PRIMARY VESTIBULAR, SECONDARY VESTIBULAR AND CUNEATE MOSSY FIBER AFFERENTS WITH PURKINJE CELL ZONES IN THE POSTERIOR VERMIS OF THE RABBIT CEREBELLUM.

N.M.Gerrits* and I.E.Thunnissen* (SPON: ENA). Dept. of Anatomy, Erasmus University, 3000 DR Rotterdam, The Netherlands.

In a study of the connections of the posterior vermis of the rabbit cerebellum the mossy fiber (mf) input from the vestibular nerve, the vestibular nuclei (VN) and the rostral part of the cuneate nuclei was investigated with anterograde tracing methods using WGA-HRP, Phaseolus vulgaris lectin and tritiated leucine. In a number of cases combinations of these tracers were injected to label different mf afferent systems simultaneously. The results obtained with the different tracers were similar.

It was found that the primary and secondary vestibular mf's share a common termination area. In the lobules IXd and X they are the dominant input. Both types of vestibular mf are present bilaterally, but primary mf are absent from the lateral two-third of the contralateral side. In the lobules IXa-c and VIIIb the number of mf terminals (mft) is very small and present in longitudinal strips: one in the midline flanked bilaterally by two others.

Cuneate mft are restricted to the lobules VIII and XIa-c. At either side of the midline two broad strips of mft are present with a strong ipsilateral dominance. The labeling is separated by narrow strips devoid of mft and also leaves a midline strip free. Rostrally in VIII the lateral strips disappear and the medial one gradually tapers off. Thus the vestibular and cuneate mft occupy complementary areas in the posterior vermis. Overlap is minimal and restricted to the lateral strips in the lobules VIII and IXa-c.

WGA-HRP injected in the VN also labeled Purkinje cells (PC) in the ipsilateral posterior vermis. In the lobules IXd and X the labeled PC's are present over the entire mediolateral extent of the cortex in a more or less continuous layer. From lobule IXc rostralward into VIII they tend to segregate into longitudinal strips. This was most obvious following injections in the medial and descending vestibular nuclei. A narrow midline PC strips matches the vestibular mf strip. Two lateral PC strips coincide closely with the cuneate mf terminal strips.

Thus, the vestibular nuclei receive inhibition from different populations of PC's. One of them driven by primary and secondary vestibular mf, and one driven by a relay in the rostral cuneate nuclei, probably mediating neck input.

69.10 THREE-DIMENSIONAL ORGANIZATION OF EYE MOVEMENTS EVOKED BY ELECTRICAL STIMULATION OF RABBIT FLOCCULUS. J. Van der Steen*, J.I. Simpson and J. Tan*. Depts. Physiol. I and Anatomy, Erasmus Univ., Rotterdam, The Netherlands and Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016.

The climbing fibers of the rabbit cerebellar flocculus that respond to rotation of the visual world can be divided into three classes on the basis of the orientations of the rotation axes associated with their best modulation (Simpson, Graf & Leonard, 1981). In the present study, the axes of the eye rotations produced by electrical stimulation (1 sec trains of 0.2 msec pulses, 200 Hz, 20 microA) of the alert rabbit's flocculus were determined and compared to the visual climbing fiber best response axes. With two orthogonal coils on each eye, the movements of both eyes were measured simultaneously in three dimensions using the methods of Robinson and Collewijn. Slow (2-5°/sec) movements were evoked most effectively from the deep granular layer and the white matter. A limited number of eye movement patterns were evoked. The most prevalent response component was rotation of the ipsilateral eye about a horizontal axis with an azimuthal orientation of 135-150° posterior to the nose. The sense of the rotation about this axis, which for covenience is called the ipsi 135° axis, was counterclockwise as viewed along the axis toward the rabbit's eye. This rotation occurred either monocularly or with a smaller, but conjugate rotation of the contralateral eye. These rotations about the ipsi 135°-contra 45° axis, and also the evoked vertical axis rotation, are consonant with the coordinate system established by the set of best response axes of the climbing fibers. However, rotations about the ipsi 45°-contra 135° axis were also evoked. For the contralateral eye, the rotation about its 135° axis was counterclockwise; it occurred either alone or as a component of an upward rotation about the roll (nasal-occipital) axis. For the ipsilateral eye, the rotation about its 45° axis was clockwise and occurred in combination with a counterclockwise rotation about the ipsi 135° axis. Given our previous measurements of the climbing fiber best response axes and the finding by Ito, Nisimaru & Yamamoto (1977) that the rabbit flocculus does not act on the ipsilateral posterior canal vestibulo-ocular pathways, rotations about the ipsi 45°-contra 135° axis were unexpected. A tentative explanation of the presence of these rotations is that the climbing fiber coordinate system can be transformed by a Purkinje cell influence on the vestibular nucleus that does eventually include posterior canal pathways. Supported by NS-13742 and FUNGO.

69.11 FASTIGIAL NUCLEUS PROJECTIONS TO BRAINSTEM AUTONOMIC AND OCULOMOTOR NUCLEI IN CATS. Y. Torigoe and R.H.I. Blanks. Department of Anatomy and Neurobiology and Department of Surgery, Division of Otolaryngology-Head and Neck Surgery, University of California, Irvine, California 92717.

Fastigial neurons are related to control of smooth pursuit and saccadic eye movements and to a number of autonomic responses. There have been, however, controversy in the pathways and projections of this nucleus, due primarily to the methodology. Older degeneration techniques invariably interrupt unrelated axons in passage within the cerebellar white matter. For this reason, projections of the fastigial nucleus were investigated using axoplasmic transport of tritiated amino acid. Experiments were conducted in 10 cats. The fastigial nucleus was approached stereotaxically with glass microelectrodes with tip diameters of 5 um, pressure injected with 4-5-^{3}H leucine, and following 2 to 7 day postinjection periods the animals were sacrificed, and brains processed for standard light autoradiography. Our injection sites covered the rostral or caudal on-half of the fastigial nucleus without involvement of the overlying cerebellar cortex or adjacent interpositus nuclei. Projections of this nucleus to the following autonomic areas were determined: contralateral rostral "vomiting center" of Borison and Wang (1949) (parvocellular reticular formation); discrete region of the bilateral, but predominantly contralateral, lateral solitary tract nucleus; moderate contralateral A5 norepinephrine group; strong bilateral inferior and superior salivatory nucleus; moderate to weak bilateral A1 norepinephrine group (in region of the ambiguous nucleus). Fastigial projections to the following oculomotor-related nuclei were found: strong to moderate contralateral nucleus reticularis tegmenti postis; strong contralateral raphe pontis; bilateral lateral and spinal vestibular nuclei and cell groups x and f; contralateral dorsal cap and beta nucleus of the inferior olive; small discrete portions of the contralateral praepositus hypoglossi nucleus; bilateral brainstem reticular formation, some of which are adjacent to the medial longitudinal fasciculus (overlaps the caudal paramediam pontine reticular formation). No labeling was found in the nucleus intercalatus, nucleus of Roller, lateral reticular nucleus (although the region around the nucleus, the A1 group, is labeled) and the medial vestibular nucleus as previously reported in cat studies using degeneration techniques.

In the present study, the fastigial nucleus projections to vestibulo-oculomotor nuclei clarifies previously described pathways. However, some pathways not described in recent studies using axoplasmic transport techniques in other species are found in this study, suggesting a possible species difference as an explanation. In addition, the fastigio-autonomic pathways demonstrated here suggest that the fastigial nucleus has a direct connections with parasympathetic outflow motoneurons (salivatory nuclei), indirect parasympathetic connections via the solitary tract nucleus and "vomiting center"and indirect sympathetic connections via A5 and A1 groups. (Supported by NASA grant #NAG2-288. YT is the recipient of a Research Associate Award from NASA #MAGN-70).

69.12 CEREBELLAR INFLUENCE UPON MEDIAL VESTIBULAR NUCLEUS NEURONS IN WILDTYPE AND WEAVER MUTANT MICE DURING VISUAL AND VESTIBULAR STIMULATION. U.Kindermann* and U.Grüsser-Cornehls* (SPON: R.F. Schmidt). Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, Germany-West.

In order to examine cerebellar influence upon medial vestibular nucleus neurons during horizontal visual or horizontal vestibular stimulation, experiments were carried out with wildtype (B6CBA) and homozygous weaver mutant mice. The defect in weaver homozygotes (wv/wv) is exclusively cerebellar and results in an almost complete loss of cerebellar granular cells. A disturbance in cerebellar integrity should be reflected in subjacent vestibular nuclei.

Single cell recordings (extracellular) of 17 wildtype and 18 mutant vestibular nucleus neurons were performed during visual (movement of a black and white striped pattern), vestibular (rotation of the animal in the dark) and visual-vestibular (rotation of the animal in light in front of the striped pattern) stimulation. All stimuli movements consisted of horizontal sinusoidal oscillations with an amplitude of ±35°. Frequencies varied from 0.01Hz to 0.6Hz. The recording sites were marked by pontamin sky blue and for normals as well as for mutants the sites were localized in the medial vestibular nucleus. Mutants could be tested under open- and closed-loop conditions, wildtypes only under open-loop.

Unexpectedly the sensitivity (difference between peak or minimum response and spontaneous frequency) of type I and type II medial vestibular neurons in the open-loop condition seemed to be unaffected by the mutation. In wildtypes as well as in mutants a considerable sensitivity increase between 0.05Hz and 0.6Hz took place during vestibular stimulation in the dark. The slope of this increase was about the same for both. In some neurons visual stimulation led to a sensitivity peak at 0.025Hz, while in others it should be assumed that the peak sensitivity occurred at lower frequencies than could be examined. For closed-loop mutant neurons visual excitability is extended to higher frequencies (up to 0.3Hz) and visual sensitivity decreased approximately at 0.4 Hz. In agreement with sensitivity values the gain for normal and mutant mice under open-loop conditions corresponded closely. Closed-loop mutants reached higher visual gain values.

In contrast to sensitivity and gain, the time relationship between peak stimulus velocity and peak response (expressed as phase shift) was considerably altered in mutants. In wildtype type I neurons the visual peak response occurred with phase leads of 17.4 imp/sec ±11 imp/sec (n=5) at 0.01Hz and 10.5 imp/sec ± 24.2 imp/sec (n=9) at 0.05Hz. Mutants showed a tremendous phase lag increase over the same velocity range.

In conclusion, cerebellar granular cell degeneration strongly affects the time relationship between stimulus and response and does not alter the sensitivity and gain under open-loop conditions.

Supported by a grant of the DFG (Gr 276/19-4)

69.13 PURKINJE CELL SPONTANEOUS ACTIVITY AND HARMALINE RESPONSES IN DYSTONIC RATS. S. Stratton, J. F. Lorden & L. E. Mays. Depts. Psychol. and Physiol. Optics, UAB, Birmingham, AL 35294.

Biochemical and pharmacobehavioral studies have emphasized the presence of cerebellar abnormalities in the dystonic (dt) rat, an autosomal recessive mutant with a motor syndrome of CNS origin. The abnormalities observed include increased norepinephrine and decreased cGMP levels in the cerebellum and increased glutamic acid decarboxylase activity in the deep cerebellar nuclei. Behaviorally, dt rats are insensitive to the tremorogenic effect of harmaline. We now report physiological correlates of the dt rat's motor syndrome. This investigation was designed to examine the functional integrity of the two major cerebellar afferent pathways in dt rats, the olivo-cerebellar and mossy fiber pathways.

We recorded extracellular single-unit activity from Purkinje cells of urethane-anesthetized (1.9 g/kg) dt rats and phenotypically normal littermates 20-28 days of age. Spontaneous complex spike (CS) and simple spike (SS) activity of Purkinje cells were discriminated off-line with two amplitude and time window discriminators. Autocorrelograms of CS and SS activity were computed for each cell. An index of spike regularity, the rhythmicity index value, was calculated for each cell's SS and CS activity by dividing the number of counts at the peak of the autocorrelogram by the total number of counts. Following 10-20 min of baseline recordings, we administered harmaline (10 mg/kg, i.v.) to induce rhythmic activation of the olivary neurons and recorded from the same cell for an additional 30-180 min.

In dt rats (n=19), the spontaneous CS firing rate was 46% lower and the SS rate, 66% lower than in controls (n=17). The pattern of SS activity in dt rats was significantly less regular than that in control rats. SS waveforms did not differ between the two phenotypes. However, CSs from dt rats showed fewer secondary wavelets. Harmaline-induced increases in CS frequency and rhythmicity and prolonged suppression of SSs were observed in 47% (8/17) of the cells in normal rats and only 17% (3/17) of the cells in dt rats. Intermittent suppression of SS activity was seen in the remaining 53% of the cells in normal rats, and 41% of the cells in dt rats. Anomalous responses occurred in 29% of the cells in dt rats. The remaining 12% of the cells from dt rats did not respond to harmaline. Thus, in dt rats, spontaneous Purkinje cell activity was slower and less rhythmic, and harmaline responses were more variable than in controls.

Lower spontaneous activity in the Purkinje cells may result from either intrinsic differences in the cells or aberrant inputs. The increased variability in Purkinje cell responses to harmaline suggests that the lack of harmaline tremor is the result of a failure in neurotransmission in the olivo-cerebellar pathway. Future studies will investigate the nature of the cortical abnormalities and their effects on cerebellar output. (Supported in part by NINCDS grant 18062.)

69.14 GABA LEVELS AND AUTORADIOGRAPHIC ANALYSIS OF GABA RECEPTORS IN THE CEREBELLUM OF THE DYSTONIC RAT. J. Lutes, M. Beales*, R. Dawson, Jr., J.F. Lorden, S.F. Hoff and G.A. Oltmans. Dept. of Pharmacology, Chicago Med. School, N. Chicago, IL 60064 and Dept. of Psychology, Univ. of Alabama, Birmingham, AL 35294.

The dystonic rat exhibits symptoms similar to human dystonia musculorum deformans. Light microscopy suggests that the nervous system of this mutant is structurally normal. Neurochemical studies have, however, revealed several cerebellar abnormalities including increased activity of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) in the deep cerebellar nuclei. The changes in GAD activity suggest increased neurotransmitter activity in the Purkinje cells terminals synapsing on the intrinsic cells of the deep nuclei. The present study examines other components of the GABAergic system in the dystonic rat to determine if additional alterations are present in this mutant.

In vitro autoradiographic studies of 3H-muscimol (MUSC) were conducted according to the method of Pan, et al. (J. Neurochem. 45:1396). Coronal cerebellar sections (10 micron) through the deep nuclei were taken from 25 day old normal and dystonic rats (N=9/group). Quantitative densitometry was used to measure specific binding in granular and molecular layers of cerebellar cortex and in lateral, interposed, and medial divisions of the cerebellar nuclei. A separate group of 20 day old normal and dystonic animals was killed using focused-beam microwave irradiation, and GABA levels in the deep nuclei determined using HPLC with electrochemical detection. It has also been shown that animals with 3-acetylpyridine (3AP)-induced lesions of the climbing fiber system develop increases in GAD activity in the deep nuclei. Therefore, as a comparison, GABA levels were also determined in the deep nuclei of rats treated with 3AP (65 mg/kg) at 20 days of age and killed 4 days post-lesion.

A significant ($p<.02$) 21% decrease in MUSC binding was found in both the lateral and interposed nuclei of dystonic rats. No differences between normals and dystonics were found in GABA levels in the deep nuclei. The normal levels of GABA, in combination with the previously reported increase in GAD activity in the deep nuclei, suggest that the current findings may be an example of receptor down-regulation in the face of increased GABA release. In 3AP-treated rats a significant 35% increase was found in GABA levels in the deep nuclei. This indicates that the lack of change found in the dystonic rats was not due to an inability to detect such a difference. The results indicate changes in multiple components of the GABAergic systems in the cerebellum of dystonic rats. Autoradiographic analysis of the benzodiazepine receptor in dystonic rats is currently being conducted. (Supported by grants from the Dystonia Foundation and NS18062.)

69.15 [^{3}H]GLUTAMATE BINDING IN NORMAL C57BL/6 AND MUTANT MOUSE CEREBELLUM. H. Ni*, A. Frostholm and A. Rotter. Department of Pharmacology, University of California, Irvine, CA 92717.

Glutamate is a major excitatory neurotransmitter in the mammalian cerebellum. Depolarization causes a Ca^{2+}-dependent release of glutamate which binds to, and activates, postsynaptic receptors in a Na^+-independent manner. The action of glutamate is terminated by high affinity Na^+-dependent uptake into astrocytes. Some glutamate may be taken up presynaptically by a Ca^{2+}/Cl^--dependent process. In order to examine the cellular localization of glutamate neurotransmitter systems, the distribution of [^{3}H]glutamate binding sites in cerebella of normal mice (C57BL/6) and Purkinje cell degeneration, staggerer and weaver mutant mice was studied by light-microscopic autoradiography. Slide mounted cryostat sections (20 μm) were preincubated for 15 min at 4°C in PBS (148 mM Na^+; 4.2 mM K^+; 4.9 mM Mg^{2+}; 0.9 mM Ca^{2+}; 148.5 mM Cl^-; 10 mM P_i; pH 7.4), containing 0.05% glutaraldehyde, then washed for 1 min at 4°C in PBS. Sections were incubated for 40 min at 4°C in PBS containing 50 nM [^{3}H]glutamate (52.4 Ci/mmole, NEN). After incubation, slides were washed twice for 1 min at 4°C in PBS and rinsed twice for 10 sec at 0-4°C in double-distilled water to remove residual salts. Slides were apposed to emulsion-coated coverslips (NTB-2, Kodak) and exposed for 10 days, after which time the coverslips were developed in Dektol (1:1). [^{3}H]glutamate binding was inhibited by 1mM glutamate and 1mM quisqualate, but not by 1 mM ibotenic acid, kainic acid, N-methyl-D-aspartic acid (NMDA) and 2-amino-5-phosphonovaleric acid (AP5).

In the cerebellar cortex of normal C57BL/6 mice, a high density of [^{3}H]glutamate binding was observed over the molecular layer, an intermediate density over the granular layer and low density over the deep cerebeller nuclei; the white matter was devoid of labeling. In the 110 day weaver mutant cerebellum, in which the number of granule cells is greatly reduced, the autoradiographic grain density over the molecular and granule cell layers remained similar to that of littermate controls, indicating that the glutamate binding sites are not located on granule cells or their parallel fibers. In the cerebellar cortex of 24-day-old staggerer mice, which is partially depleted of granule, Purkinje and Golgi cells, a reduction in labeling was observed over the molecular layer; this suggests that the remaining Purkinje or Golgi cells contain glutamate binding sites. In the 57-day-old Purkinje cell degeneration mutant cerebellum which was completely devoid of Purkinje cells, the grain density over the molecular layer was reduced to that of the granule cell layer. This finding indicates that a large proportion of the [^{3}H]glutamate binding visualized by our method is associated with Purkinje cell dendrites. These data are compatible with the generally held view that the Purkinje cells of the cerebellum are responsive to glutamate.
Supported by USPHS grants NS18089 and HL34472 to A.R.

69.16 ACUTE AND CHRONIC EFFECTS OF CLIMBING FIBER LESIONS ON CEREBELLAR cGMP LEVELS. L.E. Moss*, G.A. Oltmans, M. Beales* and J.F. Lorden. Dept. of Pharmacology, Chicago Med. School, N. Chicago, IL 60064 and Dept. of Psychology, Univ. of Alabama, Birmingham, AL 35294.

cGMP is distributed throughout the CNS, but is found in particularly high levels in the cerebellum. Within this structure, evidence suggests that cGMP may be concentrated in the Purkinje cells (PC). Initial work on the regulation of cerebellar cGMP levels indicated a direct correlation between cGMP levels and excitatory input to the PC. Activation of the excitatory climbing fiber (CF) input to the PC with the drug harmaline produced large increases in cerebellar cGMP, while loss of CF input (produced by the neurotoxin 3-acetylpyridine (3-AP)) produced decreased cGMP levels. Recent studies, however, suggest a more complex relationship may exist. Although the removal of CF input eliminates complex spike activity in the PC, simple spike (SS) activity more than doubles in the immediate post-lesion period. Then, over a period of several days, the SS activity gradually returns to pre-lesion frequencies. cGMP levels, typically measured at 3-5d post-lesion, are decreased 40-50%. PC activity at this time, however, is still well above control levels. Because of the hypothesized relationship between cGMP levels and PC activity, we measured cGMP levels at 6 post-lesion intervals selected on the basis of published reports indicating increased or normal SS activity at the selected times.

To produce CF lesions 20 day-old Sprague-Dawley rats were treated with 65 mg/kg of 3-AP. Controls were administered saline. Animals were killed by focused-beam microwave irradiation at 6h, 24h, 48h, 7d, 14d, and 20d post-3AP treatment. Cerebellar cGMP levels were determined using a commercially available RIA.

CF lesions produced time-dependent changes in cGMP levels. At both 6h and 24h post-lesion, times when SS activity is greatly increased, there was no significant change in cGMP compared to controls. At 48h and 7d post-treatment cGMP levels were significantly decreased (-28% and -45% respectively) in 3AP-treated rats. Published data indicate that at 48h SS activity is still more than doubled compared to controls. At 7d the firing rate is decreased, but is still above pre-treatment levels. At both 14d and 20d, cGMP levels were not significantly different from controls. At these periods SS activity is reduced and may have returned to pre-lesion rates.

These results do not indicate a direct relationship between cerebellar cGMP levels and SS activity in the cerebellum. The cGMP levels do, however, return to normal at the time the animals show substantial behavioral recovery from the effects of the climbing fiber lesions.
Supported in part by grant NS18062.

69.17 CONCOMITANT INCREASE OF IMMUNOREACTIVE-CHOLECYSTOKININ (IR-CCK8) AND SOMATOSTATIN(IR-SRIF) IN THE CEREBELLUM OF ATAXIC MUTANT MICE: WEAVER, STAGGERER, PURKINJE CELL DEGENERATION(PCD) AND ROLLING MOUSE NAGOYA(RMN).
A.Masui*, K.Matsui*, N.Watanabe*, K.Ando* and N.Kato (S-PON:M.Kabuto) Div. of Neurol., Natl.Ctr.Neurol.Psychiat. Kodaira, Tokyo 187; Dept. of Psychiat., Shiga Univ. Med. Sci., Otsu 520-21; Nishiyama Hosp., Kyoto 617, Japan.

A number of mutations have been described that affect the mouse cerebellum and that are characterized by ataxia. Though a recent study has revealed a favouring effect of TRH on cerebellar ataxia, neuropeptides contents in these mice have been scarcely investigated. This is partly because the cerebellum is thought to be a locus with the least concentration of nearly all peptides. The present study was conducted to study possible changes in IR-CCK8 and SRIF in the cerebellum and cerebrum in 4 types of ataxic mice with different cerebellar pathology. Among these, weavers will be of particular interest since this gene results in a marked loss of mesostriatal dopamine pathway. We have found a concomitant increase of IR-CCK8 and SRIF in the rat brain after chronic interruption of dopaminergic transmission (Soc. Neurosci. Abst.,12:232,1986).

Weaver(wet weight of cerebellum; 37%control), staggerer(21%), PCD(81%) and RMN(96%) mice were killed by microwave irradiation at 8-weeks age (N = 5-10). Unaffected littermates served as controls. The brain was dissected into cerebellum and cerebrum (whole brain except cerebellum) and the tissue was extracted in 0.1N acetic acid. The supernatant was then subjected to the assays. IR-CCK8 and IR-SRIF were measured by enzyme immunoassays developed in the laboratory as reported previously. IR-CCK8 and SRIF both were found to be unequivocally (two to five fold) elevated in the cerebellum of all ataxic mice when expressed as ng/mg wet weight. The cerebrum content of both peptides showed similar elevation yet to a lesser extent. Moreover the peptide contents proved to be increased significantly as well when expressed as total cerebellar content in PCD and RMN. In the weaver, total content of IR-CCK8 in the cerebellum was 175% higher than that in controls despite of marked cerebellar atrophy.

The present results provide another evidence of the close relationship of CCK8 and SRIF, and suggest that the cerebellum in these mice substantially contains both peptides, which may actively participate in the pathogenesis of cerebellar ataxia.

69.18 OLIVO-CEREBELLAR PROJECTION IN LURCHER MUTANT MICE.
J. A. Heckroth and L. M. Eisenman, Dept. of Anatomy, Jefferson Medical College, Philadelphia, PA 19107.

"Lurcher" is a neurological mutation in mice affecting primarily the cerebellum and, consequently, motor behavior. It is known that adult Lurcher mice are entirely lacking in Purkinje cells, and suffer a 90% deficit in cerebellar granule cells, and a 75% deficit in inferior olivary neurons. Analysis of Lurcher/Normal chimeras has demonstrated that the postnatal elimination of Purkinje cells is a direct effect of the Lurcher mutation, while the loss of granule cells and olivary neurons is not. In light of the intimate relationship between climbing fibers and Purkinje cells it is of interest to determine the fate of these afferents in this mutant. In order to demonstrate the distribution of olivo-cerebellar fibers within the cerebellar cortex of Lurcher, we have injected 10-20 nl of 1% WGA-HRP in the inferior olive of six adult Lurchers of 32(2), 67, 72, 85 and 195 days of age, and observed the pattern of terminal labeling in the cerebellar cortex using the TMB method of Mesulam for the visualization of HRP. Labeled axons can be traced from the injection site throughout their course to the cerebellar cortex. Fibers can be seen traversing the cerebellar granular layer, but do not enter the thin molecular layer. Instead they appear to accumulate at the boundary between the granular and molecular layers, forming a plexus at this interface. Interestingly, as in normal animals, the distribution of olivocerebellar fibers is not uniform. Labeled parasagittal bands alternate with unlabeled bands, giving the cortex a longitudinally striped appearance. In cases with large injections involving the reticular formation, there is additional terminal labeling in the granular layer which has the appearance of typical mossy fiber rosettes. We have also made spinal cord injections of WGA-HRP in Lurcher adults, which confirm that mossy fiber afferents terminate in the granular layer of the Lurcher cerebellum in a pattern reminiscent of that seen in normal mice. We have initiated olivo-cerebellar tracing experiments at the ultrastructural level and also studies to determine whether olivo-cerebellar fibers penetrate the molecular layer during development (9-15 days of age) in Lurcher mutants.

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69.19 EXPLORATION BEHAVIOR IN PURKINJE CELL DEGENERATION MUTANT MICE.
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Purkinje cell degeneration (pcd) mutant mice lose nearly all Purkinje cells between the third and fourth postnatal weeks. Granule cell loss occurs after the Purkinje cell loss (beginning on the third month). There is also a decrease in retinal photoreceptors in pcd mutant mice, which follows a much slower course than the cerebellar pathology.

Previous experimentation in other cerebellar mutants with Purkinje cell loss (nervous and lurcher) indicated that, in spite of ataxia, these mice are not less active than normal mice. Motor activity was therefore measured in pcd mutants. In addition, spontaneous alternation behavior was tested.

Ten pcd mutant (pcd/pcd) mice and 13 littermate controls (+/-) served as subjects. The mice were at least 4 weeks old at the start of the study. Food and water were available at all times. Exploration behavior was tested in a T-maze.

It was found that, in spite of ataxia, pcd mutant mice were not less active than normal mice in the maze. Contrary to normal mice, the pcd mutants did not alternate spontaneously in either a 2-trial or a 4-trial test. Additional experimentation indicated that whereas normal mice alternated at all three inter-trial intervals tested (15 s, 3 or 6 min), the pcd mutants did not.

These results are in agreement with previous studies of nervous and lurcher mutants in that massive degeneration of Purkinje cells in mice does not cause a reduction in motor activity. On the other hand, the spontaneous alternation rate of pcd mutants is at chance level, indicating that the cerebellum or cerebellar connections has a role in this behavior.

Spontaneous alternation is considered to be an intance of spatially-guided behavior and, in this regard, the results of this study are in agreement with recent clinical findings of impaired visuo-spatial abilities in patients with cerebellar atrophy (Botez et al. Neurology 35: 1152-1157, 1985).

Another possibility is that deficits in spontaneous alternation imply response disinhibition. In that regard, it has been found that animals with cerebellar damage have difficulty in witholding to respond to unreinforced stimuli in discrimination learning tasks. These findings indicate some similarities between animals with cerebellar as compared to limbic system damage in relation to behavior.

69.20 MODIFICATIONS IN THE CHARACTER OF A CONDITIONED RESPONSE PRODUCED BY EXTENSIVE CEREBELLAR LESIONS IN THE DECEREBRATE AMBULATING FERRET.
J.R. Bloedel, C.-C. Zuo*, R. Ferguson*, and J.-S. Lou*. Barrow Neurological Institute, Phoenix, AZ 85013 and Dept. of Physiology, U. of Arizona, Tucson, AZ 85721.

Recent studies in our laboratory have demonstrated that decerebrate locomoting ferrets can acquire a conditioned movement of the forelimb performed to avoid contacting a bar interjected into the forelimb's trajectory during swing phase. We also reported that the relationship between simple and complex spike responses recorded from cells very responsive to the perturbation was not modified during the conditioning process. The purpose of the experiments reported here was to determine if extensive lesions of the vermal and paravermal cerebellar cortex as well as the deep nuclei either modified the conditioned response of the forelimb or eliminated the animal's capacity to acquire this behavior. All animals underwent removal of the above cerebellar regions one to two months prior to the assessment of their conditioned behavior. The lesions spared only the vermal part of lobule I, rostral lobule II and the lateral-most parts of the cerebellar hemispheres; however the nuclei were removed. Following recovery from the cerebellectomy each animal was decerebrated at a supracollicular, supramammillary level, resulting in a preparation that locomoted in response to the moving treadmill. Conditioning trials consisted of interjecting a bar into the trajectory of the limb at the same phase of the locomotor cycle on each successive step. The time course and characteristics of conditioned behavior were noted, and the presence or absence of extinction was also observed. Movements were assessed quantitatively using the combination of a Watscope/Watsmart infrared emitting diode system and a standard video camera. The most striking deficit in these animals was their incapacity to successfully organize a succession of consistently performed conditioned responses over several successive trials. Although they were able to acquire a conditioned response during which they elevated the forelimb over the bar, this behavior was not performed consistently over several successive trials. Alternate strategies were used intermittently to avoid the bar, some of which would interrupt significantly the locomotor cycle. Because conditioned responses were observed clearly with appropriate acquisition and extinction time courses, the lesioned portions of the cerebellum cannot be the site of the plastic changes required for the conditioning. Rather the data suggest that the cerebellum is critical for the organization of movements required to avoid the bar in successive trials while minimizing modifications in the ongoing movement. Supported by NIH grant NS21958.

69.21 SHORT-TERM ENHANCEMENT OF SIMPLE SPIKE RESPONSES BY CLIMBING FIBERS EVOKED BY CORNEAL AIR PUFF STIMULI. C.-C. Zuo* and J.R. Bloedel (SPON: T. Tarby), Barrow Neurological Institute, Phoenix, AZ 85013 and Dept. of Physiology, University of Arizona, Tucson, AZ 85721

Specific regions of the cerebellar cortex and cerebellar nuclei have been implicated as the substrate for the plastic modifications required for conditioning the nictitating membrane reflex in rabbits. Furthermore, the induction by the climbing fiber of a long-term change in Purkinje cell responsiveness to mossy fiber-granule cell-parallel fiber inputs has been proposed as a basis for the acquisition of the conditioned behavior. However, our previous findings indicate that this afferent system produces a short-term enhancement of Purkinje cell responsiveness to parallel fibers, at least when these fibers are activated by natural cutaneous and proprioceptive stimuli. The experiments reported here were designed to determine if this kind of short-term modification occurs for the simple spike responses evoked by air puff stimuli of the cornea, the unconditioned stimulus in the conditioning paradigm alluded to above. Experiments were performed in rabbits decerebrated just rostral to the superior colliculus. Purkinje cells were recorded in the simplex and ansiform lobule. Their simple spike responses to the air puff were compared under two conditions: when the air puff stimuli were applied randomly relative to the occurrence of the cell's spontaneous climbing fiber input, and when the stimuli were applied at short intervals following the occurrence of the climbing fiber input to the Purkinje cell. By alternating the construction of histograms evoked by the randomly applied and climbing fiber triggered air puff stimuli it was possible to determine both the effect of the climbing fiber input on the simple spike response as well as the presence of any persistent changes in the simple spike responses of the neurons. The data confirmed that the simple spike responsiveness of Purkinje cells in the simplex to air puff stimuli is enhanced for short periods (up to 300 msec) following the spontaneous occurrence of their climbing fiber input. Furthermore, no persistence of this effect was observed in any of the cells studied (n=43). Since the action of the climbing fiber input on the dendrite of a Purkinje cell is not believed to be dependent on the modality by which it is evoked, the data suggest that long-term modifications in simple spike responsiveness produced by the climbing fiber system are not likely responsible for the plastic changes underlying the acquisition of the classically conditioned nictitating membrane reflex. Supported by NIH grant NS21958.

TRANSMITTERS IN INVERTEBRATES II

70.1 CLONING OF THE DROSOPHILA GLUTAMATE DECARBOXYLASE (GAD) GENE.[1] F.R. Jackson and K. Elliott.* Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of vertebrates and invertebrates, and has been implicated in the pathogenesis of a number of human neurological and psychiatric disorders. The synthesis of GABA in diverse species is controlled by glutamate decarboxylase (GAD), an enzyme which has been extensively investigated. Recently, Kaufman et al. (Science, 232: 1138, 1986) reported the isolation of feline cDNA clones encoding GAD. We have shown that these feline cDNA sequences hybridize to homologous sequences in restriction-enzyme digests of Drosophila genomic DNA. In order to pursue genetic and molecular studies of GABAergic inhibitory transmitter systems, we have used a feline GAD cDNA as a probe to isolate several homologous genomic DNA and cDNA clones from Drosophila.[2] On the basis of restriction endonuclease mapping, it appears that the Drosophila cDNA clones (3 of them) represent a single gene. Partial DNA sequence information for one cDNA clone indicates that the protein encoded by the fly gene has significant homology with feline glutamate decarboxylase. DNA sequencing, gene dosage studies, and protein expression techniques are currently being employed to verify that we have isolated a Drosophila GAD gene.---The Drosophila GAD-homologous gene has been localized to region 64A of the third chromosome by in situ hybridizations to salivary gland polytene chromosomes. This chromosomal localization will enable us to begin genetic studies to determine the role of inhibitory neurotransmitter systems in the developing nervous system. Ultimately, we wish to study the expression of GABAergic phenotypes during the development and metamorphosis of the Drosophila nervous system.

[1] This research was supported by Worcester Foundation institutional funds. F.R.J. is a recipient of a McKnight Scholars Award.

[2] We thank Allan Tobin (UCLA) for providing a feline cDNA GAD clone and Laurel Newby-Kew for technical assistance.

70.2 GABA-LIKE IMMUNOREACTIVITY IN THE PHOTORECEPTORS AND SUPRAESOPHAGEAL GANGLION OF THE BARNACLE, BALANUS NUBILUS. J.C. Callaway and J.S. Edwards (SPON: S. Hauschka). Dept. of Zoology, University of Washington, Seattle, WA 98195.

As sessile crustaceans, barnacles react to rapid changes in light intensity by withdrawing their feeding appendages into their shell behind two protective opercular plates. The photoreceptors (PRs) of the barnacle, B. nubilus are unusually large non-spiking neurons with axons 1-2 cm long. The diameter of the cell somata and axons can be up to 70um and 40um respectively (Hudspeth and Stuart, J. Physiol. 272:1-23, 1977). The photoreceptors terminate in the supraesophageal ganglion (SEG) which contains several hundred neurons. Whether GABA functions as the PR transmitter has long been debated, but recent physiological studies (Callaway and Stuart, Biol. Bull. 171:491-492, 1986) suggest that GABA may indeed serve this purpose.

An antiserum to a GABA-keyholelimpet hemocyanin conjugate (provided by J. Hildebrand: Hoskins et al., Cell Tiss. Res. 244: 243-252, 1986) was used with the peroxidase anti-peroxidase technique to label 8um paraffin sections of the SEG and ocelli of B. nubilus. About 50 cell bodies were labeled in the SEG. Two pairs of small (ca. 20um) cell somata were located in an area over the PR arbors known to contain second and third order visual interneurons. Other small neurons (<50um) were labeled in specific regions of the ganglion and many were in clusters of 4-6 cells. Some larger neuron somate (>50um, presumed motor or neurosecretory) were labeled above background, but were considerably lighter than the smaller cell bodies.

The antibody densely labeled all three PRs of each of the two lateral ocelli and all four PRs of the median ocellus. Label appeared in the soma, dendrites, axons, and terminals of the PRs. Thus, histochemical findings support physiological approaches to the question of transmitter contents in the barnacle PR.

Supported by NIH grant NB-07778 and the Murdoch Trust.

70.3 A SEROTONERGIC SYSTEM IN *DROSOPHILA MELANOGASTER*. V. Budnik & K. White*. Dept. of Biophysics and Dept. of Biology, Brandeis University, Waltham, MA 02254.

The biogenic amine serotonin (5HT) is implicated in synaptic modulation, behavioral plasticity, and development of connections in a number of animals. The fruit fly *Drosophila melanogaster* is a promising system in which to study the function of this transmitter, since mutations affecting the gene encoding for the enzyme dopa decarboxylase, generate animals deficient in serotonin and dopamine. We here combine anatomical and electrophysiological techniques to describe the pharyngeal system of *Drosophila* larva, and its modulation by serotonin.

The pharyngeal muscles of *Drosophila* are composed of small vertically oriented muscle fibers surrounding the pharynx of the larva. During feeding, these muscles contract in a rhythmic fashion. Several serotonin immunoreactive fibers are observed to project to these muscles. These projections come from the subesophageal ganglion, the brain, and two serotonin-containing cells located in a peripheral ganglion, the frontal ganglion.

Intracellular recordings of the muscle fibers reveal a rhythmic pattern of excitatory junctional potentials, probably representing the feeding motor program of the larva. This rhythmic activity requires a central input, since inputs from the frontal ganglion alone do not elicit rhythmic activity.

Bath applications of glutamate to the pharyngeal muscles suggest that this molecule is likely to be the primary excitatory transmitter at this neuromuscular junction. Serotonin, on the other hand, with a threshold as low as 10^{-8}M produced a reversible, dose-dependent increase in the frequency of EJPs. The significance of this modulation by serotonin is being studied in mutants unable to synthesize serotonin. (Supported by a Brittner predoctoral fellowship to V.B. and a NIH grant GM 31503)

70.4 DIFFERENTIAL EFFECTS OF D(+)-AMPHETAMINE AND RESERPINE ON TARSAL AND LABELLAR TASTE THRESHOLDS OF THE BLACK BLOWFLY. L.C. Sudlow* and L.L. Murdock. Department of Entomology, Purdue University, West Lafayette, IN 47907.

The black blowfly, Phormia regina Meigen, has served as a model system for studying the roles of biogenic amines in the regulation of feeding behavior. For example, injection of the octopaminergic agonist demethylchlordimeform causes a significant lowering of tarsal taste threshold, while injection of D(+)-amphetamine (a biogenic amine depleter in insects) raises tarsal threshold and alters meal size. Recent efforts in this laboratory have indicated that blowfly labellar and tarsal thresholds are regulated in a similar manner after ingestion of food. However, we noticed that flies with high tarsal taste thresholds to sucrose caused by injection of D(+)-amphetamine could still respond to low concentratons of sucrose upon labellar stimulation. To investigate this apparent differential effect of D(+)-amphetamine, groups of 3-day-old flies were injected with 150 mM saline (1 ul) or saline containing 12 ug/fly D(+)-amphetamine (1 ul). Tarsal and labellar taste thresholds were determined 1 hour later. D(+)-Amphetamine caused a significant elevation of tarsal thresholds compared to saline-injected controls. However, labellar thresholds were not significantly elevated 1 hour after injection of D(+)-amphetamine. Levels of the biogenic amines octopamine, dopamine, and serotonin were analyzed by high performance liquid chromatography with electrochemical detection using brains dissected from the D(+)-amphetamine- or saline-injected flies. Brain octopamine, dopamine, and serotonin were all significantly lowered by injection of D(+)-amphetamine. In a second set of experiments, 2 ug/fly of the amine depleter reserpine (solubilized in N,N'-dimethylacetamide then mixed 1:9 with corn oil) was injected into 2-day-old flies. The treated flies were placed in cages and were held for 24 hr. prior to testing. Reserpine caused a significant elevation of tarsal thresholds and only a slight elevation of labellar thresholds. The slight elevation of labellar thresholds may have been due to increased energy reserves resulting from the reduced locomotor activity following with reserpine treatment.

Thus, while the biogenic amines, octopmaine, dopamine, and serotonin, appear to play an important role in the regulation of tarsal taste thresholds, they may not be involved in the regulation of labellar thresholds.

70.5 CHOLINERGIC SYNAPTIC RECEPTORS AND THEIR PHARMACOLOGICAL SPECIFICITIES IN THE ANTENNAL LOBES OF THE MOTH *MANDUCA SEXTA*. B. Waldrop and J.G. Hildebrand. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721

The primary olfactory centers of the moth *Manduca sexta* are the paired antennal lobes (ALs) of the deutocerebrum of the brain. Sensory neurons in the antennae project into the glomerular neuropil of the ALs and synapse onto interneurons. Our laboratory has reported previously that acetylcholine (ACh) is synthesized and stored in the antennae and ALs (Sanes & Hildebrand, Dev Biol 52:105-120, 1976; Prescott *et al.*, Comp Bioc Physiol 56C:77-84, 1977), and that the nicotinic receptor antagonist α-bungarotoxin binds in synaptic neuropil in the ALs (Hildebrand *et al.*, PNAS 76:499-503, 1979). Thus the olfactory sensory neurons, like other arthropod chemo- and mechanosensory cells, appear to be cholinergic.

We are studying the actions of cholinergic agonists and antagonists on AL interneurons by using pressure ejection of these agents into the AL neuropil while recording intracellularly from AL interneurons. A 5-barrelled pressure ejection electrode, with each barrel controlled independently by a solenoid-activated valve, allows us to observe the actions of up to five different pharmacological agents on each neuron impaled. The solenoids are controlled from a computer-based, 14-channel stimulation system. The preparation is continuously superfused with saline, and the superfusate can be switched among reservoirs containing different pharmacological agents dissolved in saline.

All interneurons studied thus far respond to the application of ACh, as would be expected if the primary-afferent terminals release ACh. We have observed both excitatory and inhibitory responses to application of ACh. This includes both direct responses by the impaled cells to ACh and indirect responses mediated by other cells. All interneurons also respond to nicotine (applied in solution as the hydrogen tartrate salt). We have not yet found any interneurons to respond to application of the muscarinic agonist oxotremorine sesquifumarate, but these experiments are still in progress. We are also examining the actions of nicotinic and muscarinic antagonists on synaptic responses and on responses to applied agonists. By combining agonist and antagonist studies we are examining the pharmacological nature of the ACh receptors in this preparation.

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70.6 THE DISTRIBUTION AND CHARACTERIZATION OF FMRFAMIDE-LIKE PEPTIDES IN THE BRAIN OF THE MOTH, MANDUCA SEXTA. T.G. Kingan, U. Homberg, J.G. Hildebrand. ARLDN, U. of Arizona, Tucson, AZ. 85721

FMRFamide-like peptides apparently act as chemical messengers in the nervous and neuroendocrine systems of animals from diverse phyla. The distribution of FMRFamide-like immunoreactivity (FLI) was determined by immunocytochemistry (ICC) in the brain and associated neurohemal organs of the moth, Manduca sexta, using antisera provided by D. Price (U. of Florida) and T.L. O'Donohue (NIH). FLI was detected in neurosecretory cells and interneurons in the brain of Manduca. Among the neurosecretory cells of the protocerebrum, about 4 large (probably type Ia) and 10 small (Ib) lateral cells and 3-4 medial (IIa) cells were stained (nomenclature after Copenhaver and Truman, 1986). Immunoreactive (ir) fibers of these neurons were stained, particularily in the dorso-median protocerebrum, the nervus corporis cardiacis I/II, and the corpora cardiaca and allata, and on the surface of the aorta. FLI was detected in all optic neuropils, concentrated in particular strata of the medulla and lobula. FLI-containing cells at the dorsal edge of the medulla send fibers tangentially into the medulla. About 250 darkly and 500 weakly ir-cells lie in the soma rind anterior to the medulla and lobula. Numerous ir-fibers from the medulla and the lobula project into the median protocerebrum. About 100 cells in the lateral cell group of the antennal lobe exhibit FLI. Immunoreactivity appears in all glomeruli, the central neuropil, and fibers of the outer antenno-cerebral tract. Numerous small ir-somata in the pars intercerbralis send fibers into the protocerebral bridge and the central body, of which the upper division is most intensely stained. From the central body, ir-fibers project in the isthmus tracts into the lateral accessory lobes. The mushroom bodies exhibit uniform FLI in subcompartments of the α- and ß-lobes. The tritocerebrum exhibits dense fibrous FLI, and about 20-30 somata per hemisphere have immunoreactivity. The suboesophageal ganglion (SOG) contains about 40 pairs of ir-cells; ir-fibers project from the SOG into the neck connectives. A staining pattern nearly identical to that described for FLI was obtained with antisera against small cardioactive peptide$_b$ and bovine pancreatic polypeptide. We have used the same antisera employed in ICC to develop enzyme-linked immunosorbent assays (ELISAs), which have then been used to monitor the purifications of molecules showing FLI. After a batch-ion-exchange procedure followed by desalting on Sep-Paks, gel filtration chromatography revealed ir-molecules with approximate MWs of 1000 and 4250 Daltons and a minor peak at 7000 Daltons. It appears that at least some Manduca peptides are different than authentic FMRFamide (MW 599 Da.); cation-exchange and reverse-phase HPLC also reveal multiple ir-peptides differing in retention times from authentic FMRFamide. Supported by a grant from Monsanto Co. and NIH grant AI-23253.

70.7 GASTRIN/CCK-LIKE PEPTIDES IN THE BRAIN OF THE TOBACCO HAWKMOTH MANDUCA SEXTA. U. Homberg, T.G. Kingan, and J.G. Hildebrand. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Gastrin/CCK-like immunoreactivity has been previously reported in the brain of several insect species, most notably flies [Duve and Thorpe, Gen. Comp. Endocrinol. 48: 381-391 (1981) and Cell Tissue Res. 237: 309-320 (1984)].

To investigate the possible role of gastrin/CCK-like peptides as neurotransmitters in the moth Manduca sexta, we used antisera against gastrin/CCK (provided by J.M. Polak, College University of London) to study the distribution of gastrin/CCK-like immunoreactivity (GLI) in the brain and subesophageal ganglion (SEG). We are currently fractionating extracts of Manduca brains to isolate gastrin/CCK-like peptides.

The most intense GLI was consistently detected in a group of 30-40 pairs of small medial neurosecretory cells (MNCs) in the dorsal protocerebrum [IIb-cells, nomenclature according to Copenhaver and Truman, J. Comp. Neurol. 249: 186-204 (1986)]. Immunoreactive processes of these cells innervate especially the tritocerebrum, the corpora cardiaca, and the wall of the aorta. In addition, four pairs of IIa-MNCs are less intensely immunolabeled. GLI was also detected in most neuropil areas and several cell groups in the brain and SEG. Four groups of immunoreactive cells, totaling about 400 somata, are associated with the optic lobes and give rise to immunoreactive processes in the lamina, several strata in the medulla, and the lobula complex. 30-40 cells are associated with the antennal lobe (AL). GLI-containing fibers project in the AL-commissure and from the AL to the lateral protocerebrum. GLI is found in particular morphological types of neurons in the median protocerebrum, e.g. in neurons of the protocerebro-calycal tract innervating the calyces of the mushroom body, or segmental cells of the central complex. Scattered cells in the tritocerebrum and SEG also exhibit GLI, and fibrous immunoreactive staining is distributed throughout the neuropil.

Colocalization experiments suggest that virtually all gastrin/CCK-immunoreactive neurons are also immunoreactive with antisera against a crustacean pigment dispersing hormone (provided by K.R. Rao, U. of West Florida), a peptide that is structurally not related to gastrin/CCK. Particular groups of gastrin/CCK-immunoreactive cells in various parts of the brain show FMRFamide- or GABA-immunoreactivity.

The gastrin/CCK antisera have been used in the development of a sensitive enzyme-linked immunosorbent assay. We have used this assay to monitor the factionation of tissue extracts for GLI. Immunoreactivity in boiled tissue extracts is retained on anion-exchange resins (DEAE) in 0.1M NaCl and can be recovered during a salt gradient. Supported by a grant from Monsanto Co.

70.8 **ASSOCIATION OF A PROCTOLIN-LIKE PEPTIDE WITH THE OVIPOSITOR MUSCLES OF *LOCUSTA MIGRATORIA*.** Jim H. Belanger and Ian Orchard*. Dept. of Zoology, Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A1.

The innervation and musculature of the ovipositor of *Locusta migratoria* are similar to those previously described for *Schistocerca americana* (K.J. Thompson, *J. Exp. Biol. 122:* 387 - 411). As part of an investigation of pattern generation in this system, we have examined for the possible association of the pentapeptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) with the ovipositor muscles. Proctolin-like bioactivity (PLB) was determined using the oviducal muscles of *L. migratoria* as a bioassay. Authentic proctolin, in amounts from 2 to 200 fmol, produces a dose-dependent tonic contraction in this preparation (A.B. Lange, I. Orchard, and M.E. Adams, *J. Comp. Neurol. 254:* 279 - 286).

Ganglion and muscle extracts were assayed for PLB following Sep-Pak purification and chromatography on RP-HPLC. PLB, co-eluting with ^{3}H-proctolin, was found in the ventral opener, closer, retractor and protractor muscles, in amounts ranging from 0.68 to 1.97 pmol proctolin/mg protein (means of 4 - 5 determinations). Abdominal ganglia VII and VIII, which contain the cell bodies of the motorneurons supplying the ovipositor muscles, contained, respectively, 3.79 ± 0.71 and 9.39 ± 2.63 pmol proctolin/mg protein. When the fractions containing PLB were rechromatographed on a second (isocratic) HPLC system, the majority of the PLB co-eluted with ^{3}H-proctolin.

We conclude that proctolin, or a very similar peptide, is involved in the control of the ovipositor muscles and are currently using immunohistochemistry to further substantiate this.

70.9 PEPTIDE ACTION ON INSECT CARDIAC MUSCLE IS MEDIATED BY INOSITOL TRISPHOSPHATE (IP_3). N.J. Tublitz and P.Q. Trombley*. Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

The CNS of the tobacco hawkmoth, Manduca sexta, contains two bioactive neuropeptides that are released into the blood immediately after adult emerges from its pupal skin. These two peptides, known as the Cardioacceleratory Peptides (CAPs), have been previously shown to facilitate inflation of the adult wings by directly increasing contraction rate of the Manduca heart in vivo. That the CAPs induce a relatively long-term change in cardiac activity prompted a search for the mode of action mediating this physiological response. We report here our evidence suggesting that the CAPs may be acting by stimulating inositol phosphate metabolism.

Initial experiments showed that the response of the heart to pulse applications of either CAP_1 or CAP_2 was markedly reduced in the presence of 10mM Li+. This result implicated the inositol phosphates as a potential second messenger system in this preparation because others have demonstrated that extracellular Li+ blocks the terminal dephosphorylation of inositol 1-phosphate to inositol. This leads to a decrease in the concentration of phosphotidylinositol, the precursor of IP_3. Direct measurements of IP_3 using anion exchange chromatography showed that the CAPs elicit a demonstrable and rapid rise in IP_3 levels. Intracellular IP_3 concentrations rise almost 2-fold within 30 seconds in response to CAP treatment.

To determine whether IP_3 directly affected heart rate, hearts were either permeabilized with 0.5% saponin and pulsed with inositol 1,4,5-trisphosphate (Ins1,4,5P) or intact heart cells were treated with Ins1,4,5P in a solution containing 5% DMSO. Either procedure elicited similar responses, namely that Ins1,4,5P produced a dose-dependent increase in contraction rate of the in vitro heart. Neither IP_2, IP_1, nor inositol were cardioactive under these conditions. Threshold for the IP_3 response was between 0.1 and 1 micromolar. Support for the hypothesis that IP_3 exerts its effect through a rise in cytosolic calcium comes from 2 separate experiments: (1) the IP_3-induced cardioexcitatory response is totally abolished in the presence of EGTA; and (2) A23187, a calcium ionophore, causes an increase in heart rate.

We conclude from these data that the cardioexcitatory effect of the CAPs on the Manduca heart is mediated by intracellular changes in the levels of inositol trisphosphate, which in turn acts to regulate the levels of cytosolic free calcium. Supported by NIH grant #NS-24613.

70.10 NEUROPHARMACOLOGICAL EVIDENCE THAT ACETYLCHOLINE IS A NEUROTRANSMITTER IN THE CRAYFISH VISUAL SYSTEM. C.L. Pfeiffer and R.M. Glantz. Department of Biology, Rice University, Houston, TX 77001

Immunocytochemical studies in this lab have localized Ach in the columnar transmedullary neurons of the second optic ganglion (medulla external, ME) of the crayfish, Procambarus clarkii (Wang-Bennett et al., Neurosci. Abst. 12:243, 1986) with the Ach antibody of Geffard et al. (Science, 229:77, 1985). Other evidence suggests that the transmedullaries convey visual excitation from the lamina columnar projections to the noncolumnar cells of the ME (Wang-Bennett et al., J. Comp. Phys., 1987, in press). Three such noncolumnar cells can be functionally distinguished with intracellular recording in the ME neuropil. These include 14 identified sustaining fibers (SFs), (tonic "on" spiking interneurons) amacrine cells (nonspiking inhibitory interneurons) and tangential cells (hyperpolarizing medulla to lamina neurons).

Each of these cell types was tested for Ach sensitivity in the ME using Ach or carbachol. The SFs respond to light with a sustained compound EPSP, a tonic discharge and a hyperpolarizing "off response." Of 40 SFs tested, 21 exhibited exclusively depolarizing responses to carbachol (10^{-6} to 10^{-8} M) either presssure injected in the ME neuropil or bath applied. 19 SFs exhibited a hyperpolarizing component in the response. Amacrine cells respond to light with a graded depolarization. Carbachol applied to these cells depolarized 9 out of 13 neurons. The remaining 4 cells exhibited a hyperpolarizing component. Tangential cells hyperpolarize in response to light. Of three tested, 2 cells exhibited purely hyperpolarizing carbachol responses. One cell contained a diphasic response.

Cobalt experiments indicate that Ach acts directly on postsynaptic receptors. In 6 preps, cobalt was applied to the ME and synaptic visual responses were blocked by 80 to 100% in SFs and amacrine cells. In each of these cases, the effect of pressure injected carbachol was unchanged. These cobalt experiments also suggest that 2 classes of Ach receptors may coexist on the SFs and amacrine cells. In 5 out of the 6 cells tested, SFs and amacrine cells responded to carbachol with both depolarizing and hyperpolarizing potentials following cobalt treatment. Furthermore, the anticholinesterase, neostigmine (10^{-5} M) acts to potentiate the SF light response by 200% (N = 3).

These results further support the hypothesis that Ach is the neurotransmitter of the columnar transmedullary neurons which provide visual signals to the SFs, amacrine and tangential cells of the ME. Supported by NSF Grant BNS83-12296.

70.11 CHOLINERGIC TRANSMISSION AT SENSORY SYNAPSES OF CRAYFISH LATERAL GIANT ESCAPE REACTION CIRCUIT. F.B. Krasne and M.W. Miller. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Biochemical analyses of sensory nerves and pharmacological experiments on transmission at selected sensory neuron synapses indicate that ACh is probably a major sensory transmitter in decapod crustaceans such as lobsters and crayfish. We demonstrate here that ACh is the transmitter at junctions between mechanosensitive afferents and first order sensory interneurons in the well studied lateral giant (LG) tailflip escape reaction pathway of the crayfish. Arterially perfused cholinergic agonists depolarize and fire sensory interneurons and depolarize LG dendrites, while cholinergic antagonists depress normally evoked EPSPs in both sensory interneurons and LGs. The pharmacological depolarization of the LGs is due in part to input (via electrical synapses) from interneurons and in part to a direct action of the cholinergic agonists on the LGs themselves (even though they receive no known excitatory chemical synaptic input). The postsynaptic ACh receptors are pharmacologically similar to the nicotinic ACh receptors of sympathetic ganglia; selective skeletal muscle nicotinic agonists and muscarinic agonists are without postsynaptic effects. However, muscarinic agonists depress EPSPs in sensory interneurons and (therefore) also in the LGs. Therefore, it appears that ACh release from the cholinergic terminals of mechanosensory axons is subject to muscarinic down regulation by ACh. We consider it of particular interest that these cholinergic junctions, like the glutamatergic junctions of hippocampus, are subject to cooperativity-dependent long-term potentiation. Supported by USPHS grant NS08108.

70.12 IMMUNOCYTOCHEMICAL LOCALIZATION OF A NEUROTENSIN-LIKE PEPTIDE IN THE LOBSTER, *HOMARUS AMERICANUS*. *S.R. Kirschenbaum, H. Mekeel*, B.S. Beltz and E.A. Kravitz.* Biology Dept., Boston Univ., Boston, MA 02215 and Neurobiology Dept., Harvard Medical School, Boston, MA 02115.

Neurotensin, a tridecapeptide originally isolated from the bovine hypothalamus, serves an important role in gut function in vertebrate species. Recently a neurotensin-like peptide was identified in tissue extracts from the East Coast lobster. By radioimmunoassay, neurotensin-like immunoreactivity (NT-LI) was detected in nervous tissue, hepatopancreas, and cardiac tissue (Soc. Neuro. Abstr. 1984). An 11 residue peptide from lobster hepatopancreas has been purified and sequenced. The peptide has approximately 36% homology to mammalian forms of neurotensin with 4 identical residues at the COOH terminus. Here we report the immunocytochemical localization of NT-LI to the connective tissue sheath surrounding nerves of the stomatogastric system by light and electron microscopic immuncytochemical methods.

Wholemount preparations of the stomatogastric nervous system were processed for immunocytochemistry using a polyclonal antibody that recognizes the COOH terminus of mammalian neurotensin (kindly supplied by Dr. R.E. Carraway). At the light microscopic level, NT-LI is observed in the sheath of the superior oesophageal nerve, the inferior oesophageal nerve, and the proximal portion of the stomatogastric nerve. The label is localized to varicosities 1-2 um in diameter that form a network in the connective tissue sheaths surrounding these nerves. Since the labeled structures look like neurosecretory endings electron microscopy was used to determine if the staining was localized to particular classes of nerve terminals. NT-LI was selectively localized to terminals showing the typical appearance of neurosecretory endings. The terminals, which contain dense-cored granules of approximately 100 nm diameter are adjacent to hemolymph sinuses that may serve to collect released peptide.

Biochemical analyses were performed to determine if the peptide labeled in nervous tissue is similar to the peptide originally purified from the hepatopancreas. A connective tissue sheath extract was compared to synthetic peptide using reverse phase high pressure liquid chromatography. The major peak of NT-LI in the sheath extract elutes with the same retention time as synthetic lobster neurotensin.

The localization of this peptide to neurosecretory endings in nervous tissue of the lobster stomatogastric system raises the possibility that neurotensin like peptides play roles in gut function in species as diverse as primates and decapod crustaceans. In lobsters, peptide released from terminals in the sheath could modulate the activity of axons within the sheath or could, via circulation in the hemolymph, act as a hormone on nearby or distant targets. Physiological studies currently are underway exploring these possibilities. (This work was supported by NIH.)

70.13 THE PRESENCE OF ENKEPHALIN IN THE EYESTALK AND BRAIN OF THE LAND CRAB, GECARCINCUS LATERALIS. M.K. Leung, K. Whitfield,* M. Murray,*E.A. Martinez*and G.B. Stefano. Depts. of Chemistry and Biological Sciences, SUNY/Old Westbury, Old Westbury, NY 11568.

Recent work with opioids point to their involvement with color change regulation in Gecarcinus lateralis. The present study provides additional evidence for the presence of endogenous opioids within the central nervous system of this particular species. Current study revealed binding profile of ^{3}H-etorphine to eyestalk membrane suspensions to be monophasic, stereopspecific and saturable. Scatchard analysis showed a single class of high-affinity binding sites with K_D of 2.3 nM and B_{max} of 15.0 pmole/g protein. The eyestalk neural tissues and cerebral ganglia from 30 crabs were extracted with 1 M acetic acid containing 1% 2-mercaptoethanol and the protease inhibitors; PMSF, pepstatin A, bestatin, phosphoramidon, amastatin and EDTA. The extracts were clarified by centrifugation and purified by Sep-Pak C_{18}. The samples were lyophilized, redissolved in 10% acetonitrile and injected into a HPLC reverse-phase column. The column was eluted with a flowrate of 1 ml/min and a gradient using 0.1% TFA as solvent A and 80% acetonitrile in A as solvent B. One-ml fractions were collected and lyophilized. Fractions 10-17 were dissolved in 1 ml water and 200ul aliquots were used for quantification of Met- and Leu-enkephalin via radioimmunoassay. The results showed that the eyestalk extract contained several fractions with Met-enkephalin activity. Three fractions (10,11,12) in particular exhibited high Met-enkephalin activity with levels beyond the limit of the assay (5000 pg/ml). No Leu-enkephalin was detected in the fraction that corresponded to the retention time of Leu-enkephalin; however, fractions 11 and 12 showed the presence of material with Leu-enkephalin-like activity equivalent to 2000 and 4000 pg/ml, respectively. The cerebral ganglia extract contained both Met- and Leu-enklephalin in the amounts, 550 and 800 pg/15 brains, respectively. In addition, fraction 16 also contained met-enkephalin-like immunoreactive material. This and previous studies demonstrate that these chemical signal molecules have diverse roles in invertebrates. The present study is the first to demonstrate a neural-endocrine role for opioids in invertebrates. Supported by ADAMHA-MARC MH-17138 and NIH-MBRS RR 08180.

70.14 DISTAL RETINAL PIGMENT OF THE FIDDLER CRAB, UCA PUGILATOR: EFFECTS OF HISTAMINE AND GABA ON THE RELEASE OF THE LIGHT ADAPTING AND DARK ADAPTING HORMONES. G. K. Kulkarni* and M. Fingerman. Dept. of Biology, Tulane Univ., New Orleans, LA 70118.

Histamine (HA) and gamma-aminobutyric acid (GABA) inhibit pigment migration in melanophores of Uca pugilator, apparently by inhibiting release of melanophorotropic hormones (Hanumante, M. M. and Fingerman, M., Biol. Bull., 162-256, 1982; Quackenbush, L. S. and Fingerman, M., Comp. Biochem. Physiol., 79C:77, 1984). The current study determined whether HA and GABA also affect release of the light adapting hormone (LAH) and dark adapting hormone (DAH) that regulate migration of the distal retinal pigment, a screening pigment. To analyze the effect of HA, crabs on a white background exposed to 637 lx of light were used. HA (125 nmol/crab) produced dark adaptation of the distal pigment. An H_2 receptor agonist, 4-methyl histamine (4-MeHA)(20 µg/crab), also produced dark adaptation, but an H_1 receptor agonist, 2-methyl histamine, did not. An H_2 receptor blocker, metiamide (20 µg/crab), given 10 minutes prior to injection of 4-MeHA inhibited the action of 4-MeHA. Metiamide alone had no effect. These results suggest HA stimulates DAH release or inhibits LAH release by activating H_2 receptors. Because the study of the melanophores favored the interpretation that HA inhibits release of black pigment dispersing hormone, the hypothesis that HA inhibits LAH release is favored. Presumably, when the distal pigment is in a position intermediate between fully light adapted and fully dark adapted, as herein, both LAH and DAH are released to hold the pigment in that position. Inhibition of LAH release would then cause a shift of the position of the pigment toward dark adaptation. GABA (50-125 nmol/crab) produced light adaptation that was directly related to the dose in crabs in white pans exposed to light, 175 lx. GABA (125 nmol/crab) also produced light adaptation in crabs exposed to 88,532 and 700 lx. Crabs transferred from dim (88 lx) to bright (700 lx) illumination and given 125 nmol/crab of GABA exhibited more rapid proximal migration of their distal pigment, toward the fully light adapted position, than did saline injected controls. But GABA (25-125 nmol/crab) inhibited distal migration of the pigment, toward the fully dark adapted position, in crabs transferred from 700 lx to 88 lx, the amount of inhibition being directly related to the dose. A GABA antagonist, picrotoxin, inhibited the action of GABA when injected 15 minutes prior to the GABA injection. Picrotoxin alone had no effect. A GABA agonist, muscimol, produced a small but significant light adaptation. When muscimol was injected with GABA, the effect was additive. The simplest interpretation of these results with GABA, consistent with its well known role as an inhibitory neurotransmitter, is that GABA inhibited DAH release, resulting in light adaptational responses.

70.15 AN EFFERENT NEUROPEPTIDE IN THE EYE OF *LIMULUS*.
H.K. Lehman and R.B. Barlow, Jr. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

A circadian clock in the *Limulus* brain generates efferent optic nerve activity at night. The efferent input to the retina exerts multiple physiological and morphological changes which combine to increase visual sensitivity. Octopamine and a neuropeptide appear to act together to mediate these changes, and we have undertaken this study to identify the neuropeptide and to define its actions in the retina.

Biological activity that increases ERG amplitude in a dose dependent fashion can be extracted with acidified aqueous acetone from the retina, brain and circumesophageal ganglia. Moreover, the activity is destroyed by carboxypeptidase Y, trypsin and pronase and thus appears to be a neuropeptide. All tissues have a similar elution profile from Sephadex G-25, however, several peaks elute from CM-Sephadex. The brain and ganglia extracts contain a predominate peak of bioactivity that is strongly retained by the ion exchange resin, whereas the retinal extract elutes much earlier but is more potent.

HPLC has also been used to fractionate the efferent neuropeptide. The brain/ganglia extracts contain activity that is slightly more hydrophobic than the retinal extracts when eluted from a C-18 reverse phase column with a 10 to 35 % acetonitrile gradient with TFA as a counter ion. Only a single peak of activity elutes from HPLC from either of the ion exchange purified extracts. We are currently isolating and sequencing the retinal peptide.

We have also characterized the actions of the purified neuropeptides on the response of single optic nerves. In brief, the peptide decreases the spontaneous optic nerve discharge and increases the response at high light intensities. The latter effect appears to be correlated with an increase in the ERG amplitude.

This work has been supported by NIH grants EY-05861 (HKL), EY-00667, and NSF grant BNS-8320315 (RBB).

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY I

71.1 THE ROLE AND REGULATION OF CARBONIC ANHYDRASE-II IN EARLY EYE DEVELOPMENT.
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In most vertebrate species, the neural retina is one of the most concentrated sources of the enzyme carbonic anhydrase-II (CA-II). In many species including birds, the primary cellular compartment of CA-II in retina is the Muller glial cells. However, during embryonic development, the enzyme is first expressed in very high levels in all retinoblast cells later becoming restricted to the glia as cell differentiation and maturation occurs. Previous studies showed that high levels of CA-II are measured in the neural ectoderm of the embryonic eye as early as day 3 of development in the chicken. We have examined a potential role of this enzyme in early eye development as well as the timing and inductive influences which control its early expression. In mature eye, CA-II activity is directly involved in the regulation of intraocular pressure. Hence we have tested whether or not CA-II activity influences the spherical expansion of the embryonic eye during early morphogenesis. The inhibitor of CA-II activity, methazolamide, was applied to 3-7 day embryos in shell-free culture by two different methods: topically via microosmotic pumps; and by injection into the extraembryonic cavity around the head. In both cases, significant reduction of eye growth was measured.

Our efforts to understand the regulated timely expression of CA-II have focussed on defining the earliest time and location of CA-II expression and attempting to perturb the expression by blocking normal tissue and cell interactions. Using monoclonal antibody immunohistochemistry we have found that the earliest expression of CA-II occurs in the region of neuroectodermal-skin ectodermal interaction and induction around stage 14 (Hamburger and Hamilton) of development. The antigen appears simultaneaously in a few cells of both the neural retina and lens primordia and thus it seems that CA-II expression is one of the early events following embryonic induction of retina and lens. Even as early as day 3 of development (stage 20-22) the distribution of CA-II distinguishes neural ectoderm destined to become retina or PE from contiguous cells destined to become optic nerve. Perturbation of CA-II expression has only been accomplished thus far by either preventing the interaction of the two ectodermal layers or later in development by dissociating neural cells and plating in sparse tissue culture.

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71.2 ADHESIVE MOLECULES IN THE DEVELOPING MAMMALIAN RETINA.
J.A. Robson and S. Carbonetto. Dept. Anat. & Cell Biol., SUNY Health Science Ctr., Syracuse, NY, 13210, and Montreal General Hospital Res. Inst., McGill Univ. Montreal, Quebec, Canada, H3G 1A4.

We are using in vitro methods to study histogenesis in the embryonic rat retina. Of particular interest are adhesive molecules important for cell-substrate attachment (e.g. laminin and its receptors) and cell-cell attachment (e.g. NCAM). We have screened whole eyes and organ cultured retinas from embryonic rats for several of these adhesive molecules using immunocytochemistry.

Embryonic rats between 15 and 21 days post-conception have been used. Eyes were dissected and the retinas were cultured. In some cases, ^{3}H-thymidine (plus or minus cytosine arabinoside as a control) was added to the medium. After 24 hours, these retinas were solubilized and counted with a scintillation counter. Other cultured retinas and non-cultured whole eyes were fixed with paraformaldehyde, embedded in polyethylene glycol and sectioned.

The cultured retinas incorporate as much as 100 times more ^{3}H-thymidine than the controls. In thionine stained sections, the neuroepithelium appears healthy although degenerating cells are present in the region of the future ganglion cell layer. Antibodies to cell-substrate adhesive molecules have not labeled the cultured retinas. However, an antibody to NCAM produces staining throughout them. It is prominent in the inner plexiform layer which appears around embryonic day 19. In the whole eyes, anti-NCAM labeling is like that in the cultured retinas. In addition, antibodies to cell-substrate adhesive molecules label tissue, but not in the neural retina. For example, anti-laminin labels the internal limiting membrane adjacent to the growing axons in the optic fiber layer. It also labels the external limiting membrane, while anti-heparan sulfate proteoglycan labels the choroid. These results are consistent with those from other labs suggesting a role for NCAM in cell migration and retinal differentiation, while laminin may be important for the elongation of ganglion cell axons. (Supported by NIH grants EY03940 and NS19068 and NATO grant 0235/87).

71.3 **CYTOGENESIS OF MONKEY RETINA: COMPARISON OF THE GENERATION OF RETINAL PIGMENT EPITHELIUM AND NEURAL RETINA**. D.H. Rapaport[1], D. Yasumura[2*], M.M. LaVail[2] and P. Rakic[3]. [1]School of Anatomy, University of New South Wales, Kensington, NSW 2033 AUSTRALIA, [2]Department of Anatomy, University of California, San Fransisco, CA. and [3]Section of Neuroanatomy, Yale University, New Haven, CT.

The optic vesicle, an embryonic outgrowth of the diencephalon, generates the neural retina and the retinal pigment epithelium (RPE), two tissues which although quite distinct both structurally and functionally remain closely related throughout life. Both their common embryological origin and spatial proximity suggest that the development of one tissue could be influenced by that of the other. The purpose of this investigation was to compare the timing and pattern of cell birth in the RPE with that of the neural retina which was reported previously (Invest. Ophth. Vis. Sci. 24 (Suppl.): 7, 1983). Pregnant female Rhesus monkeys (Macaca mulatta) were injected with ^{3}H-thymidine on selected post-conception days, and the fetuses allowed to come to term and survive for 2-5 postnatal months. At the time of sacrifice, the animals were deeply anesthetized and perfused. An eye was removed, hemisected along the horizontal meridian, embedded in polyester wax, and processed for autoradiography. Tissue was viewed with incident, polarized light in order to resolve silver grains over the pigmented RPE cells. Three sections from each eye were analyzed by counting all labelled cells in 240μm bins sequentially, from the center of the fovea to the nasal and temporal edges.

No labelled cells were found in either the RPE or the neural retina at E27. At E30, a significant number of labelled cells were found in the RPE (mean=8.3 cells/section) and neural retina (ganglion and horizontal cells only). In both tissues labelled cells were only found in an area from the temporal edge of the fovea extending approximately 2mm into nasal retina, encompassing only ~18% of the retinal length. This region expanded gradually, 1.5 mm into temporal retina and 5.0 mm into nasal retina at E43 (~38% of retinal length), and 3.6mm into temporal and 6.7mm into nasal retina at E56 (~69% of retinal length). Concurrently, labelled cells disappeared from an ever expanding central area. As a result there is an annulus of labelled cells centered on the fovea. This is true for ganglion and horizontal cells; however labelled cells of different classes (amacrine, bipolar etc) are still found in central retina, and as a result the annulus is not apparent unless cell class is taken into account. Other cells in the neural retina are generated in the rough, overlapping sequence: amacrine (E38-E110), cone (E38-E102), bipolar (E38-P17), Müller (E45-E110), and rod (E45-P17) cells. The generation of each cell class follows the same spatial pattern described above. The last labelled cells in the RPE were seen at the edge of the retina, at P17. Thus, the period of RPE cell birth spans the entire sequence of birth of the neural retina, from E30 to P17. These data suggest that whatever mechanism controls the onset, spatial pattern, and cessation of cytogenesis in the retina operates simultaneously on both the neural retina and RPE.

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71.4 CULTURED RETINAL NEURONS FORM CLUSTERS IN THE PRESENCE OF NEUROEPITHELIAL CELLS. I. Mody, L. Brandes*, M. Deverill* and J.F. MacDonald. Playfair Neuroscience Unit, Dept. of Physiology, Univ. of Toronto and The Toronto Hosp., Toronto, Ont. M5T 2S8.

Monolayer cultures of CNS neurons (e.g., hippocampus) do not form organized cellular laminations as would be characteristic of these regions. We report here that dissociated cultures of cat, guinea pig and mouse retina differ significantly in this regard. Mammalian retinal cultures demonstrate a period of cellular migration during which "clusters" of neurons are formed (as shown in the chick[1]).

Retinae were dissected from 18 day old mouse (the majority of experiments), 120 day old cat, and 50 day old guinea pig foeti. The eye was removed and the retina micro-dissected. Cells were dissociated and cultured using standard techniques for one to two weeks. In some cases, retinal neurons were grown on feeder plates (that included fibroblasts, etc. instead of neurons) derived from retina, hippocampus, spinal cord or cerebral cortex.

Cultures were photographed under phase contrast microscopy. Neuronal somata were located with respect to each other by assigning each cell to the element of a reference grid. The randomness of the spatial arrangement of neurons was determined by fitting observed data to a Poisson distribution with the same mean. Using an adaptation of Lloyd's Indices of Mean Patchiness[2] it was possible to objectively quantify "clustering" of the neurons.

The following observations were made: 1) Retinal cultures consistently demonstrated significant clustering, whereas mouse hippocampal, cerebral cortical, spinal cord or midbrain cultures did not. 2) When retinal cells were grown on feeder plates derived from other CNS regions, clusters were no longer formed. 3) In contrast, when retinal cells were grown on feeder plates derived from the retina, clustering was again observed. 4) If retinal cells were cultured on half of the same dish with hippocampal neurons (no mixing of retina and hippocampus, but shared media) clustering occurred only on the retinal side. 5) If non-neuronal retinal tissue (including the epithelium) was included with hippocampal neurons, they also could be induced to form clusters. 6) In the guinea pig retina, clustering was related to the inclusion of the pigmented epithelial cell layer. These results suggest that clustering of retinal neurons is dependent upon physical contact with support cells such as the neuroepithelium.

Supported by the Medical Research Council of Canada.

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[2]Lloyd, M., 1967, J. Anim. Ecol., 36:1-30.

71.5 **NEUROTRANSMITTER-RELATED ACTIVITIES IN DISSOCIATED CULTURES OF MOUSE RETINA NEURONS AND PHOTORECEPTORS** L.E. Politi* and R. Adler. Wynn Center, Wilmer Institute, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

Culture systems recently developed in this laboratory allow growth of purified populations of retinal neurons and photoreceptors from both normal and "rd" (retinal degeneration) mice. The differentiation of these cells in serum-free, chemically defined medium has already been investigated by immunocytochemistry and electron microscopy (Invest. Ophthalmol. Vis. Sci. 28:345, 1987). We report here further characterization of these cultured cells using biochemical and autoradiographic methods for neurotransmitter-related activities. High affinity uptake mechanisms were studied by incubating cultures of normal mouse retinal cells for 15 min at 37° in 10^{-7}-10^{-9} M ^{3}H-γ-aminobutyric acid (GABA), glutamate, aspartate, taurine, or glycine, in the presence or the absence of Na^+. The cultures were lysed to determine intracellular radioactivity by liquid scintillation counting, or fixed in glutaraldehyde and processed for autoradiography.

The cultures exhibited uptake mechanisms for all the putative neurotransmitters mentioned above. Most of these uptakes were almost completely inhibited in Na^+-free medium, although glycine uptake was inhibited only 40-60% under the same conditions. GABA uptake was inhibited 90% by excess unlabeled diaminobutyric acid (DABA), but only 10% by β-alanine. Autoradiographic analysis showed that the uptake mechanisms were differentially distributed among different cell types. For example, GABA was accumulated by more than 95% of the multipolar neurons, but was not taken up by photoreceptors. On the other hand, glutamate and aspartate were taken up by 85-95% of the photoreceptor cells, while labeling was seen in only 40% of the other retinal neurons. This labeling was generally less intense than that associated with photoreceptors. Choline acetyltransferase was also present in these cultures.

These studies demonstrate that differentiating retinal neurons and photoreceptors from normal mice can express characteristic neurotransmitter-related activities in serum free cultures. Work is in progress to compare these normal cells with cultured retinal neurons and photoreceptors from "rd" (retinal degeneration) mice.

Supported by USPHS grant EY05404.

71.6 IN SITU HYBRIDIZATION TO LOCALIZE NEUROFILAMENT mRNA IN THE GOLDFISH RETINA. P.S. Jones*, P. Tesser* and N. Schechter (SPON. I. Fand) Depts. of Biochemistry, Pharmacology, and Psychiatry, SUNY at Stony Brook, NY 11794.

In situ hybridization experiments are being performed to determine the sites of synthesis of the goldfish visual pathway neurofilament proteins ON_1 and ON_2. Ex vivo protein synthesis and in vitro translation studies have determined that ON_1 and ON_2 are synthesized in the retina, but not in the optic nerve. We have synthesized and cloned cDNAs for ON_1/ON_2 from mRNA isolated from the retina. We have subcloned a 150 nucleotide Eco R1 fragment encoding a portion of the carboxyl variable region of ON_1/ON_2 into a T_3/T_7 transcription vector and have used this system to synthesize ^{35}S and biotin labeled RNA probes. These transcripts are being used to localize the ON_1/ON_2 message in the retina. Previous experiments using polyclonal antibodies directed against ON_1 and ON_2 revealed high amounts of immunoreactivity in the retinal ganglion cell layer, and lower levels of reactivity elsewhere in the retina. In situ hybridization analysis will determine whether ON_1 and ON_2 are synthesized in various other cell types in the goldfish retina, for example, the amacrine cells. Furthermore, this type of analysis will be able to determine whether the regeneration-induced increase in synthesis of ON_1 and ON_2 occurs in all ganglion cells, or whether it is restricted to a specific subpopulation of cells, for example, the younger, peripherally located retinal ganglion cells. This research is supported by a grant from the NIH (EY-05212 to N.S.).

71.7 RNA SYNTHESIS IN THE GANGLION CELL LAYER OF THE DEVELOPING CAT RETINA. H.E. Pearson, D.L. Smith*, N.K. Rosenblatt*, B.R. Payne and T.J. Cunningham. Depts. of Anatomy, Temple Univ. Sch. of Medicine, Philadelphia, PA 19140, Boston Univ. Sch. of Medicine, Boston, MA 02118 and Medical Coll. of Pennsylvania, Philadelphia, PA 19129.

Ablation of visual cortex in the cat results in primary retrograde degeneration of cells in the dorsal lateral geniculate nucleus (dLGN) of the thalamus. Such degeneration deprives many retinal ganglion cells of their principal target neurons. In cats which survive to adulthood, the long-term effects of the ablation can be seen in the transneuronal retrograde degeneration of retinal ganglion cells, particularly in peripheral retina, and in the reorganization of the axon projections of the surviving cells. We were interested in determining any short-term metabolic changes in retinal ganglion cells which may provide some insight into the mechanisms underlying these morphological effects.

Retinae were obtained from intact kittens and from kittens in which all the contiguous visual cortical areas had been ablated bilaterally on the day of birth (P1). All kittens received bilateral intraocular injections of tritiated uridine 24 hours prior to sacrifice and were perfused with aldehyde fixative on postnatal days 2,4 and 7 (P2, P4 and P7). The area centralis was dissected from each retinal wholemount and the resulting sample embedded in plastic. Sections one micron in thickness were processed for autoradiography and stained through the emulsion with toluidine blue. The density of overlying silver grains was determined for 100 neurons within the ganglion cell layer of each retina.

Within each retina, the grain densities showed a unimodal distribution. There was therefore no evidence of any separation of the different neuronal populations, such as displaced amacrine and ganglion cells, on the basis of differences in uridine uptake. No differences in average grain density were found between intact kittens and those with neonatal visual cortex ablation at any of the ages studied. However, the average grain density in both intact and ablated kittens was significantly higher at P4 than at either P2 or P7, when the densities were not different from each other. These results show that developmental changes occur in the uptake and incorporation of uridine into RNA in neurons of the retinal ganglion cell layer during the first week of postnatal life. The results suggest that this increase in RNA synthesis at P4 relates to developmental events intrinsic to the retina and is independent of interactions with target neurons in the dLGN.
(Supported by Biomedical Research Support Grant #RR05417-25 awarded to Temple University by the Division of Research Resources, NIH)

71.8 MATERNAL SUPPLY OF N-3 ESSENTIAL FATTY ACIDS TO THE DEVELOPING MOUSE RETINA. B.L. Scott, J. Moises and N.G. Bazan (Sponsored by H.E.P. Bazan), LSU Eye Center, 2020 Gravier Street, Suite B, New Orleans, LA 70112

The n-3 essential fatty acid family comprises a series of polyunsaturated fatty acids possessing a double bond at the n-3 carbon -- the third carbon from the methyl terminus of the fatty acyl chain. The major n-3 fatty acid found in membranes is docosahexaenoic acid (22:6), which contains twenty-two carbons and six double bonds and is found in high concentrations in synaptic membranes and in the outer segment disk membranes of retinal photoreceptor cells. The function of 22:6 in photoreceptor membranes is not known although the fatty acid may facilitate phototransduction. 22:6 is retained tenaciously by retinal membranes when animals are placed on an n-3 deficient diet. Long-term n-3 deficiency, however, is known to alter the normal electroretinogram (Wheeler et al, Science 188:1312, 1975) and compromise visual acuity (Neuringer et al, J Clin Invest 73:272, 1984). Animals cannot synthesize 22:6 de novo, rather it must be formed by elongation and desaturation of other members of the n-3 family, the most common precursor being linolenic acid (18:3, n-3).

We are interested in the metabolism of n-3 fatty acids and their accumulation in the developing retina. In order to determine in the mouse, the form in which n-3 fatty acids are supplied by the mother to her pups, we have determined the fatty acid composition of the stomach contents and blood plasma of developing mouse pups. Analysis of stomach contents of the pups reflects the composition of the mother's milk. Analysis of plasma obtained from the pups reflects any n-3 metabolism carried out by the developing animals.

Stomach contents and plasma were obtained from 1- to 27-day-old normal C57BL/6J mouse pups. Total lipid extracts were prepared using 2:1 (by vol) chloroform-methanol. Fatty acyl groups were converted to methyl esters and resolved by capillary gas-liquid chromatography, using a 30m x 0.25 mm capillary column containing a 10% SP-2330 stationary phase (Supelco, Inc.).

For the first two weeks of post-natal development, the stomach contents of pups exhibited an 18:3/22:6 molar ratio ranging from 2 to 4, indicating that the maternal supply of n-3 fatty acids was primarily in the 18:3 form. At about 14 days of age, the pups became able to feed on the adult hard-pellet diet, which was found to possess an 18:3/22:6 molar ratio of about 750. Clearly, throughout development, the primary supply of n-3 fatty acids to the developing pups was in the form of 18:3. The plasma samples taken from the pups revealed an 18:3/22:6 molar ratio of less than one at all ages sampled. Thus, the developing mouse pup has the ability to rapidly desaturate and elongate 18:3 supplied by the mother, converting it to 22:6 for subsequent incorporation into retinal lipids. Supported by EY04428.

71.9 THE PRENATAL DEVELOPMENT OF THE A-TYPE HORIZONTAL CELL OF THE CAT'S RETINA: AN ELECTRON MICROSCOPIC AND AUTORADIOGRAPHIC STUDY R.P. Zimmerman, R.L. Fortney & R.H.Polley, Rush Medical College and University of Illinois, Chicago 60612

The development of a specific class of interneuron of the mammalian retina, the A-type horizontal cell (AHC), was studied in the retina of the cat using transmission and scanning electron microscopy and light microscopic tritiated thymidine autoradiography. Where possible, observations were made in central retina; and all times quoted refer to this region.

Previous autoradiographic studies of the retinas of adult cats injected during fetal development have established that AHC undergo their final cell division during the earliest period of neurogenesis, from E22 (embryonic day 22) to E30. Retinas examined electron microscopically during the third through fifth week of development (E20, E24, E28, E35) do not show a distinct morphological population that might represent committed AHC neuroblasts. However, by E40 AHC (labelled by a tritiated thymidine injection at E26) have migrated away from the outer limiting membrane, and their somata are larger than those of either the cones or the ganglion cells produced on the same day.

While the AHC somata form a distinct layer after E40, their processes do not form a continuous outer plexiform layer (OPL) for about another 10 days. The infolded nucleus and electron-lucent cytoplasm characteristic of the adult AHC appear by E49. At this time the AHC dendrites are directed radially as well as horizontally. The dendrites of the AHC become predominantly horizontal and form a continuous OPL at E51 (central retina) or later. By E56 these dendrites are postsynaptic to photoreceptors at flat contacts and dyadic ribbon synapses. The ribbon synapses are complete with arciform densities, presynaptic vesicles and postsynaptic membrane specializations. Thus, morphologically, and perhaps functionally, maturing synapses are found in the OPL more than a week before birth. Supported in part by EY 6163 and EY 4593.

71.10 DEVELOPMENT OF GABA-LIKE IMMUNOREACTIVITY IN HORIZONTAL CELLS AND THE OUTER PLEXIFORM LAYER OF THE RAT RETINA. B-A. Battelle and T. W. Hilton*. C. V. Whitney Laboratory and the Department of Neuroscience, University of Florida, St. Augustine, Florida 32086.

GABA-like immunoreactivity (GABA-LI) has recently been identified in subpopulations of horizontal cells (H-cells) and in the outer plexiform layer (OPL) of a number of mammalian species. We reported the presence of GABA-LI in the rat retina during early postnatal development. Here we describe the development of this GABA-LI during the first two postnatal weeks. GABA-LI was detected in 15 um frozen sections of glutaraldehyde-fixed retinas using antibody purchased from Immunonucler and the PAP technique of Sternberger. Staining was completely blocked when the antiserum was preabsorbed with BSA-conjugated GABA. Sections were cut along the superior-inferior axis and most sections analyzed either passed through or were close to the optic nerve head in the nasal half of the eye.

GABA-LI develops in the H-cells and the OPL with a distinct central to peripheral gradient. On postnatal day 2 (P2) a few cells lightly stained for GABA-LI were observed in the outer 1/3 of the retina near the optic nerve head. At this time strong GABA-LI was already present in the developing ganglion cell, inner plexiform and amacrine cell layers. On P4 a short row of cells and fibers containing GABA-LI was observed near the optic nerve head in the region of the developing OPL. Peripheral to this, other GABA-LI cells were seen scattered in the outer 1/3 of the developing retina extending 3/4 of the distance toward the peripheral edge of the retina. By P-6 a row of cells and fibers containing GABA-LI extended 3/4 of the distance toward the peripheral edge of the retina in the region of the developing OPL. GABA-LI reached the peripheral edge of the retina by P10. The OPL of retinas from P10 rat retinas also stained with antibodies directed against glutamic acid decarboxylase.

GABA-LI also fades from the H-cells and the OPL with a central to peripheral gradient. At P12, it is less intense in the central retina compared to that seen in the periphery, and by P14, it was no longer detected in this layer in the central retina, but faint staining persisted in the periphery. We were unable to detect GABA-LI in this layer in the adult rat retina. Our observation that the GABA-LI appears just as the OPL develops and then declines, suggests that GABA may have a special role to play in OPL development.

Others (Mosinger et al. 1986 and Osborn et al. 1986) using different antibodies directed against GABA have reported GABA-LI in horizontal cells and the OPL of the adult rat retina. It is not clear whether the cells containing GABA-LI described here are the same or different from those found in the adult rat retina.

71.11 A DEVELOPMENTAL STUDY OF GAMMA-AMINOBUTYRIC ACID IMMUNOREACTIVE CELLS AND PROCESSES IN THE EMBRYONIC CHICK OPTIC TRACT. R.H.Granda* and W.J. Crossland, Dept. Anatomy and Cell Biology, Wayne State Univ. Schl. Med., Detroit, MI 48201.

We have previously shown the existence of cells that are immunoreactive to an antibody to gamma-aminobutyric acid (GABA+ cells) in the chick optic tract. These cells have neuronal morphology at the light and electron microscopic level and stain positively with an antibody to neuron-specific enolase. To further understand the origin of these cells, we undertook a study of their morphology and distribution in the chick embryo.

Chick embryos ranging in age from day 10 of incubation (E10) to day 18 (E18) were perfused with a mixed aldehyde solution. Frozen (40-60u.) or polyethylene glycol embedded (10-20u.) sections were incubated in a 1/1000 rabbit anti-GABA antiserum (provided by Dr. R. Pourcho) and visualized with an avidin-biotin system (ABC kit, Vector Labs) using diaminobenzidine as a chromagen.

GABA+ cells were seen in the tract as early as E10. Antibody staining demonstrated neuron-like morphology (bipolar and multipolar) of the GABA+ cells at all stages examined. Many cells near the GLv had one stout process perpendicular to the tract fibers, while other cells lacked the stout process and gave off finer processes parallel to the fibers. At E10, GABA+ cells were seen in the optic tract near the ventral lateral geniculate nucleus (GLv), while at later stages these cells shifted medially toward the chiasm. Thus the cells may originate from the more laterally placed visual centers such as the GLv or optic tectum.

GABA+ growth cone-like enlargements were seen at the ends of GABA+ (presumably axonal) processes between E10 and E14 in the optic tract and could be followed into the optic nerve or into the contralateral optic tract. It is unlikely that the processes originate in the isthmo-optic nucleus because its cells and processes are not GABA+ in the mesencephalon, and therefore the growth cone-like enlargements may originate from the GABA+ tract cells or from "aberrant" projections from other visual centers.

This study was supported in part by grants from the Michigan Eye Bank (W.J.C.) and by an NIH Core Grant (EY-04068). R.H.G. was supported by a fellowship from the Neuroscience Program at Wayne State University.

71.12 MONOCLONAL ANTIBODIES SPECIFIC FOR INTERMEDIATE FILAMENT PROTEINS OF NEURONAL AND NON-NEURONAL ORIGIN IN THE GOLDFISH VISUAL PATHWAY. J. Borchert*, P. Jones*, F. Salles*, and N. Schechter (SPON. W. Quitschke) Depts. of Biochemistry, Pharmacology, and Psychiatry, SUNY at Stony Brook, NY 11794.

We have generated monoclonal antibodies which can distinguish between neuronal and non-neuronal intermediate filament (IF) proteins in the goldfish visual pathway. The IF proteins of this pathway do not match the conventional classification as found in mammalian neural tissue. The predominant IF proteins of the goldfish optic nerve have a molecular weight of 58K, and can be separated into a series of isoelectric variants, designated as ON_1 - ON_4. ON_1 and ON_2 are neurofilament proteins, whereas ON_3 and ON_4 are non-neuronal IF proteins. Peptide mapping analysis indicates that ON_1/ON_2 are similar to each other but differ from ON_3/ON_4 which are also similar to each other.

BALB/c mice were immunized with goldfish optic nerve cytoskeletal proteins. After the fourth immunization, the spleen cells were fused with 8653 mouse myeloma cells. Approximately 500 clones were obtained from the fusion. The clones were screened by reacting the supernatents against cytoskeletal proteins using the ELISA assay which we developed. Forty-five clones produced positive signals. Of these, four clones which were extremely reactive were used to probe the cytoskeletal proteins by Western immunoblot analysis of 2D gels. Three clones reacted specifically with proteins ON_1/ON_2, and one clone reacted with ON_3/ON_4 and a 48K protein which was previously reported to be of non-neuronal origin.

IF proteins consist of a conserved 40K core region which is flanked by two variable domains. These variable domains are responsible for the observed diversity associated with this class of proteins. Therefore, monoclonal antibodies specific for particular IF proteins more than likely recognize epitopes in the variable domains. The monoclonal antibodies against the goldfish IF proteins differentiate a homologous class of proteins from two different cell types, i.e., retinal ganglion cells and glial cells, the former of which displays a remarkable capacity for nerve regeneration. This research is supported by a grant from the NIH (EY-05212) to N.S.

71.13 cDNA CLONING OF THE INTERMEDIATE FILAMENT PROTEINS OF NEURONAL AND NON-NEURONAL ORIGIN IN THE GOLDFISH VISUAL PATHWAY. S. Giordano*, P. Tesser*, and N. Schechter. Departments of Biochemistry and Psychiatry, SUNY at Stony Brook, New York 11794.

We are investigating the structure of the predominant intermediate filament proteins of the goldfish visual pathway. These proteins have a molecular weight of 58K and can be separated into a series of isoelectric variants which have been designated as ON_1-ON_4. ON_1 and ON_2 are similar to each other but differ from ON_3 and ON_4 which are also similar to each other. In vivo and in vitro studies indicate that the levels of synthesis of ON_1 and ON_2 are linked to the regeneration of the optic nerve, whereas the synthesis of ON_3 and ON_4 is rather stable during the process. ON_1 and ON_2 are neurofilament proteins and ON_3 and ON_4 are non-neuronal intermediate filaments. cDNA cloning experiments were initiated in order to compare these proteins at the structural level and also to compare them with other higher vertebrate intermediate filament proteins which may be involved in cell differentiation and neurogenesis.

In addition to a previously reported cDNA library from retina which contained positive clones for ON_1 and ON_2, we have now prepared a cDNA library from optic nerve. The glial cells are the predominant source of RNA from the optic nerve. Although we are only able to obtain approximately 1 microgram of total RNA per optic nerve, we have been successful in translating total optic nerve RNA and have specifically immunoprecipitated ON_3 and ON_4 with anti-ON_3/ON_4 polyclonal antibodies. Furthermore, total optic nerve RNA provided satisfactory templates for cDNA synthesis in the presence of oligo(dT) as a primer. Total RNA was reverse transcribed using RAV reverse transcriptase followed by second strand synthesis using RNase H and DNA polymerase. The cDNA was subsequently methylated, tailed with Eco RI linkers, and cloned into the lambda gt 11 expression vector. A library of over 1.5×10^6 recombinants was generated containing cDNA inserts of approximately 1 KB. We are now employing both polyclonal and monoclonal antibodies to screen this library for positive clones for proteins ON_3 and ON_4.

Intermediate filament proteins contain a 40K core region which is highly conserved with respect to its amino acid composition. The diversity of this class of proteins is generated by variable regions which are adjacent to either side of the core. It has been suggested that these variable regions may be involved in special functions associated with the differentiated state of specific cells. A structural analysis of the goldfish ON proteins will help to determine what the contribution of these variable regions are to the function of intermediate filament proteins in neurogenesis and nerve regeneration. This research is supported by a grant from the NIH (EY-05212) to N.S.

ALTERED RETINAL AMINE NEUROTRANSMITTERS IN FORM-DEPRIVATION MYOPIA. R.A. Stone, T. Lin*, A.M. Laties and P.M. Iuvone. Dept. of Ophthalmology, Scheie Eye Inst., Univ. Pa. Sch. of Med., Philadelphia, PA 19104 and Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Deprivation of form vision in the newborn chick causes exaggerated postnatal ocular growth. As a result, distant images focus in front of the plane of the retina, and a myopic refractive error results. The paramount anatomical changes, an increase in axial and equatorial dimensions, resemble common human myopia. We induced form deprivation myopia in day-old male White Leghorn chicks under aseptic conditions and ether anesthesia using one of three procedures, all unilateral:
1) eyelid suture; 2) translucent plastic goggle; or
3) transparent plastic goggle.

At two or four weeks of age, the chicks were killed by decapitation. The visually deprived eyes were significantly larger in both axial and equatorial dimensions than contralateral control eyes. By high performance liquid chromatography with electrochemical detection, the following statistically significant alterations in the concentration of biogenic amines expressed as percent of contralateral control were measured in post-equatorial retinas of the myopic eyes:

	Two Weeks - Percentage Change			Four Weeks - Percentage Change		
	Eyelid Suture	Translucent Goggle	Transparent Goggle	Eyelid Suture	Translucent Goggle	Transparent Goggle
Dopamine	-23%	-20%	-25%	-29%	N.S.*	-23%
DOPAC	-62%	-37%	-42%	---	N.S.	N.S.
Serotonin	+67%	+23%	N.S.	+53%	N.S.	N.S.

*N.S. = Not Significant

Thus, deprivation of form vision in the chick by all three procedures induces a reduction in retinal dopamine concentration at two weeks. This tendency persists at four weeks, at least for two of the experimental conditions. In contrast, serotonin concentrations are elevated after eyelid suture at two and four weeks and after translucent goggles at two weeks. Further work is necessary to seek a role, if any, for biogenic amines in the exaggerated ocular growth that accompanies form deprivation.

71.15 INCREASED POTENTIATION OF POSTSYNAPTIC RESPONSES CORRELATES WITH SENSITIVE PERIOD DURING OPTIC NERVE REGENERATION IN GOLDFISH.
J.T. Schmidt, Dept. Biol. Sci., SUNY Albany, NY 12222.

In this study, I tested for an increased capacity for long term potentiation in the regenerating projection and its possible relationship to the activity driven sharpening of the retinotopic map on tectum. In the normal optic pathway, supramaximal stimulation gives rise to very stable field potentials in tectum (approx. 4 mV maximum negative response in the retinal synaptic layer). After moderate or high frequency trains of stimuli, there are only very small increases (20-30%) in response amplitude (see also Lewis and Teyler, Brain Res. 375:246, 1986). The regenerating projection first yields small field potentials around day 20 postcrush (0.1 mV), and the amplitude becomes larger at longer times postcrush reaching normal by 5 months. At 20 to 30 days postcrush, the small (0.1-1 mV) responses fatigue rapidly during repeated stimulation. However, administering as few as 6 to 20 supramaximal stimuli at 0.1 Hz typically results in a 100 to 200% increase in response amplitude when tested 15 to 30 minutes later. (To test without potentiating, I stimulated only once per 30 min. which resulted in stable potentials.) Subsequent trains of stimuli resulted in smaller percentage gains in response amplitude, but the cumulative gain could be very large, and the increased responses were both stable for many hours and less susceptible to fatigue. At later times during the sensitive period (40-80 days), smaller but significantly enhanced potentiations could still be elicited. I am currently testing whether the NMDA blocker APV can prevent potentiation without decrementing ongoing responses. Doses less than 50 μM cause little reduction in response amplitude, but larger ones in the mM range block transmission. In one fish, the 50 μM dose prevented the potentiation suggesting a possible role for NMDA receptors.

During regeneration the newly formed retinotopic map goes through an activity dependent sharpening process that can be disrupted either by blocking activity with tetrodotoxin or by synchronizing activity with strobe illumination (Schmidt, Cell & Molec. Neurobiol. 5:65, 1985). The lack of sharpening could be seen in electrophysiological maps which indicate uncorrected errors in targeting of regenerated arbors. HRP stained regenerating optic arbors make early widespread branches that are usually eliminated (Schmidt et al., Neurosci. Abstr. 10:667, 1984). In the current model, retinotopically appropriate synapses (and branches) may become more effective (and therefore stabilized) because normally correlated firing of neighboring ganglion cells can cause summation of postsynaptic responses. A requirement for coincident activation of converging inputs bears a formal similarity to associative conditioning, which is thought to involve a change in synaptic circuits, either an increased strength of existing synapses or an increased number of synapses. (Supported by NIH grant EY03736).

CORTEX: MOTOR CORTEX I

72.1 MOTOTOPIC ORGANIZATION OF BABOON PRIMARY MOTOR CORTEX: FACE AND FORELIMB REPRESENTATIONS.
S. McFarlane*, D.D. Samulack, R.S. Waters[1], R.W. Dykes, P.A. McKinley, S.S. Leclerc* and N.J. Kabani* (SPON: D.W. Baxter). Depts. of Physiology, Surgery, Neurology and Neurosurgery, Physical and Occupational Therapy, McGill Univ., Montreal, Quebec H3A 1A1, and [1]Dept. of Anatomy and Neurobiology, Col. of Med., Univ. of Tennessee, Memphis, TN 38163.

Re-representations of muscles, movements, and body parts in primary motor cortex have been described in several primate species, emphasizing the need for a re-examination of the principles of organization for this motor region. Intracortical microstimulation (IMCS) was used to elucidate these principles.

Four female adult baboons (Papio c. anubis) were anesthetized with a mixture of halothane and nitrous oxide. A craniotomy was performed over the primary motor cortex and the dura reflected. A well of dental impression wax was formed and filled with silicon fluid. After surgery the level of gas anesthesia was reduced and spontaneous movements were attenuated with small doses of sodium pentobarbital. Trains of cathodal stimulus pulses (12-13 pulses, 0.2 ms duration, 300Hz) were delivered at 200 μm steps through a tungsten-in-glass microelectrode inserted into the motor cortex. Whenever a motor response was observed, the effective muscle(s) was noted and the threshold for activation was recorded.

The degree of variability observed among the four animals studied was sufficient to preclude pooling the data from individual animals. An overall mototopic progression was apparent in each animal, with muscles of the tongue located most laterally followed in a medial direction by a regular progression of muscles of the face, hand, forearm, and shoulder. The areal extent devoted to any one of these major muscle groups was significantly different among animals. As well, the sequence of individual muscles encountered within any region varied.

Previous reports of a dual forelimb representation were not confirmed in this study. Efforts to subdivide the data into a rostral and caudal map were unsuccessful and it became clear that the same muscle could be represented three or four times at distinctly different sites within the cortex. In all animals, numerous penetrations failed to elicit muscle contractions, suggesting that activation of some portions of motor cortex fail to produce overt contractions. These regions were termed silent zones. The consistent location of silent zones among animals, and the repeated confirmation of their lack of excitatory effect within animals ruled out the possibility of artifact.

Contrary to the situation in other primates and man, most of the effective motor cortex was located on the anterior wall of the central sulcus, with little or none of the cortex located on the gyral crown displaying low threshold motor foci.
(Supported by MRC MA5522 and NSF BNS 83-17662).

72.2 SILENT ZONES AS A CONSISTENT AND INTEGRAL COMPONENT OF THE MOTOTOPIC ORGANIZATION OF BABOON MOTOR CORTEX.
D.D. Samulack, R.S. Waters[1], R.W. Dykes, P.A. McKinley and S. McFarlane*. Depts. of Physiology, Physical and Occupational Therapy, McGill Univ., Montreal, Quebec H3A 1A1, and [1]Dept. of Anatomy and Neurobiology, Col. of Med., Univ. of Tennessee, Memphis, TN 38163.

In an accompanying report we described the organization of the face and forelimb representation of baboon (Papio c. anubis) motor cortex as being composed of a regular progression of body parts, with the tongue region located most laterally, and shoulder regions more medially. However within this pattern of organization, individual muscles were often re-represented as many as four times. While collecting the data for these maps, points in the penetration sequences were often encountered where intracortical microstimulation (ICMS) failed to elicit observable muscle contractions. Initially we assumed that the absence of an observable motor response was the result of electrode failure, level of anesthesia, state of the animal, and/or location of the affected muscle deep within the limb.

Evidence that the silent zones were not an artifact comes from several observations: (i) the silent zones were located consistently at junctions between major muscle groups, (ii) the silent zones were found at comparable locations in each of the four animals studied, (iii) the silent zones were found both earlier and later in the experiment and their locations could be verified on subsequent days by reinsertion of another electrode into a region mapped earlier, (iv) the silent zones were observed at several anesthetic levels. Evidence that the silent zones did not reflect activation of muscle groups that escaped detection, comes from data collected with EMG electrodes. By inserting Teflon coated silver wires into muscles of the hand, forearm, and face, it was possible to check for EMG activity correlated with ICMS that did not elicit overt movements. The results showed that any time an EMG was observed, it was closely related to an observable muscle contraction. Thresholds for EMG and visible muscle contractions seldom differed by more than a few microamperes. Silent zones were characterized by a lack of ICMS-related EMGs as well as a lack of overt contractions.

The role of these silent zones remains an enigma, but their large size and consistent relationship to other parts of the motor cortex suggests that they comprise an important feature of this tissue.
(Supported by MRC MA5522, NSF BNS 83-17662, and UPF, Inc.).

72.3 MOTOR CORTICAL REPRESENTATION PATTERNS SHIFT RAPIDLY FOLLOWING MOTOR NERVE SECTION. J. P. Donoghue, S. Suner*, J. F. Lando* and J. N. Sanes. Center for Neural Science, Brown University, Providence, RI 02912 and Human Motor Control Section, Medical Neurology Branch, NINCDS-NIH, Bethesda, MD 20892

Peripheral nerve injury in neonatal or adult rats alters somatotopic representation patterns in primary motor cortex (MI, Donoghue and Sanes, 1987; Suner et. al., 1986). MI reorganization was observed as early as 8 days following nerve lesions and could be obtained when lesions were restricted to a motor nerve. The present study was designed to determine the shortest interval in which MI representations reorganize following a motor nerve injury.

Intracortical electrical stimulation techniques were used to map MI in 7 rats. Rats were anesthetized with ketamine HCl and PtIr microelectrodes were used for MI stimulation. The electromyogram (EMG) was recorded from the biceps and wrist extensor muscles contralateral to the mapped cortex. Vibrissa and forelimb representations were located by stimulating, with standard pulse trains (60 μA) at 12-16 sites 500 μm apart. A single site within the vibrissa region, about 500 μm medial to the forelimb area, was chosen. The microelectrode was located at this test site prior to transection of the marginal mandibular and buccal branches of the facial nerve and was kept in place for as long as 10 hours in 4 rats. At 15-60 minute intervals forelimb muscles EMG activity was recorded during presentation of 30 stimulation trains. After repeated testing at this site, several of the previously mapped MI sites were stimulated a second time.

At each test site electrical stimulation showed a shift from vibrissa to forelimb movements between 45 and 90 minutes following transection of the facial nerve. Prior to the nerve section, no biceps or wrist extensors EMG was evoked in 2 rats, but in the other 2 rats the averaged EMG revealed a small amount of activity in either biceps or wrist extensors. After nerve section, peak forelimb EMG evoked from the test site was 30-80% of the maximum EMG evoked from stimulation in the normal forelimb area. The maximal muscle activity occurred from 1-7 hours after the facial nerve transection. By 10 hours, muscle activity could still be evoked in 5 of the 8 muscle recordings. When border sites were retested after the nerve transection, different movements (either forelimb or eye) were evoked from some of these sites. In 3 control rats, an electrode was placed in the MI vibrissa region near the forelimb border, the facial nerve was not transected, and muscle activity was recorded every 15 to 30 minutes for the next 6-7 hours. In none of these controls did stimulation within the vibrissa region evoke forelimb EMG.

These experiments demonstrate that one region of MI can assume control over muscle groups that are ordinarily allocated to other MI areas. Within 1-2 hours of a facial nerve transection, electrical stimulation within the MI vibrissa region evoked activity in forelimb muscles. The rapid time course of this shift in MI representation suggests that the basis for reorganization is not anatomical (e. g., collateral sprouting), but is related to mechanisms that regulate the strength of existing synapses. These results further suggest that MI representation patterns are dynamic, and that these changes could be related to allocation of MI cortical tissue according to motor output exigencies.

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72.4 FUNCTIONAL GROUPINGS OF MUSCLES IN THE FORELIMB AREA OF PRIMATE MOTOR CORTEX. S.J. Leibovic*, T. S. Buchanan*, J.P. Donoghue, and J.N. Sanes (SPON: V. A. Jennings). Dept. of Brain and Cognitive Sciences, M.I.T. Cambridge, MA 02139, Dept. of Orthopedics, Children's Hospital, Boston, MA 02114, Center for Neural Science, Brown University, Providence, RI 02912, Section of Human Motor Control, NINCDS, NIH Bethesda, MD 20892.

Numerous studies have reported that the primary motor cortex (MI) is organized in terms of movements about a given joint. However, the precise representation of individual and groups of muscles in MI remains unclear. We have combined intracortical electrical stimulation and EMG recording to identify muscle representation patterns in MI. Muscle representations were mapped during ketamine anesthesia (i.v.) in one squirrel monkey and two rhesus monkeys using trains of electrical stimuli (10 pulses, each 200 μsec long, at 333 Hz) delivered through PtIr microelectrodes inserted at 250 to 500 μm intervals in the MI forelimb area. Muscle activity was recorded from 12 arm, forearm, or hand muscles. At each MI stimulation site the EMG evoked in each muscle was simultaneously recorded. Surface maps of integrated EMG were constructed; in the rhesus monkeys this required unfolding of the cortex in the posterior bank of the central sulcus.

Cortical maps showed that most muscles were represented in single contiguous territories, although multiple separate sites where maximal EMG activity could be elicited within this representation were present. For some muscles maximal activity could be evoked from disparate areas separated (>2 mm) by regions of minimal EMG activity. Multiple, completely separate representations were only found for some distal muscles, such as the intrinsic thumb and hypothenar muscles. In all monkeys studies muscles that act synergistically across a single joint have highly overlapping representations in cortex. This includes the elbow flexors, wrist flexors and extensors, and the intrinsic muscles of the thumb. Wrist muscles that can act either as synergists or antagonists occupy largely separate cortical territories. For example, extensor carpi ulnaris and flexor carpi ulnaris which can act as antagonists (to extend or flex the wrist) or as synergists (to produce ulnar deviation), had little overlap in the regions from which maximal EMG was evoked. Pairs of muscles which commonly act together to move the digits have a high degree of overlap in their cortical representation. These include the thumb and finger extensors, as well as the thumb and finger flexors.

These data indicate that regions of motor cortex may be organized to participate in the action of particular muscle groups. The cortical contribution to some movements must therefore require the activation of multiple cortical areas because the muscles are represented in spatially separate zones. Other movements, in particular those involving synergistic muscles, might require cortical activation of only a single site where muscle representations are overlapping. However, our recent findings (Donoghue et al., this meeting) suggest that motor cortex is able to shift representation patterns in short periods of time, at least following nerve lesions so that these relationships may be altered by changing peripheral conditions. Supported by NIH 22517, 25074, 09343, AM26710, and United Cerebral Palsy Foundation.

72.5 SYNAPTIC INTERACTIONS BETWEEN IDENTIFIED PRIMATE MOTOR CORTEX CELLS DURING WRIST ACTIVITY W.S. Smith* and E.E. Fetz, Dept. of Physiology & Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195

We tested the hypothesis that the active response properties of identified motor cortex cell pairs are related to the synaptic interactions between them. Pairs of neighboring neurons were recorded with independently controlled microelectrodes in precentral cortex of Macaque monkeys performing alternating ramp-and-hold isometric wrist torques. Neurons were characterized by their relation to the task, their relative cortical location, and classified as pyramidal tract (PT), non-PT, or corticomotoneuronal (CM) cells. CM cells were identified by post-spike facilitation in spike-triggered averages of rectified forearm EMG activity. *Cross-correlation histograms* were compiled for 233 pairs of identified neurons; 95 of these correlograms exhibited peaks that straddled the origin, indicative of common synaptic input. The peaks had a mean width of 21.8 ± 11.1 (SD) ms, an average mean percent increase of firing above baseline of 21.4 ± 21.2%, and a mean normalized peak area of 0.055 ± 0.041. The widths of the correlogram peaks did not differ significantly for the six possible combinations of cell types; however the mean percent increase and peak areas were larger for pairs that included non-PT cells. The peaks of correlograms between CM cells and non-CM cells were too broad and shallow to artifactually mediate the long-latency, sharply rising post-spike facilitation in spike-triggered averages from non-CM cells.

To determine whether the strength of short-term synchrony between cell pairs was related to the similarity in their firing properties, we also quantified their *covariation*. The degree of covariation was measured as the correlation coefficient (r) of the the smoothed instantaneous firing rates of the two units, using a running 200 ms average. These r values ranged from -0.7 (for inversely varying pairs) to +0.9 (for strongly covarying pairs). The degree of covariation of the cells was not related to their short-term synchrony - measured either by the mean percent increase or the normalized peak area. This result indicates that a significant amount of common input to these cells is not task related; moreover, the covariation of cell pairs appears to be mediated more by covarying parallel inputs than by common synaptic input.

72.6 INPUT-OUTPUT RELATIONS OF NEURONES IN PRECENTRAL AND POSTCENTRAL OROFACIAL CEREBRAL CORTEX OF AWAKE MONKEYS (MACACA FASCICULARIS). C.-S. Huang, H. Hiraba*, G.M. Murray and B.J. Sessle. Faculty of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

Little information is available of the cortical mechanisms contributing to sensorimotor integration of orofacial movements. This study was designed to delineate the functional organization of the orofacial sensorimotor cortex in terms of (a) afferent inputs, as determined by electrophysiological recording of superficial and deep inputs to single neurones, and (b) efferent outputs, as revealed by intracortical microstimulation (ICMS) delivered to the same recording sites.

In two awake monkeys, a series of microelectrode penetrations was made at 0.5-1.0 mm intervals across the precentral and postcentral cortex; it extended rostrocaudally and mediolaterally 2.0-4.0 mm beyond the boundaries of the orofacial motor cortex (MI), defined as the area within which ICMS (≤20 μA) could induce twitch movements of face, jaw or tongue muscles. At 250 μm intervals within each penetration, light tactile and muscle stretch stimuli were delivered to the orofacial region to test for neuronal responsiveness, and then ICMS was applied to test for orofacial muscle activation. Jaw position and EMGs of temporalis, masseter, genioglossus, and digastric muscles were continuously monitored during the experiment. Electrolytic lesions were placed at selected intracortical sites to aid histological reconstruction.

We confirmed our earlier findings that MI is characterized as a laterally facing horseshoe-shaped facial muscle representation partially enclosing the cortical representations of the jaw and tongue, and that multiple representation of individual orofacial muscles occurs in MI. Sensory inputs were tested for 685 neurones in MI, 609 neurones in primary somatosensory cortex (SI), and 426 neurones in premotor cortex (PM). Deep, and especially light tactile, stimuli were effective in activating 361 neurones in MI, 506 neurones in SI, and 116 neurones in PM. Mechanoreceptive fields were predominantly localized to the perioral region (77% in MI, 73% in SI, 68% in PM) and tongue (19% in MI, 12% in SI, 25% in PM). Sensory input from the same peripheral site was represented multiply in several different areas within MI. While ICMS was ineffective in SI and PM, it evoked orofacial muscle activity at the site of 237 MI neurones receiving sensory input; the majority (86%) of these neurones received their input from the same peripheral region in which the ICMS-induced movement occurred.

The results suggest that multiple representations of orofacial sensory inputs as well as motor outputs are a feature of the functional organization of MI neurones. The close spatial relationship noted between sensory input and motor output may be of fundamental importance in the neural substrate for cortical integration of orofacial movements. (Supported by the Canadian M.R.C.)

72.7 ACTIVITY OF PRIMATE MOTOR CORTEX NEURONES IN RELATION TO TRAINED AND UNTRAINED OROFACIAL MOVEMENTS. G.M. Murray, H. Hiraba*, C.-S. Huang and B.J. Sessle. Faculty of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

Recent work from this laboratory has defined input-output relations of the orofacial motor cortex in terms of sensory inputs recorded in single neurones, and the motor effects produced by intracortical microstimulation (ICMS, $\leq$ 20 μA) at the site of recording. The aim of this study was to examine the activity patterns of these neurones in relation to functional orofacial movements.

Extracellular single unit recordings were made from the ICMS-defined orofacial motor cortex in a monkey (Macaca fascicularis) while the animal repeatedly performed each of several test movements. These were untrained movements (e.g. mastication, licking, swallowing and tongue protrusion), as well as trained movements in which the animal was operantly conditioned either to protrude its tongue against a force transducer or to bite onto a force transducer. In both trained tasks, the animal was rewarded for maintaining a pre-selected force level for a 1-2 sec period. Masseter, anterior digastric and genioglossus EMGs and the force outputs from the transducers were recorded. Neurones were classified as being task-related if they consistently altered their firing rates, in comparison with control resting levels, during the period of the test movement. The presence of sensory inputs from cutaneous, intraoral or deep receptors was tested for each neurone.

Thirty-three neurones showed increases or decreases in activity in association with untrained and/or trained orofacial movements, and ICMS at the neuronal recording sites evoked twitch movements of the tongue, face or jaw. For 8 of the neurones, a receptive field could be definitively identified in the awake animal: the receptive fields were either cutaneous (n=4) or intraoral (n=4). Seven of these 8 neurones received their input from the same peripheral region in which the ICMS-induced movement occurred. All 8 of them exhibited phasic increases in firing in association with orofacial muscle activities occurring during the untrained movements. Six of these 8 neurones also altered their firing rates during a trained task: 3 showed an increase in firing rate and 3 exhibited a decrease. An additional 2 neurones with identified input-output relations did not alter their firing rates during any of the test movements.

These findings reveal a close relationship in the orofacial motor cortex between orofacial sensory inputs and ICMS-defined motor outputs in neurones which alter their firing rates in association with a variety of trained and untrained movements. Such observations point to an important role for these sensory inputs to the motor cortex in the control of orofacial movements.

(Supported by the Canadian Medical Research Council)

72.8 ORIGIN OF CORTICOSPINAL PROJECTIONS TO THE IPSILATERAL SPINAL CORD. K.D. Hutchins* and P.L. Strick. V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-HSC, Syracuse, NY 13210.

The corticospinal tract in macaques contains an **ipsilateral** component which does not cross at the pyramidal decussation. A portion of the ipsilateral pathway travels in the dorsolateral funiculus along with the contralateral component of the tract. Clinical and experimental evidence suggest that the recovery of motor function which follows damage to contralateral motor pathways depends in part on the integrity of this ipsilateral pathway. We have used retrograde transport of horseradish peroxidase (HRP) to define the origin of neurons in the ipsilateral hemisphere which contribute to the ipsilateral corticospinal tract. HRP (Sigma, Type VI) was placed in the dorsolateral funiculus at C2 in 2 monkeys (Macaca nemestrina).

All of the cytoarchitectonic areas of cortex which project via the contralateral corticospinal tract also contribute to the ipsilateral corticospinal tract. This includes five cortical areas in the frontal lobe (e.g., the primary motor cortex, the supplementary motor area, the arcuate premotor area and two areas in the cingulate sulcus) and five regions in the parietal lobe (e.g., the primary somatosensory cortex, the secondary somatosensory cortex, area 7b, granular insular cortex and several portions of area 5). In each cortical area, the size range for cells which contribute to the ipsilateral tract is the same as that for cells which contribute to the contralateral tract. Within the ipsilateral primary motor cortex, substantial numbers of corticospinal neurons were found in regions of forelimb representation located on the crest of the precentral gyrus and in the rostral bank of the central sulcus. Thus, projections to the ipsilateral corticospinal tract include cortical regions involved in the control of distal limb movements. Furthermore, some of these projections originate from the largest cells in the ipsilateral primary motor cortex.

The overall density of corticospinal neurons in the contralateral hemisphere is 20 times that in the ipsilateral hemisphere. However, the peak density of corticospinal neurons in the contralateral frontal lobe is only 7 times greater than the peak density of corticospinal neurons in the ipsilateral frontal lobe. In contrast, the peak density of corticospinal neurons in the contralateral parietal lobe is at least 16 times greater than that in the ipsilateral parietal lobe. These observations illustrate that, compared to the contralateral hemisphere, proportionally more of the ipsilateral projection originates from the frontal lobe than from the parietal lobe.

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72.9 DIRECT FACILITATION BY CORTICOSPINAL NEURONS OF SINGLE MOTOR UNITS IN THE HAND MUSCLES OF THE CONSCIOUS MONKEY R.N. Lemon* and G.W.H. Mantel*. (SPON: M. Sofroniew). Anatomy Dept., Cambridge University, Cambridge CB2 3DY, England.

A distinguishing feature of the primate motor system is the presence of direct cortico-motoneuronal (CM) connections, which are particularly well-developed for motoneurones of the intrinsic hand muscles. In the conscious monkey, we have made cross-correlations between discharges of single, identified CM cells and activity recorded from single motor units of the thumb muscle, abductor pollicis brevis (AbPB) (Mantel & Lemon, Neurosci.Lett, in press). For 12/15 selected CM cells, cross-correlation revealed a clear peak. This correlation peak probably reflects the direct, monosynaptic excitation of the motor unit by the CM cell: first, the correlation peaks had brief half-widths (mean 1.9ms $\pm$ 1.1ms); indirect, oligosynaptic effects would be expected to produce broader peaks. We rarely observed any secondary tail-effects in our correlograms. Second, peak latency was consistent with conduction time over the CM pathway: the latency of the correlation peak with a given motor unit was comparable with the latency at which the same motor unit was activated from the pyramidal tract. Third, utilizing the study of Cope, Fetz and Matsumura, (J. Physiol. in press), we predicted that the amplitude of the unitary CM-EPSPs which would underlie the observed correlation peaks would be 50-200uV, consistent with observed values at the CM synapse.

If a correlation was found between a CM cell and one AbPB motor unit, it was found with all concurrently sampled AbPB motor units. Correlations were found with motor units of different sizes: with low-threshold motor units that fired tonically during the gentle (0.5N) precision grip exerted by the monkey and with phasic motor units which were only recruited during the onset of precision grip. These results suggest widespread connections of the CM cell within the motoneuron pool of AbPB. For a given CM cell, the strength of correlation with different motor units was similar, and was directly related to the amplitude of the post-spike facilitation (PSF) seen in spike-triggered averages of surface e.m.g. from AbPB. Our results suggest that strong PSF (peak percentage modulation of 20% or more) reflects stronger, individual CM connections, rather than being due to the recruitment of additional motor units. When PSF was less than 10%, we found no significant correlations between CM cell and motor unit activity.

72.10 CORTICOSTRIATAL NEURONS IN MOTOR CORTEX REFLECT THE PATTERN OF MUSCLE ACTIVITY RATHER THAN THE DIRECTION OF HAND MOVEMENT T.D. Ferguson*, M.P. Heyes* and S.P. Wise (SPON: C. Asanuma), Lab. Neurophysiology, NIMH, Bethesda MD 20892

Putamen neurons discharge at or after the onset of EMG activity and reflect the direction of movement (Crutcher and Delong, Exp. Brain Res. 53: 244, 1984), whereas many neurons in the primary motor cortex (MI) discharge prior to the onset of EMG activity and reflect individual muscle activity independent of the direction of movement. To investigate the type of motor information conveyed to the basal ganglia by MI corticostriatal (CS) neurons, stimulating electrodes were implanted in the putamen of two rhesus monkeys. CS neurons were identified in MI by antidromic activation and studied while monkeys performed operantly conditioned wrist flexion/extension movements over a 50° arc between three equally spaced 8° windows. Neuronal activity was studied when loads were applied that altered tonic muscle activity during maintained wrist position and phasic activity during movement. The EMG activity of selected prime-mover muscles preceded the onset of movement by 70 $\pm$ 24 ms.

Ninety-four CS neurons (antidromic latency = 1.24 $\pm$ 0.47 ms) were antidromically identified. At least 19 of these CS neurons were movement related and verified as antidromically activated by the collision method. Fifteen movement-related non-CS neurons, located near the CS cells were also studied. Eighteen of the 19 CS and 13 of the 15 non-CS neurons increased discharge rate before movement. The others decreased their discharge rate before movement. The time from the pre-movement change in firing rate (unit activity onset) to the onset of movement was 101 $\pm$ 30 ms for CS neurons and 136 $\pm$ 26 ms for non-CS neurons in our sample. Except for one CS cell that was inhibited before both directions of movement, all movement-related CS neurons showed greater activity modulation before one direction of wrist movement (the preferred direction) than the other. Nine of the 19 movement-related CS neurons either showed no change or decreased activity in the nonpreferred direction (directionally specific neurons). During steady holding and movement, tonic and phasic discharge rates, respectively, were increased by loads against the preferred direction of wrist movement in all 9 directionally specific CS neurons. Of the directionally nonspecific CS neurons, 6 were significantly affected by the load.

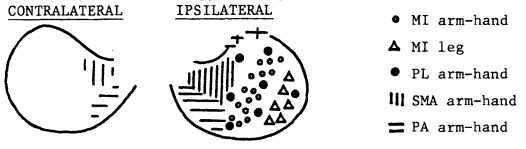
We conclude that: (i) motor information is conveyed via the CS pathway and (ii) because they are affected by loads, CS neurons do not closely resemble putamen cells.

72.11 TERMINAL FIELDS OF CORTICORUBRAL PROJECTIONS FROM SEPARATE ARM-HAND REGIONS IN THE MONKEY'S MOTOR AND PREMOTOR CORTEX.
D. Bulyalert* and D.R. Humphrey, Lab. of Neurophysiology, Emory Univ. Sch. Med., Atlanta, GA 30322

The parvocellular red nucleus (pRN) of the monkey receives substantial projections from the primary motor cortex (MI), the supplementary motor area (SMA), the periarcuate cortex (PA), and area 5 of the posterior parietal lobe (PL) (Humphrey et al, J. Comp. Neurol., 225:75, 1984). Each of the latter three zones projects to the arm-hand area of MI, and also to the cervical spinal cord (Muakassa and Strick, Brain Res., 177:176, 1979; and unpublished observations). The purpose of this study was to determine if the arm-hand regions of these four cortical areas project to separate or to common target areas within the pRN.

Cortical arm-hand (or leg) areas were identified by microstimulation in lightly anesthetized monkeys (cynomolgus, N=6). Two, 0.1 uL injections of 2% WGA-HRP, spaced 1 mm apart, were then made into one of the following arm-hand regions in each animal: MI (N=1), SMA (N=2), PA (N=1), PL (N=2); similar injections were placed in the MI leg area of one animal. After a survival period of 72 hr, the animals were sacrificed, and 50 um (frozen) coronal sections through the red nuclei were processed with TMB (modifed method of Mesulam) for identification of anterogradely labeled cortico-pRN terminals. All sections were examined under both bright- and darkfield microscopy.

Our major findings were as follows. (1) All injected areas sent convergent terminals to a common prerubral zone, just anterior to the rostral pole of the pRN. (2) Regions of the mesencephalic reticular formation dorsal to the pRN also received convergent projections from all injected cortical zones. (3) Within the pRN, however, the various cortical areas tended to project to separate territories, as is summarized in the following drawings of coronal sections through the this division of the RN. Only the projection from PL tended to overlap substantially with those from other (MI) cortical zones. (4) Projections from PL and MI were ipsilateral only, whereas those from the SMA and PA were bilateral, with heaviest termination ipsilaterally. These findings suggest that the pRN contains at least three, perhaps functionally different arm-hand regions, each controlled by a different set of corticorubral inputs. (Supported by NIH Grant NS 10183).



72.12 SYNAPTIC ACTIONS EXERTED BY DIFFERENT ARM-HAND REGIONS OF THE MONKEY'S MOTOR AND PREMOTOR CORTEX ON THE PARVOCELLULAR RED NUCLEUS (pRN).
D.J. Reed, D. Bulyalert* and D.R. Humphrey, Lab. of Neurophysiology, Emory Univ. Sch. Med., Atlanta, GA 30322.

Using anterograde tracing methods, we have shown that the arm-hand regions of the primary (MI) motor cortex, the supplementary motor area (SMA), the periarcuate area (PA), and area 5 of the posterior parietal lobe (PL) project to different subregions of the pRN (Bulyalert & Humphrey, this volume). In the present study, we used recordings of the field potentials evoked in the pRN by stimulation of these different cortical loci to estimate the early postsynaptic responses that are evoked in pRN neurons by each projection system.

Bipolar cortical stimulating electrodes and pRN recording chambers were implanted chronically in two monkeys (one rhesus, one cynomolgus). Under very light ketamine sedation (1.5-2.0 mg/Kg/hr averaged evoked responses (10-20/average) were recorded throughout the pRN (300-320 sequential recording sites) during stimulation of each cortical site; a complete mapping of the nucleus required 4-7 daily recording sessions. The evoked response data were then used to estimate the potential fields that would have existed throughout the pRN at a particular point in time, following stimulation of each cortical site (8-10 and 15-20 msec after stimulation). Average (instantaneous) directions of current flow within the nucleus were then estimated from these voltage contour maps, by drawing vectors orthogonal to computed isopotential lines.

From these data, and from:(1) knowledge of intranuclear projection zones; (2) the fact that pRN neurons are stellate in shape, and thus generate 'open' extracellular potential fields; and (3) observed, extracellular single unit responses, we have estimated the following, initial postsynaptic responses.

CORTICAL SITE	MAJOR DIRECTION OF CURRENT FLOW IN RELATION TO CELL SOMA	PROBABLE PSP
MI hand area	Radially outward	Dendritic EPSP
SMA hand area	Radially outward	Dendritic EPSP
PA arm area	Radially inward-outward	Somatic EPSP
PL arm area	Radially inward-outward	Somatic EPSP

Reversals in the net direction of current flow 5-10 msec after the initial postsynaptic response to stimulation of PA and PL arm-hand zones suggest that the initial somatic EPSP is followed by either a dendritic EPSP, or a somatic IPSP, in the response evoked in pRN neurons by these two corticorubral projections. Thus, cortical 'premotor' zones may have a stronger influence on pRN neurons than do cortical motor areas (MI and SMA). (Supported by NIH Grant NS 10183).

72.13 RELATIONS BETWEEN THE AMPLITUDE OF 2-DIMENSIONAL ARM MOVEMENTS AND SINGLE CELL DISCHARGE IN PRIMATE MOTOR CORTEX.
Andrew B. Schwartz and Apostolos P. Georgopoulos. Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

We studied the relations between the amplitude of arm movement and the frequency of discharge of 48 arm-related single cells recorded in the motor cortex of two rhesus monkeys. The animals moved an articulated manipulandum over a two-dimensional working surface and captured lighted targets on the plane in reaction time tasks. For every cell, the animals performed a "direction" task, followed by an "amplitude" task. In the "direction" task the animals made movements of equal amplitude but in 20 different directions (from 0 to 360 deg at a 18 deg angular interval); in the "amplitude" task they made movements of four amplitudes (1.5, 3, 6, and 12 cm) in the cell's preferred direction. Fourteen cells were also studied in the "direction" task using movements of different amplitudes. The results were evaluated using an analysis of variance (ANOVA) and a linear regression analysis performed separately on the discharge rates observed during the reaction time and the total time (from the onset of the target until the end of the movement). In the reaction time the discharge rate of 37% of the cells studied showed a statistically significant relation to the amplitude of the movement (ANOVA, F-test, $p<0.05$) whereas 88% of the cells showed a significant relation to the direction of the upcoming movement; all amplitude-related cells were also related to the direction of the movement. In the total time, the percentage of cells that showed a significant amplitude effect increased to 65% (31/48), and in 29/31 cells the linear regression provided a good fit (F-test, $p<0.05$). However, the strength of this effect was rather weak: most (82%) of the statistically significant slopes were below 2 imp/sec/cm, and 43% were below 1 imp/sec/cm. Finally, 82% of the amplitude-related cells were also related to the direction of the movement; moreover, the preferred direction remained very similar in those cells studied in the "direction" task using various movement amplitudes. We conclude, first, that the major input to the arm area of the motor cortex in relation to the initiation of the movement (ie, during the reaction time) concerns the direction of the movement and to a lesser degree its amplitude; second, that the amplitude-related input is weak in magnitude; third, that the amplitude effect on cell discharge is superimposed on the directional effect; and fourth, that the directionality of the cells remains very similar across different movement amplitudes. (Supported by USPHS Grants NS17413 and MH18030.)

72.14 COMPARISON OF MOVEMENT-RELATED NEURONAL ACTIVITY IN PRIMATE MOTOR CORTEX AND PUTAMEN.
M.D. Crutcher and G.E. Alexander. Dept. of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

It is widely assumed that the motor cortex plays a direct role in the control of limb musculature. However, Crutcher and DeLong (Exp. Brain Res., 53:244, 1984) recently found that neurons in the putamen were preferentially related to the direction of movement rather than the pattern of muscular activity. The aim of the present study was to compare directly the motor cortex and putamen by studying both structures in the same monkeys in order to determine whether movement-related activity patterns in the putamen can be accounted for on the basis of activity in motor cortex.

Rhesus monkeys were trained to perform a visuomotor step-tracking task which required elbow flexion/extension movements with assisting or opposing loads. This paradigm thus dissociated the direction of arm movement from the pattern of muscular activity required to perform the movement. Neurons were categorized as "directional" if they were differentially related to the two directions of movement and had identical patterns of activity for all load conditions. Neurons were categorized as "muscle-like" if they had a static and/or dynamic load effect in the appropriate direction (e.g. enhanced firing when the load opposed movements in the preferred direction). Similar patterns of neural activity were observed in the motor cortex and putamen. Thirty-four percent (36/105) of motor cortex cells and 42% (90/216) of putamen cells were classified as "directional". Thirty-seven percent (39/105) of motor cortex cells and 28% (61/216) of putamen cells were classified as "muscle-like". While there were relatively more "muscle-like" cells in the motor cortex than the putamen, this trend was not significant. The motor cortex and putamen did, however, have different distributions of neural response onset latencies. For the motor cortex, the median time of onset of the neural response was 60 ms before movement onset (range -175 to +125). For putamen, the median onset time occurred at the time of movement (range -240 ms to >300 ms). Although the distribution of putamen neural responses lagged behind that in the motor cortex by 60 ms, there was a small population of neurons in the putamen which became active earlier than neurons in the motor cortex.

The similarity of patterns observed in the motor cortex and putamen, and the fact that the neural onset times in the putamen generally followed those in motor cortex, suggest that much of the movement-related activity in the putamen may be attributable to the dense, somatotopically organized inputs from motor cortex. On the other hand, the earliest movement-related activity in the putamen may result from the overlapping inputs from premotor cortex and/or the supplementary motor area. Neurons in the putamen that manifest such extremely early movement-related activity may contribute to the often suggested role of the basal ganglia in the initiation of movement. (Supported by NIH grant NS - 17678)

72.15 PREPARATORY ACTIVITY IN PRIMATE MOTOR CORTEX AND PUTAMEN CODED IN SPATIAL RATHER THAN LIMB COORDINATES. G.E. Alexander and M.D. Crutcher. Dept. of Neurology, Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

There are a number of reports of neuronal activity in motor cortex related to the preparation for planned limb movements. Recently, we have also found evidence of such activity in the putamen (Alexander, Exp. Brain Res., in press). In both areas, the directionally selective preparatory activity has appeared to be related to the intended direction of forthcoming limb movements, suggesting that this type of activity might represent a neural correlate of "motor set". In all of these studies, however, the directions of the targeted limb movements covaried systematically with the locations of the spatial targets. Thus, to be certain that the directionally selective preparatory activity was actually linked to the intended direction of limb movement, rather than to the spatial location of the target, we devised a motor preparation task that dissociated the direction of the intended limb movement from that of the anticipated target shift.

Three rhesus monkeys were trained to perform a visuo-motor step-tracking task in which elbow movements were made both with and without a preceding preparatory "set" concerning the impending direction of the forthcoming movement. In some blocks of trials, the target shifts and the required elbow movements were in the same direction, while in others the target shifts and the required movements were in opposite directions. While the monkeys performed these two different types of preparatory trials, neuronal activity was recorded from the arm areas of motor cortex and putamen. In the putamen, 22 cells that showed directionally specific preparatory activity were tested with both types of trials. For the majority of these cells (12/22), the preparatory activity was found to depend upon the expected direction of the forthcoming target shift, irrespective of the intended direction of the limb movement required to align the cursor with the target. In motor cortex, 11 neurons that showed directionally selective preparatory activity were tested with both types of trials. In all but one of these cells, the preparatory activity was found to depend upon the expected direction of the forthcoming target shift, rather than the direction of the limb movement.

Thus, both in motor cortex and putamen, much of the preparatory neuronal activity related to targeted limb movements may be coded in terms of spatial rather than limb coordinates. These results suggest that while both of these structures are strongly implicated in the direct control of movement execution they may also play a role in the highest levels of motor preparation.
(Supported by NIH grant NS - 17678).

72.16 COMPARISON OF POSTSPIKE AND POSTSTIMULUS FACILITATION OF FOREARM MUSCLE EMG ACTIVITY FROM RED NUCLEUS SITES IN THE MONKEY. K. Mewes*, G.W. Widener* and P.D. Cheney. Dept. of Physiology, Univ. of Kansas Medical Center, Kansas City, KS 66103.

The sign, distribution and strength of effects on motoneurons of different muscles from single corticomotoneuronal (CM) and rubromotoneuronal (RM) cells can be estimated from spike-triggered averages of rectified emg activity. Whereas spike-triggered averaging reveals the output effects of a single neuron, microstimuli applied to the sites of CM or RM cells activate a collection of neurons near the electrode tip and averages computed from these stimuli reveal the summated output effects of the recorded cell and its neighbors. Using these methods, Cheney and Fetz (J. Neurophysiol. 53:786-804, 1985) showed that the patterns of poststimulus facilitation (PStF) evoked from CM sites closely match the patterns of postspike facilitation (PSpF) produced by individual CM cells at the same sites, suggesting that neighboring CM cells form functional aggregates in which each cell of the aggregate has a similar set of target muscles. The purpose of this study was to: 1) document the distribution of RM-PStF across muscles in comparison to PSpF obtained at the same site, and 2) compare RM-PStF with CM-PStF. Spike and stimulus-triggered averages of emg activity from twelve forearm wrist and digit muscles were computed at 31 RM cell sites in two monkeys during alternating, ramp-and-hold wrist movements. 55% of RM cells facilitated extensors exclusively, 13% facilitated flexors exclusively and 32% cofacilitated flexors and extensors. This clear extensor preference was even more evident in stimulus-triggered averages; at all RM cell sites the strongest PStF was of extensor muscles - even at sites where the PSpF was exclusively of flexors. The mean magnitude of the greatest PSpFs from RM cell sites expressed as PEAK/NOISE RATIO (P/N) was 6.8 compared to 14.6 for 23 CM-PSpFs (normalized to 12K triggers; noise = standard deviation of the baseline points). The corresponding P/N ratios for RM-PStF at 5, 10 and 20 μA stimulus intensity were 26, 56 and 76 respectively; the comparable 10μA CM-PStF was 69. Although CM-PSpF was more than double RM-PSpF, CM-PStF was not significantly larger than RM-PStF. At 3 of 31 RM sites (10%) where 20 μA averages were computed, the muscle with the strongest PSpF was also the muscle with the strongest PStF (35% at 10μA; 42% at 5μA). In comparison, PSpF and PStF (10μA) from motor cortex was strongest in the same muscle at 22 of 23 (96%) sites. At RM cell sites, the muscle field (all muscles with PStF) for 10μA matched the PSpF muscle field at only 5 of 20 (25%) sites, whereas at CM cell sites 86% of muscle fields matched. We conclude that RM cell output preferentially facilitates forearm extensor muscles and that clustering of cells into functional aggregates with common muscle fields is much less clear in red nucleus than in motor cortex. (Supported by NSF grant BNS-8216608)

72.17 DISCHARGE PATTERNS OF PYRAMIDAL TRACT NEURONES IN MOTOR CORTEX DURING A LOCOMOTOR TASK REQUIRING A PRECISE CONTROL OF LIMB TRAJECTORY. T. Drew. Centre de recherche en sciences neurologiques, Univ. de Montréal, Québec, CANADA

To test the hypothesis that the motor cortex plays an important role in locomotion under conditions requiring a precise control of limb trajectory, a task has been devised in which cats have to adjust their gait in order to step over (or between) obstacles of different heights and widths fixed to a treadmill.

Single units were recorded from the forelimb area of the motor cortex of two unrestrained, intact cats using glass-insulated tungsten microelectrodes held in a microdrive attached to the head; cells were identified by stimulation of their axon in the pyramid (AP -7) with chronically implanted microwire electrodes. EMGs were recorded from flexor and extensor muscles acting around the shoulder, elbow, wrist and digits of the contralateral forelimb. Recordings were made initially when the cat walked normally on the treadmill and subsequently as it adjusted its gait to step over the obstacles.

Recordings were made from 46 fast and 11 slow pyramidal tract neurones lying principally around the lateral edge of the cruciate sulcus. Thirty of the 57 neurones changed their discharge patterns when the cat stepped over the obstacles; 16 of these showed a marked increase in their discharge rate. Normally one of the types of obstacles (high or wide) was more effective than the other. Temporal relationships between cell discharge and muscle activity were made on the basis of raster displays. All 16 cells which increased their discharge rate were best related to flexor muscle activity. Although the discharge rate of some cells was linearly related to the amplitude of flexor muscle EMG, others were better correlated with changes in the relative time of the onset of the flexor muscle activity.

In 9 of the loci from which cells were recorded that showed an increase in discharge during the task, an attempt was made to determine the output relations of the cell by using the technique of single pulse microstimulation (Cheney, P.D.and Fetz. E.E., J. Neurophysiol., 53:, 786, 1985). Facilitation of flexor muscle activity was seen from 5 sites; in all 5 cases the results were consistent with the correlations made on the basis of temporal relationships.

The results demonstrate that identified pyramidal tract neurones show marked changes in their discharge patterns during a locomotor task which requires visuo-motor guidance and fine control of limb trajectory. It is suggested that these cells may be implicated in the control both of flexor muscle amplitude and of changes in muscle timing. (Funded by the Fonds de la recherche en santé du Québec (FRSQ) and by the Canadian MRC).

72.18 THE ACTIVITY OF CLOSELY SPACED SINGLE NEURONS RECORDED FROM DIFFERENT REGIONS OF THE CAT MOTOR CORTEX DURING LOCOMOTION. C. I. Palmer. Institute of Physiology, Fribourg University, Switzerland.

This investigation was carried out to examine the motor cortical organization of units modulated with respect to the step cycle. Several neurons were recorded at the same cortical location to ascertain the possibility that units modulated during this movemen-t are organized in a columnar fashion in the motor cortex. These neurons were recorded from chronically implanted microwires on different postoperative days.

Results were obtained from three cats chronically implanted with 7 to 14 electromyographic (EMG) electrodes in muscles activated from intracortical microstimulation (ICMS) at each microwire. They were also implanted with fine wires looped around the distal phalynx of claws in the forepaw and wires set in a sylastic sheet so-wn to skin at the back of the wrist, to electrically activate cortical cells from the periphery. A recording cuff electrode was implanted around the median nerve to record this afferent volley and a bipolar Pyramidal tract (PT) electrode to antidromically identify cortical cells projecting into the PT.

Single units (n94) were recorded from 14 implanted microwires with an average of 7 per electrode site. Units recorded at the same site on different days could be differentiated from each other by their latency and threshold to antidromic or synaptic activation from PT stimulation, size and location of their peripheral receptive field, latency and configuration of their responses to electrical cutaneous stimulation, responses to tapping in their receptive field, also their level of tonic discharge and type of modulation during locomotion. The stability of the position of the electrode in the brain during the weeks of recording was ascertain-ed from the stability of the field potentials from cutaneous and PT stimulation and the ICMS responses in muscles.

The responses of units during locomotion at the different cortical recording sites could be divided into four classes I) the majority of units not modulated, 2) the majority of units modulated during one phase of the step cycle, 3) the majority modulated and their combined activity covering the complete step cycle, 4) the majority activated as would be predicted from the location of their cutaneous receptive field. These regions were found to occur independently of the strength or location of ICMS muscle responses or the number of units projecting into the PT.

Some evidence was found for a columnar organization in unit activity modulated during locomotion but this was not always associated with the activity of a single or synergistic group of muscles.

72.19 **PRIMATE MOTOR CORTEX: LOAD COMPENSATION MECHANISMS AND LIMB STIFFNESS.** John F. Kalaska, Ted E. Milner & Martha L. Hyde. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

We have been investigating the response of shoulder-related motor cortex cells in a 2-dimensional reaching task. The distribution of cell preferred movement directions was uniformly distributed in all directions. The task also requires the monkey to hold its arm in 9 different postures while compensating for loads in 8 different directions. These loads typically caused large continuously-graded changes in the tonic discharge of the cells, centered on one direction called the cell's maximum load axis (MLA). The spatial distribution of MLAs was not uniform, but was strongly bilobed, skewed in the directions perpendicular to the long axis (i.e., hand to shoulder) of the arm. The bilobed distribution tended to be more sharply skewed, the further the arm was held away from the body. Moreover, the MLA of single cells measured at the 9 different postures often varied, so that the spatial orientation of the skewed MLA distributions shifted with the degree of external/internal rotation of the arm in the different postures.

It has been reported that the stiffness of the limb to external perturbations is non-uniform, being greatest along its long axis and least perpendicular to its long axis. This property can be described by an ellipsoidal "stiffness field" whose eccentricity and orientation varies with limb posture (Mussa-Ivaldi et al., J.Neurosci. 5: 2732-2743, 1985). Based on a 3D analysis of limb movement in the task, we estimated the stiffness field of the monkey's arm at the 9 different postures. We likewize found that it tends to be more eccentric when the arm is held further away from the body, and that its orientation shifted with different postures is such a way as to remain approximately perpendicular to the bilobed MLA distributions of our motor cortex sample measured at each posture. We propose that the skewed bilobed MLA distributions in the motor cortex reflect a neural mechanism to compensate for the inherent non-uniform stiffness of the arm. Shoulder-related motor cortex cells play a greater active role in the compensation for static loads pulling the limb perpendicular to its long axis than parallel to its long axis. Changes in skew and orientation of the MLA distribution indicate that the response properties of cortical cells are tuned continually to match changing biomechanical properties of the limb skeletomuscular system in different postures. This also raises the possibility that the movement direction-related discharge of motor cortex cells may be modified with changes in the posture of the limb. This work supported the Medical Research Council of Canada.

72.20 DISCHARGE OF MOTOR CORTEX NEURONS IN CEBUS MONKEYS FOLLOWS KINEMATICS OF IMPOSED AND VOLUNTARY LIMB DISPLACEMENTS. D. Flament and J. Hore, University of Western Ontario, Dept. Physiology, London, Canada.

Recent experiments in cats trained not to resist imposed limb displacements showed that some motor cortex neurons (MCNs) preferentially responded to the acceleration and jerk of the displacements (1). In the present study the question was asked whether neurons responding to the kinematics of arm movement were also present in the primate motor cortex, in response to torque pulse perturbations or when making voluntary, goal-directed movements. The relation of MCN discharge to limb kinematics was analyzed in terms of timing and average firing frequency of neural discharge relative to timing and peak magnitude of the related variable. Only neurons with reciprocal responses to oppositely directed perturbations and movements were analyzed in detail.

Neurons were found that predominantly followed the velocity, acceleration or jerk of elbow movement, in response to perturbations. The majority of neurons followed acceleration (i.e. were acceleration-like). For perturbations of different durations these neurons followed the timing of the acceleratory phases of the displacement. They were sensitive to very small perturbations (< .5 deg) and their discharge was proportional to the acceleration of the displacement, saturating for large accelerations. During voluntary movements their discharge also followed acceleration. Velocity-like neurons were the second most common category. For perturbations eliciting an early burst of neural activity, the duration of this burst was proportional to the duration of velocity. For perturbations of the opposite direction, the latency of the discharge was proportional to the latency of the return velocity. No relationship was found between firing frequency and peak velocity. Velocity-like neurons also followed velocity during voluntary movements.

The results indicate that some MCNs in monkeys are related to, and follow, the derivatives of limb displacement whether it is produced passively or actively. The results support the hypothesis that predictive (derivative) feedback is important in the control of arm movements by motor cortex.

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(1) Bedingham, W., Tatton, W.G., (1985) J. Neurophysiol. 53:886-909.

72PO REPRESENTATIONS OF INDIVIDUAL MUSCLES WITHIN THE MOTOTOPIC ORDER OF BABOON FORELIMB MOTOR CORTEX. R.W. Dykes, R.S. Waters[1], P.A. McKinley, D.D. Samulack, S. McFarlane*, N.J. Kabani* and S.S. Leclerc*. Depts. of Physiology, Physical and Occupational Therapy, McGill Univ., Montreal, Quebec H3A 1A1, and [1]Dept. of Anatomy and Neurobiology, Col. of Med., Univ. of Tennessee, Memphis, TN 38163.

Detailed mapping of motor cortex of four baboons (Papio c. anubis), as described elsewhere in this volume, uncovered numerous cases of multiple efferent zones for a single muscle. In some cases the re-representation of an efferent zone was located in a place consistent with the general mototopic order of the representation. A second efferent zone could be interpreted as a contribution to a second map of the body musculature. However, the multiple efferent zones were inconsistent with a second map because either (i) they were clearly contained within the area devoted to the first map, or (ii) they represented a third or fourth efferent zone for the same muscle. The area devoted to any one efferent zone was rarely larger than 3 mm^2 and often less than 1 mm^2.

The hypothesis that there were zones of overlap and co-activation of several muscles within some efferent zones was an adequate explanation for only a limited number of cases. Generally, in situations where more than one muscle could be activated from a single stimulus site, the threshold for activation of one muscle was distinctly greater than for activation of the other. This suggested the possibility that the additional muscles activated at higher stimulus intensities were driven indirectly. These observations suggest that 'lowest threshold' stimulation of a single site in baboon motor cortex activates only one muscle or muscle compartment.

Evidence for low-threshold muscle co-activation was seldom obtained. Co-activation alone, is inadequate to explain the re-representation of muscles. The role that each efferent zone plays in motor control is not apparent from the currently available information.

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73.1 INTRINSIC CIRCUITRY OF THE CEREBRAL CORTEX: THE LOCAL AXONAL PROJECTIONS OF CORTICOCORTICAL PROJECTION CELLS. E. L. White and Edith El-Hanany*. Dept. of Morphology, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva, Israel.

Horseradish peroxidase (HRP) was injected into the vibrissal area of the primary motor cortex (MsI) in young adult, male CD/1 mice. The next day, the injected region of cortex was lesioned. Two days later, the mice were perfused with aldehydes, their brains were removed, tissue chopped at 100 μm and reacted with diaminobenzidine to demonstrate the HRP. The result of this procedure was to label with HRP, the cell bodies, dendrites and local axon collaterals of corticocortical (CC) projection cells within the vibrissal region of SmI cortex, and in the same region, by lesion induced degeneration, the axon terminals of cells having somata in MsI cortex. Examination with the light and electron microscopes of single sections through SmI cortex showed HRP-labeled cell bodies to occur in layers II/III and V; HRP-filled terminals of local CC axon collaterals occurred in layers III-VI, but predominated in layer V and to a lesser extent in layer III. Examination of serial thin sections through layer V showed that local CC axon collaterals were presynaptic only at asymmetrical synapses; postsynaptic elements were nearly all spines which are presumed to belong to pyramidal cells, but some were shafts of nonspiny dendrites which are considered to belong to nonspiny nonpyramidal cells. Occasionally, local CC axon collaterals synapsed with spines belonging to HRP-labeled dendrites of CC cells. The preference for local CC axon terminals in layer V to synapse with spines, differs from the situation in layer III where these terminals synapse in roughly equal numbers with spines and nonspiny dendrites, and contrasts sharply with the marked tendency in layers IV-VI of axon collaterals of corticothalamic (CT) cells to synapse onto nonspiny dendrites (White and Keller, J. Comp. Neurol., 1987). These results suggest that in the lower layers of SmI cortex, the local output of CC cells has a direct excitatory effect on pyramidal cells, whereas in the same layers, the local output of CT cells, is directed instead to nonspiny nonpyramidal cells whose activation probably acts to decrease activity within neighboring pyramidal neurons. In the upper layers, the local output of CC cells is evenly split between similar excitatory and excitatory/inhibitory pathways. Supported by N.I.H. grant to ELW # NS 20149-04.

73.2 SYNAPTIC INTERACTIONS INVOLVING THALAMIC AFFERENTS, CORTICOTHALAMIC CELLS, AND GABAERGIC NEURONS IN THE MOUSE BARREL CORTEX. A. Keller and E. L. White Dept. of Morphology, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva, Israel.

Horseradish peroxidase (HRP) was injected into the vibrissal area of the ventrobasal thalamus in young adult, male CD/1 mice. The next day, electrolytic lesions were placed in the injection site. Four days later, the mice were perfused with aldehydes, blocks of barrel cortex were removed, sectioned coronally and processed for HRP histochemistry. Corticothalamic (CT) projection cells, labeled by HRP, were identified and photographed, and then the sections were reacted for GABA immunocytochemistry. The result of this procedure was to label, in the same preparation, the cell bodies, dendrites, and axon collaterals of CT projection cells (by the retrograde transport of HRP), the axon terminals of thalamocortical (TC) relay cells (by lesion induced degeneration), and the cell bodies, dendrites, and axon terminals of GABAergic neurons (by HRP). HRP-filled dendrites, previously determined by light microscopy as belonging to CT cells, were examined in serial thin sections. These dendrites posessed large numbers of spines, whereas GABAergic dendrites were sparsely-spined or nonspinous. HRP labeled GABAergic axon terminals were differentiated from HRP labeled axon terminals of CT collaterals because the GABAegic terminals form only symmetrical synapses, whereas the CT axon terminals form exclusively asymmetrical synapses. Dendrites of GABAergic neurons received TC synapses, and many dendrites also received synapses from CT axon collaterals. All the dendrites belonging to CT cells received both TC synapses and GABAergic synapses. These synaptic interactions provide direct evidence for the existance of a cortical "triad" which has long been hypothesized but not yet supported by anatomical data. This circuitry involves reciprocal synaptic interactions between pyramidal neurons (here, CT cells) and nonspiny, nonpyramidal neurons (here, GABAergic neurons), with both classes of neurons directly receiving TC input. We suggest that this synaptic triad, identified previously in subcortical regions, is a basic feature of the synaptic organization of the cerebral cortex.

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73.3 MEMBRANE PROPERTIES AND THALAMOCORTICAL SYNAPTIC RESPONSES IN MOUSE BARREL CORTEX NEURONS: AN INTRACELLULAR STUDY IN VITRO. A. Agmon and B.W. Connors. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We previously reported the development of an in vitro preparation of the mouse barrel system, in which the neuronal pathway between the ventrobasal nucleus of the thalamus (VB) and the SmI cortex is preserved and functional in a 400 μM slice (Agmon, A. and Connors, B.W., Soc. Neurosci. Abstr. 10:493, 1984 and 11:750, 1985). We now report some of the membrane properties of barrel cortex neurons and their thalamus-evoked synaptic responses.

Thalamocortical slices were prepared as described. Intracellular recordings revealed all three firing-mode classes of cells previously described in the guinea pig neocortex (McCormick et al., J. Neurophysiol. 54:782-806, 1985): slow-spiking accomodating, bursting (restricted to the middle layer of the cortex) and fast-spiking non-accomodating. In most (> 80%) impaled cells a focal stimulus in VB evoked synaptic responses. The extracellular field potential in layer IV, monitored simultaneously, exhibited an early negativity of constant amplitude and latency that was not abolished in a low Ca, high Mn solution, and that followed stimulation frequencies of up to 20 Hz without failure. This negativity was interpreted as arising from the presynaptic volley, and served as point zero for calculating the synaptic latencies of the PSP's. Virtually all cells in layers VI to lower II-III showed a sequence of a monosynaptic EPSP (latency of 1.0-1.1 ms) followed by a disynaptic IPSP (latency of 2.0-2.2 ms). In upper II-III some cells received a disynaptic IPSP alone, while a small fraction showed a disynaptic EPSP followed by a trisynaptic IPSP, or a trisynaptic IPSP alone. The strength of the excitatory inputs varied considerably from one cell to another.

Since all pyramidal cells in layers VI to lower II-III have some part of their dendritic arbor within the thalamic termination zone, our data suggests that, in the thalamocortical system, spatial confluence of dendrites and axons is a sufficient condition for synapse formation. Our electrophysiological results agree with the EM studies of White and co-workers, who found that thalamocortical synapses occur, albeit in different densities, on virtually all the dendritic elements that pass through layer IV of mouse SmI cortex (E.L. White, in Cerebral Cortex, Vol. 5, p. 275, E.G. Jones and A. Peters, eds, Plenum Press, N.Y., 1986).

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73.4 **THE FINE GRAINED ORGANIZATION OF THE PROJECTIONS FROM THE BARREL CORTEX OF THE MOUSE; A PHASEOLUS VULGARIS LEUCO-AGGLUTININ (PHA-L) STUDY.**

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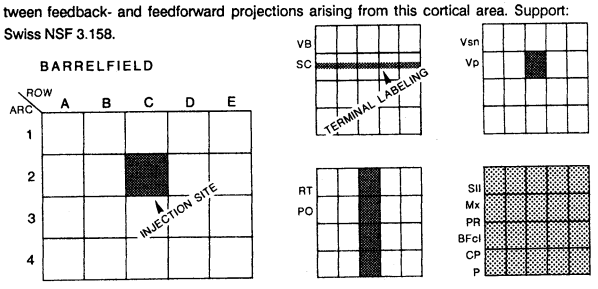
The whisker-to-barrel pathway has been used because of its property that in all stations of the pathway the representation of individual vibrissal follicles can be visualized: in the three subnuclei of the descending trigeminal tract (Vsn), the principal trigeminal nucleus (Vp), the nucleus ventro-basalis of the thalamus (VB) and the barrelfield (BF). While it has been demonstrated that signals from single follicles are kept separated from those of neighboring follicles within these regions, it is unknown whether separation persists in projections beyond BF.

To describe the organization of the efferent projections of single barrel columns (BC, i.e. barrel in layer IV plus the cortical tissue above and below), iontophoretic injections of the anterograde tracer PHA-L were made in the BF's of 21 adult mice. On the basis of reconstructions of the sites of terminal labeling, the brain regions receiving BF projections could be divided into four groups, each one characterized by the topography of the distribution of efferents arising from a single BC (*see schema*). The distribution of terminal labeling varies from punctiform in Vsn, Vp, VB and superior colliculus (SC; in the latter two stations one BC projects to an "arc" of units), via an overlap of projections arising from one row of BCs in the reticular (RT) and the posterior thalamic nucleus (PO), towards a completely overlapping projection within restricted zones of SII, motor cortex (Mx), perirhinal cortex (PR), contralateral (CL) BF, caudo-putamen (CP) and pons (P). These differences in organization of the efferents of BF demonstrate a contrast between feedback- and feedforward projections arising from this cortical area. Support: Swiss NSF 3.158.



73.5 PARALLEL PATHWAYS FOR THE FACIAL COMMON FUR AND MYSTACIAL VIBRISSAE OF THE RAT FROM THE BRAINSTEM TO THALAMUS AND CORTEX. F.R. Sharp, M.F. Gonzalez, M.T. Morton*, J.W. Sharp* and C.W. Morgan. Department of Neurology, University of California, San Francisco, and V. A. Medical Center, San Francisco, CA 94121.

The mystacial vibrissae of rows A,B,C were stimulated in awake rats and local cerebral glucose utilization (LCGU) measured quantitatively using the [^{14}C] 2-deoxyglucose method. Ventral portions of ipsilateral Sp5c, Sp5i, Sp5o, and Pr5 were activated. Whereas stimulation of the common fur above the A,B,C vibrissae activated primarily laminae II and III of the most ventral parts of Sp5c, A-C vibrissae stimulation primarily activated laminae III and IV of more dorsal and rostral parts of Sp5c. Sp5i, Sp5o, and Pr5 were minimally activated by stimulation of the common fur above the vibrissae. Common fur stimulation between the vibrissae activated laminae II-V of Sp5c, Sp5i, Sp5o, and Pr5 possibly because of common fur stimulation as well as unavoidable stimulation of the remaining stubs of the mystacial vibrissae.

A-C mystacial vibrissae stimulation increased LCGU in the rostral, dorsal parts of VPM nucleus of thalamus, whereas stimulation of the common fur above the vibrissal pad increased LCGU in caudal, dorsal VPM. A-C vibrissae stimulation increased cortical LCGU in two separate regions, one corresponding to the rat posteromedial barrel field (PSMBF), the other presumed to be in SII. Stimulating the common fur above the vibrissae increased LCGU in a distinct column which was located ventral to the PSMBF and slightly rostral to it in accordance with physiological studies by Welker, Van der Loos, and others. Though all cortical layers were metabolically activated, layer IV had the greatest LCGU increase for both whisker and common fur stimulation.

These results suggest that the mystacial vibrissae and the common fur above them are processed in parallel pathways from the brainstem through thalamus to cortex. They also suggest that the common fur pathways are primarily processed at the brainstem level in the substantia gelatinosa of Sp5c whereas vibrissae inputs are processed primarily in deeper layers of Sp5c as well as all other subdivisions of Sp5 and Pr5.

73.6 LAMINAR AND SYNAPTIC ORGANIZATION OF TERMINALS FROM THE VENTROBASAL AND POSTERIOR THALAMIC NUCLEI IN RAT BARREL CORTEX. C.-S. Lin, S. M. Lu*, and R. M. Yamawaki*. Dept. of Anatomy, Duke Univ. Med. Ctr. Durham, NC 27710.

We have previously reported that, in layer IV of rat barrel cortex, axons from the ventrobasal complex (VB) terminate in the center of individual barrels, while axons from the posterior nucleus (PO) terminate in the surrounding septa. Also, there are differences in the laminar distribution of these two thalamocortical projections. For example, in the infragranular layers, button-like swellings were observed only in upper layer VI for the VB projection and only in layer V for the PO projection. In this report, we describe these two projections at the ultrastructural level. The purpose of the study was to determine (1) whether the button-like swellings observed in different layers at the light microscopic (LM) level actually form synaptic contacts, and (2) whether there are differences in the synaptic organization of axon terminals from VB and PO.

Phaseolus vulgaris leuco-agglutinin (PHA-L) was deposited iontophoretically into either VB or PO. After 4-5 days survival, rats were perfused with a periodate-lysine-paraformaldehyde fixative. Somatosensory cortex and the dorsal thalamus were cut with a vibratome into 50-70 um sections. In order to increase the penetration of the immunoreagents, sections were frozen and thawed in the presence of one of the following cryoprotective agents: dimethyl sulfoxide (DMSO, 5%, 10%, and 20%) or a mixture of sucrose and glycerine (10%+5%, 15%+10%, and 20%+10%) in 0.1 M phosphate buffer. PHA-L was localized by avidin-biotin-complex (ABC) immunoperoxidase methods. After the peroxidase linkage, the sections were treated with diaminobenzidine with heavy metal intensification. Sections with a high density of labeled terminals were processed for ultrastructural studies.

At the ultrastructural level, labeled axon terminals were studied in every layer of the barrel cortex. There are three similarities in the VB and PO projections: (1) Labeled button-like swellings in all layers observed at the LM level are indeed forming synaptic contacts, (2) All the labeled terminals form asymmetrical synapses, and (3) The most common postsynaptic structures observed so far are dentritic spines and small dendrites. So far, the only difference is in layer I, in which we have observed both labeled myelinated and unmyelinated axon segments after PO injections and only labeled unmyelinated axon segments after VB injections.

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73.7 INTERNAL ORGANIZATION OF ANATOMICAL AND PHYSIOLOGICAL REPRESENTATIONS OF THE FOREPAW IN RAT SOMATOSENSORY CORTEX. D.R. Dawson, J.T. Wall, H.P. Killackey, and J.H. Kaas. Dept of Psychobiology, U.C. Irvine, CA, and Dept of Psychology, Vanderbilt University, Nashville, TN. (SPON: A. Frostholm)

Rat somatosensory cortex which has been reacted for succinic dehydrogenase (SDH) contains a series of discrete clusters representing the entire body surface. The region corresponding to the forepaw has a particularly well defined, stereotypic pattern of internal organization, consisting of four large SDH bands surrounded by several smaller clusters. The following studies were designed to ascribe functional properties to specific SDH clusters within the forepaw region of cortex.

In the first study, fetal rats resulting from timed matings were exposed in utero on E16 or 17, and specific forelimb digits were removed. Pups were born naturally, on E22 or E23. On PND15, pups were sacrificed, and processed for SDH.

Prenatal removal of specific forelimb digits results in the disappearance of specific SDH clusters in the cortical representation; the loss of ulnar digits is reflected by a loss of the more posterior clusters, whereas loss of radial digits results in disturbances of the anterior clusters.

In a second study, twelve normal adult rats were subjected to standard neurophysiological mapping procedures within the forepaw region of somatosensory cortex, generating comprehensive maps (40-50 penetrations). The forepaw representations of twelve additional rats were mapped in fewer penetrations (10 or less) labelled with either HRP or fast blue fluorescence. Animals with labelled penetrations were sacrificed and the brains processed for SDH. Sections superficial and deep to the SDH pattern were reacted for HRP, using the TMB reaction. The HRP thus served as a marker for a given penetration, which could then be localized within the SDH clusters on the adjacent section. In this manner, penetrations with well defined peripheral receptive fields on the forepaw were related to specific SDH clusters. Fast blue labelled penetrations were visualized directly on SDH sections.

Cells responsive to stimulation of specific structures on the forepaw could be localized to discrete regions of cortex. Radial digits were represented anteriorly and ulnar digits posteriorly. Responses to stimulation of palm pads were evoked medially, and hairy dorsal skin laterally. Animals with labelled penetrations confirmed these findings, permitting a precise association of specific receptive fields with specific SDH clusters.

In summary, the anatomical and physiological maps of the rat forepaw representation in primary somatosensory cortex are in register with each other, such that discrete subdivisions within one can be reliably related to subdivisions within the other.

73.8 **THALAMOCORTICAL RESPONSE TRANSFORMATION IN THE VIBRISSA/BARREL SYSTEM.** **G.E. Carvell* and D.J. Simons. Program in Physical Therapy and Department of Physiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.**

Extracellular recordings were used to examine the response properties of > 150 single cells in the thalamic ventrobasal complex (VB) of adult rats maintained with a slow infusion of fentanyl. Electromechanical stimulators were used to deflect vibrissae in controlled spatial and temporal patterns. Results are compared with data obtained from > 250 cortical barrel neurons. Results show that: 1) the cyclic pattern of stimulus evoked excitation/inhibition that characterizes responses in the barrels is considerably less pronounced in VB; 2) slowly-adapting responses are observed more often in VB than in the cortex; 3) inhibitory interactions between adjacent whiskers are observed less frequently and are less potent in VB; 4) facilitation is observed commonly in VB but rarely in cortex; 5) spontaneous discharge rates are on average 6X greater in VB; 6) VB cells tend to respond more vigorously to whisker deflections at their 'best' angle; 7) in VB, ON responses are more 'tuned' for deflection angle and more cells discharge selectively to either stimulus onset or offset; 8) unexpectedly, many VB cells (~30%) are strongly excited by more than one whisker; in the barrels fast-spike units are more likely than regular-spike units to be strongly multi-whisker.

These functional differences may reflect: a) the known absence of inhibitory interneurons in the VB barreloids and the presence in the cortex of inhibition provided by fast-spike/smooth barrel neurons; b) the ~10:1 ratio of barrel to barreloid neurons whereby, presumably, a single barreloid axon contacts many barrel neurons and a single barrel cell is contacted by axons of many barreloid neurons; and c) the fact that thalamocortical synapses occur more proximally on smooth vs. spiny barrel cells (White, JCN, 181:627-661, 1978). Barrel circuitry transforms the thalamocortical input into a temporal code that underlies operations performed by the rest of the cortical column. **Supported by NIH grant NS 19950.**

73.9 DIFFERENCES IN GAD AND GABA IMMUNOREACTIVITY IN THE SOMATOSENSORY CORTEX OF THE RAT. R.Spreafico, G.Battaglia, C.Frassoni, S.De Biasi* (Spon.ENA) Dept. of Neurophysiology, Neurol Institute C.Besta and Dept. of Physiol and Biochemistry, Section of Hystology and Human Anatomy, 20133 Milano Italy

Aim of the present work is to verify if GAD and GABA antisera label the same neuronal population in different brain areas. Eight deeply anesthetized albino rats were perfused with a 4% paraformaldehyde and 0.1% glutaraldehyde solution in phosphate buffer. Brains were cut in 40 um thick coronal serial sections. Alternate serial sections were than processed for immunohistochemistry according to the pre-embedding paroxidase-antiproxidase (PAP) method (Steinberger 1972) using two different antisera agonist GAD (Oertel et al. 1981) and GABA (Immunonuclear Corp.). Adjecent sections were counterstained with Cresilect Violet to identify the different cortical areas and layers. Two of these animals received colchicine injections in somatosensory cortex or in the ventro basal complex, two days before sacrifice. In two additional animals small blocks of identified somatosensory cortex were dissected out, cut in 10 um thick serial sections by means of a cryostat, and processed with the same procedure described above. Labelled neurons from both anti-GABA and anti-GAD reacted sections, were plotted by means of an x-y plotter interfaced to a Leitz microscope. A total count of 15396 labelled neurons in colchicine treated and untreated animals show that GAD positive neurons in the cortex are approximately the 60% of GABA labelled neurons counted in the adjecent sections. This difference seems to be restricted to the cortex. In fact, in the same animals the ratio between GAD and GABA thalamic labelled neurons in the lateral geniculate nucleus and in the thalamic reticular nucleus was 1:1. Moreover the histogram distribution of areas of immunoreactive neurons in the cortex, calculated on cells with the nucleus evident on the plane of the 10 um thick sections,showed that in the GABA positive neuronal population is present a group of large neurons not present in GAD positive population. These data, although could imply the different specificity of the two antibody used, could suggest the presence in the cortex of a neuronal population in which GABA is synthesized from methabolic way other than glutamate.

73.10 **THE CHOLINERGIC INNERVATION OF SOMATIC SENSORY CORTEX IN THE CAT.** Kristin Barstad* and Mark F. Bear (SPON: C. Elbaum), Center for Neural Science, Brown University, Providence, RI 02912

The cat visual cortex can be readily modified by sensory experience during a critical period of postnatal development. These changes depend critically on the pattern and amount of retinal activity. However, they also appear to require that the animal attends to visual stimuli and uses vision to guide behavior. The neural substrate of these "extra-retinal" influences appears to be the noradrenergic projection from the pons and the cholinergic input from the basal telencephalon. Destruction of these two projections interfere with normal experience-dependent modifications in the visual cortex. We are interested in the possibility that similar mechanisms contribute to the use-dependent shifts in the adult cortical somatotopic map. As a first step in testing this hypothesis, we have begun to characterize the anatomical organization of the cholinergic projection to the somatic sensory cortex in the cat. Previous work has shown that large excitotoxin lesions of the basal telencephalon will deplete acetylcholinesterase-containing axons throughout the neocortex, including the somatic sensory areas. In the present investigation, we sought to identify with greater precision the location of cholinergic neurons that project to S1 cortex. Injections of either HRP or WGA-HRP were made in area 3 and after a 36-48 hour survival period, the brains were processed for routine HRP histochemistry. The distribution of retorgradely labelled neurons was compared with the distribution of cells labelled by choline-acetyltransferase immunocytochemistry. The distribution of labelled neurons in the basal telencephalon after large cortical injections of ~0.75 µl of 30% HRP was surprisingly circumscribed. Most back-filled neurons were found on the border between the globus pallidus and adjacent structures: the internal capsule medially, the putamen laterally, and the substantia innominata ventrally. In addition, labelled neurons were sometimes also found in both limbs of the diagonal band of Broca. As a rule, small electrophoretic injections confined to area 3b yielded very few labelled neurons in the basal telencephalon; however, those that did label usually fell in the zone bordering the globus pallidus. These findings predict that it will be possible to deplete S1 cortex of its cholinergic innervation with relatively small excitotoxin lesions of the basal forebrain. This prediction is currently being tested. (Supported by U.S. ONR contract N000-14-81-K-0041)

73.11 PROCESSING OF INPUTS FROM HAIRY AND GLABROUS SKIN WITHIN THE DIGIT REPRESENTATION OF CAT SI CORTEX. N.E. Fletcher* and P. Zarzecki. Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Primary somatosensory cortex forms an orderly representation of the body, but in addition contains neurons with inputs from more than one body location (topographic convergence) or of more than one modality. An issue which we have been pursuing is how these convergent inputs shape cortical neuronal activity. When forelimb nerve trunks are stimulated electrically, there are a variety of interactions among the effects evoked in cortical neurons from separate nerves (Kang et al., Exp. Brain Res., 1985). A problem with using electrical stimulation of nerve trunks is that there is considerable overlap of zones of innervation of paw skin by superficial radial (SR), ulnar (UN), and median (MeN) nerves. However, each nerve also serves autonomous zones of skin on the cat paw where cutaneous stimuli evoke primary afferent activity only in that nerve (Kitchell et al., J. Comp. Neurol., 1982). Therefore, we delivered punctate electrical cutaneous stimuli through loops of stainless steel wire sewn into hairy skin of digits three (D3) and five (D5), and glabrous skin of the metacarpal pad (MCP). These are typical autonomous zones for SR, UN and MeN, respectively. Stimulation of D3, D5 and MCP gave rise to primary afferent volleys only in the expected peripheral nerves and to cortical surface potentials of characteristic latency, waveform and distribution across SI cortex. Intracellular recordings were obtained from neurons within the cortical representation of the digits. Most impalements were made in the focus for D5. As expected, epsps were evoked in most neurons (35 of 39) by D5 stimuli. However, sources of input were not restricted to D5. Epsps were also evoked in many of these same neurons by electrical cutaneous stimulation of D3 and MCP, or both. Convergence of inputs from D3 and D5 were found for 24 of these neurons (62%), and 16 of 32 (50%) also had inputs from MCP. The selectivity of paw stimuli was verified electrophysiologically at the end of recording sessions. Cortical surface potentials evoked from D3, D5 and MCP disappeared one-by-one when the forearm nerves carrying afferent volleys from each skin stimulus site were severed sequentially. Thus, to detect neurons with topographic convergence does not require electrical stimulation of nerve trunks with overlapping peripheral distributions. Furthermore, we predict that neurons in the digit zone of SI cortex will generate psps in response to natural stimulation of hairy as well as glabrous skin of the cat paw. Supported by the MRC of Canada.

73.12 MULTIPLE REPRESENTATIONS WITHIN THE FERRET FIRST SOMATIC SENSORY CORTEX (MUSTELA PUTORIUS FURO). S.S. Leclerc,*[1] F.L. Rice[2] and R.W. Dykes[1]. [1]Depts. of Physiology, Neurology and Neurosurgery and Surgery, McGill University, Montreal, Quebec, Canada, H3A 1A1 and [2]Dept. of Anatomy, Albany medical College, Union University, Albany, NY 12208.

In the present study, anatomical and electrophysiological methods were combined to examine the organization of the somatosensory cortex in the ferret (Mustela putorius furo).

Experiments were performed on 16 animals using sodium pentobarbital anesthesia (35mg/kg). A craniotomy over the left frontal and parietal poles permitted access to the somatosensory region. Multiunit responses were recorded using single barrel carbon fiber electrodes. Cutaneous stimuli were delivered using hand-held glass probes while inputs from deep tissues were activated by tapping or manipulating muscles and joints. The adequate afferent stimulus, receptive field location and threshold were determined for each recording site. A total of 2,904 penetrations were obtained with individual penetrations spaced 50 to 150um apart. Data was recorded from the cortical depth which presented the best multiunit response. In two and four animals respectively, over 500 and over 200 penetrations were made. These results were combined with the data from the remaining animals, to construct the full body and facial representation of the ferret.

The overall features of the somesthetic representation were similar to those observed in other mammalian species. Caudally located body parts were found most medially, adjacent to the longitudinal fissure, while head and face representations were located most laterally. Details of the facial representation were based on a total of 1139 electrode recordings obtained in four experiments. Grouping of recording sites according to receptive field location revealed a dual representation for most facial cutaneous areas. Long rows of recordings across this projection area illustrated that the representations were arranged in a roughly mirror-image pattern. Conclusions concerning body and limb representations were based on data acquired in six cases. Data analysis suggested a dual representation of the forelimb. These conclusions were based upon examination of receptive fields limited to single digits and from comparison between projections arising from both glabrous and hairy surfaces. One of the forelimb representations appeared to receive a larger proportion of small, well-defined receptive fields thereby suggesting a different type of sensory processing. These results support the hypothesis that multiple representations exist within the primary somatosensory cortex of nonprimate mammals.

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73.13 ORGANIZATION OF CUTANEOUS INPUT FROM HAND TO PRIMARY SOMATIC SENSORY (SI) HAND AREA IN AN OLD WORLD PRIMATE, ERYTHROCEBUS PATAS. Susan Warren and Mary Carlson. Depts. Psychiatry, Anatomy and Neurobiology and Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

The organization of cutaneous input from the hand to primary somatic sensory cortex (SI) in Old World prosimian primates is comprised of a single topographic pattern. In forming this pattern, three organizing principles ('P') are followed: P1) input from glabrous and hairy surfaces are separately organized; P2) the distal-proximal axes of both surfaces are oriented serially in the rostral-caudal plane; and P3) the ulnar-radial axis lies in the medial-lateral plane. These principles of organization are violated to varying degrees in Old World anthropoids in which a major expansion of the distal digit tip projection occurs in concert with a reduced projection from the remaining cutaneous surfaces of the hand.

In previous studies of Macaca, Cercopithecus, Miopithecus, we found considerable interdigitation of glabrous and hairy receptive fields (rfs) in violation of P1. Rfs on the palm are compressed to the medial and lateral margins of the digit area, and frequent proximal-distal axis shifts occur within and between cytoarchitectonic areas 3b and 1 in violation of P2. However, in both area 3b and 1, P3 was maintained.

In our recent attempts to characterize the topographic pattern of the hand area in the Old World anthropoid, E. patas, using micro-mapping techniques, we found consistent violations of these principles. Unique to E. patas are the appearance of rfs covering multiple glabrous digit tips (25% of rfs) in area 3b and the presence of mixed glabrous/hairy fields on the dorsal digit (5%)in area 3b and 1. The finding of mixed glabrous/hairy fields violates P1. With more than 60% of the total mapped rfs restricted to the distal tips, and 10% of the remaining rfs located on more proximal portions of the glabrous hand, shifts along the proximal-distal axis are scarce and in violation of P2. Similiar to the Old World anthropoids studied thus far, E. patas preserves a shift from d5 to d1 in the medial-lateral plane of area 1. However, for any given medial-lateral point in area 3b of E. patas, this ulnar-radial axis of orientation appears rotated slightly with ulnar rfs located more superficial and radial rfs located deep in violation of P3.

By expanding these studies to include single unit recordings, we will attempt to determine what part of the organizational differences are reflections of the response properties of the underlying individual neurons, are specific to the species studied, or are attributed to other factors such as development. (Supported by MH 14677 and MH 40157).

73.14 THE FUNCTIONAL TERRITORY OF THE PRIMATE MEDIAN NERVE IN THE HAND SKIN AND CORTICAL MAP OF THE HAND. M. F. Huerta and J. T. Wall. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

The skin on the primate hand contains a dense sheet of low threshold mechanoreceptors. These receptors activate ascending central circuits in an organized fashion and, as a result, somatosensory cortical area 3b contains a somatotopic map of the hand surface. The receptor sheet is connected to central circuits via the median, ulnar, and radial nerves. Two questions were asked in the present experiments. First, what parts of the receptor sheet are innervated by the median nerve? Second, what are the spatial features of the representation of this skin in the area 3b map of the hand?

These questions were addressed in ketamine anesthetized squirrel or owl monkeys in which multiunit recordings were made from the median nerve, or in which the hand cortex was mapped with multiunit recordings following acute transection of the ulnar and radial nerves in the contralateral wrist.

There are two main results. (1) Skin areas that were innervated by low threshold mechanoreceptor inputs in the median nerve were found to be similar when assessed with median nerve fiber recordings, and with cortical neuron recordings in the absence of inputs from the other hand nerves. The median nerve territory consistently included the radial (thumbward) 3/4 of the palm and glabrous skin on digits 1-4. Small areas on glabrous digit 5, the tips or edges of dorsal digits 1-4, and the radial edge of the dorsal hand were also innervated but with more variability. (2) The area 3b hand map normally represents all or most of the hand surface. Following transection of the ulnar and radial nerves, the hand was incompletely represented. The median nerve skin was represented laterally near the face representation and was somatotopically organized to a large extent. This representation was sometimes interrupted by small zones that were unresponsive to cutaneous stimuli. Unresponsive zones were also found between the representation of the median nerve skin and the wrist skin.

These findings indicate that median nerve inputs are sufficient to support a topographically ordered representation of a major fraction of the glabrous surface of the hand. Considered with other findings, these results further indicate that the peripheral innervation fields of the hand nerves overlap. This suggests that the composite topography in the normal hand map emerges from an overlaying of orderly maps of the partially shifted cutaneous field of each nerve. (Supported by Grant NS21105; present address of M. F. H.: Department of BioStructure and Function, University of Connecticut Health Center, Farmington, CT 06032)

73.15 DISTRIBUTION OF GAD-LIKE IMMUNOREACTIVE NEURONS IN THE FIRST (SI) AND SECOND (SII) SOMATOSENSORY CORTEX OF THE MONKEY. E.H. Chudler, S. Pretel and D.R. Kenshalo, Jr. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in mammalian neocortex. The distribution of glutamic acid decarboxylase (GAD), the synthesizing enzyme of GABA, has been investigated in SI, but not in SII. The present study used immunocytochemical methods to describe the distribution of GAD-like immunoreactive (GAD-LI IR) somata and axonal varicosities in SII and to compare it with that in SI.

Four monkeys, two of which were pretreated with 5% colchicine intraventricular (25 µl) or intracortical (30 µl), were examined. The survival time ranged from 1-4 days after which the monkeys were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde or 4% paraformaldehyde alone. SI (area 3b) and SII tissue blocks were cut into 30 or 50 µm serial parasagittal or coronal sections. Alternate tissue sections were then incubated in GAD antiserum* for 48 hrs at 4°C and processed for GAD immunoreactivity using the PAP immunocytochemical method. Adjacent sections were stained with cresyl violet. GAD-LI IR somata were counted within vertical traverses of 540 µm and their major and minor diameters were measured.

GAD-LI IR neurons and axonal varicosities were found in all layers of SII and SI. The total number of GAD-LI IR neurons was similar in SII and SI. The mean major and minor diameters, respectively, of GAD-LI IR somata in SII were as follows: layer I, 11.0 x 8.2 µm; layer II, 11.8 x 9.6 µm; layer III, 13.0 x 10.3 µm; layer IV, 12.7 x 10.0 µm; layer V, 17.6 x 13.0 µm; layer VI, 15.3 x 10.8 µm. These values are similar to those obtained from GAD-LI IR somata in SI. Differences existed however, in the density distribution of GAD-LI IR somata and axonal varicosities. The highest density of GAD-LI IR cell bodies was found in layer III of SII and in layer IV of SI. The greatest density of GAD-LI IR axonal varicosities was in layers IV and VI of SI. In SII, the greatest density of GAD-LI IR axonal varicosities was in layer III. Moreover, GAD-LI IR axonal varicosities in SII were less dense in all layers when compared to those of SI.

These observations demonstrate differences in the distribution of GAD-LI IR somata and axonal varicosities in SII and SI. Of particular interest is the smaller amount of GAD-LI IR axonal varicosities in SII compared to SI. Previous studies have shown that receptive field sizes are generally larger in SII than in SI. Since GABA is an inhibitory transmitter that is known to affect receptive field size, it is possible that the difference in GABAergic inhibitory processes may account for the larger receptive fields in SII than in SI. *Anti-GAD kindly provided by Dr. I.J. Kopin, Lab. Clinical Science, NIMH, NIH.

73.16 AN IMMUNOCYTOCHEMICAL STUDY ON GLUTAMATERGIC CORTICO-CORTICAL NEURONS IN THE SOMATIC SENSORY AREAS OF MONKEYS. F. Conti, M. Fabri*, T. Manzoni*. Institute of Human Physiology, University of Ancona, I-60131 Ancona, Italy.

Previous studies on cats showed that cortico-cortical neurons of the somatic sensory areas might use the amino acid glutamate (Glu) as neurotransmitter. The evidence was obtained by using the specific retrograde axonal transport of tritiated D-Aspartate (Barbaresi et al., J. Comp. Neurol., 1987, in press) and by combining the immunocytochemical visualization of Glu with the horseradish peroxidase (HRP) retrograde labelling of cortico-cortical cell bodies (Conti et al.; Manzoni et al.: Abstr. 2nd World Congr. Neurosci., 1987). Since previous immunocytochemical studies (Conti et al., J. Neurosci., 1987, in press) showed that the cerebral cortex of monkeys contains numerous Glu positive neurons, we performed the present experiments to test the hypothesis that also in monkeys cortico-cortical neurons might be glutamatergic. HRP was injected in the somatic sensory areas of adult monkeys (M. Fascicularis). Animals were then perfused transcardially with saline followed by 4% carbodiimide in 0.1M phosphate buffer (pH 7.4) and 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Selected regions of the ipsilateral and contralateral first (SI) and second (SII) somatic sensory areas were cut in blocks and sectioned with a Vibratome (25 µm thick sections). Sections were reacted for HRP histochemistry using a cobalt-enhanced protocol and then for immunocytochemistry using an anti-Glu serum previously characterized (Hepler et al., J. Histochem. Cytochem., 1987, in press). Three types of neurons were observed in both ipsilateral and contralateral hemispheres: i) neurons with only black HRP granules (cortico-cortical neurons); ii) neurons with only pale-brown, diffuse labelling (Glu-positive neurons), and iii) neurons with HRP black granules and pale-brown diffuse labelling (Glu-positive, cortico-cortical neurons). In SI and SII double-labelled neurons were found in both supragranular and infragranular layers. These results show that also in monkeys a large fractions of cortico-cortical neurons may use Glu as neurotransmitter.

73.17 WIDESPREAD THALAMIC PROJECTIONS TO LAYER I OF PRIMATE CORTEX. D.P. Friedman[1,2], J. Bachevalier[1], L.G. Ungerleider[1], and M. Mishkin[1] Lab Neuropsychology[1], NIMH, Bethesda, MD 20892 and Neurosciences Research Branch[2], NIDA, Rockville, MD 20857

A system of "nonspecific" thalamic fibers, which terminate widely in layer I of cerebral cortex and may help to control cortical excitability has long been postulated, but never demonstrated in primates. By contrast, many components of the "specific" system, which projects to layers III and IV of restricted regions of cortex and transmits sensory information, have been extensively described. We now present evidence for a widespread layer I projection system in the macaque that arises from nuclei on or near the thalamic midline.

In two cynomolgus monkeys, isotope injections were made that involved the entire anteroposterior and dorsoventral extent of the thalamic midline, but that did not spread more than 1.5mm from it. Five rhesus monkeys received smaller injections largely restricted to either the magnocellular division of the mediodorsal nucleus (MDmc), nucleus reuniens (Re), or the anterior group. All surgery was performed under deep anesthesia while aseptic procedures were observed. After a survival period of one week, the brains were prepared for autoradiography. Exposure times were 16-69 weeks.

The large injections revealed an extensive projection system that ran in long bands in the outer half of layer I and commonly crossed the borders between cortical fields. This labeling pattern was seen in the ventrolateral, orbital, and subcallosal regions of prefrontal cortex, virtually the entire extent of the temporal lobe ventral to the superior temporal gyrus, the visual fields of the occipital and parietal lobes, the entire extent of the insula, much of the frontoparietal operculum, including gustatory and somatic fields, and in the auditory areas that border the ventral insula. In addition, cortical limbic areas, including the cingulate gyrus, entorhinal and perirhinal areas, the subicular fields, and the hippocampus proper were also labeled.

Injections into MDmc and the adjacent midline nuclei led to dense label in layer IV of orbital cortex and some patches of label in the overlying layer I as well. The injection in Re produced layer I label in subcallosal and orbital cortex. The injection into the anterior group, which involved mainly the dorsal and medial nuclei, and also spread to the adjacent midline nuclei, led to label in both layers I and III of the subcallosal region.

These findings supply evidence for a widespread system of midline thalamic projections to layer I of sensory and limbic cortex. Because the hippocampus and amygdala both project to the midline thalamus, and because damage to this region leads to memory deficits, these layer I projections may form part of a limbic-cortical feedback circuit involved in the formation of memories.

73.18 **POST-ROLANDIC CORTICAL PROJECTIONS OF THE SUPERIOR TEMPORAL SULCUS IN THE RHESUS MONKEY.** Benjamin Seltzer and Deepak N. Pandya. V.A. Hospital, Bedford MA 01730, Boston Univ. Schl. of Medicine.

Previous architectonic and connectional studies identified uni- and polymodal sensory-related regions in the superior temporal sulcus (STS) of the rhesus monkey (Seltzer and Pandya, '78). The present study addresses the efferent projections of these discrete architectonic zones to more distant regions of post-Rolandic cortex, as shown by autoradiographic techniques.

The proisocortex of the rostral STS (area Pro) projects to insular proisocortex, prorhinal cortex, and areas TF and TH of the parahippocampal gyrus (PHG). Polymodal cortex of the upper bank (area TPO) projects to the posterior parietal lobe, insula of Reil, PHG, and cingulate region. The rostral portion of area TPO projects to the inferior parietal lobule (IPL) (areas PFG, PFop, PG, and Opt; Pandya and Seltzer, '82) and lower bank of the intraparietal sulcus (IPS) (area POa; Seltzer and Pandya, '80) while caudal TPO projects to area PG, the depth of the IPS (area IPd; Seltzer and Pandya, '86), and medial parietal lobe (area PGm). Rostral area TPO projects to agranular and dysgranular insula; caudal TPO projects to granular insular cortex. Rostral TPO has the more extensive projections to the PHG whereas caudal TPO projects to the cingulate region (area 23).

Unimodal visual-related sectors of the STS (areas TEa and TEm), located rostral to area "MT" in the lower bank, project only to the PHG and perirhinal cortex. A unimodal somatic sensory area (IPa) in the rostral depth of the sulcus projects to the IPL and lower lip (area PG) and bank (area POa) of the IPS. Auditory-related unimodal cortex in the STS (area TAa) has projections similar to those of the adjacent superior temporal gyrus, viz. PHG, caudal insula, and area 23.

Thus polymodal cortex of the STS has extensive projections, organized differentially according to the rostrocaudal dimension. Rostral polymodal cortex has connections with rostral paralimbic areas (i.e. prorhinal, perirhinal, and rostral insular cortex) while caudal polymodal cortex projects to more caudal paralimbic regions (i.e. area 23, caudal insula). Rostral TPO also projects to previously demonstrated polymodal regions of the PHG (Seltzer and Pandya, '76) and caudal IPL. The ventral temporal and parietal projections of caudal TPO, by contrast, resemble those of the IPL. Finally, unimodal zones of the STS have restricted post-Rolandic projections which are the reciprocal of their afferent cortical connections.

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MORPHOGENESIS AND PATTERN FORMATION I

74.1 PATTERN DUPLICATIONS IN THE PNS OF L(1)GIANT GAP MUTANT IN DROSOPHILA MELANOGASTER. J. P. Petschek*and A. P. Mahowald* (Spon; W. D. Lust)Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio,44106.

Zygotic lethal mutations at the 1(1)giant locus (1(1)gt; 3A1), a member of the gap class of pattern mutations in Drosophila melanogaster, produce embryos with two regions of segmental defects. Previous work indicates that 1(1)gt+ gene activity is required by the time of segment formation for differentiation of the labial and thoracic T1 and T2 segments in the anterior, and abdominal segments A5, 6, and 7 in the posterior of the embryo. In 1(1)gt mutants, localized cell death within the neural and mesodermal tissue is correlated with the segmental defects observed in the posterior region at 7 hours of development. Later, neuromeres A5-7 fail to form, producing a gap in the ventral nerve cord of the mature embryo. We have examined 1(1)gt embryos to determine the effect of early cell death on development of the central and peripheral nervous systems in the gap region of the mutant. 1(1)gt embryos were immunostained with polyclonal antibody probes directed against Drosophila segmentation gene products fushi tarazu (ftz) and engrailed (en), and neuronal cell surfaces (antibody to HRP). Also, embryos were examined with a set of monoclonal antibodies directed against peripheral nervous system components (Mab 22C10, 5B12, T45; kindly provided by S. Benzer). Our results indicate that despite extensive neuronal cell death, longitudinal connectives are maintained over the gap region, and the pattern duplication of peripheral sensory structures occurs in some mutant embryos.

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74.2 NEUROMUSCULAR TRANSFORMATION IN BITHORAX DROSOPHILA MELANOGASTER. A. J. Benson* and D. G. King (SPON: W. Yau). Dept. of Zoology and Dept. of Anatomy, Southern Illinois Univ., Carbondale, IL 62901

Bithorax complex mutations are known to affect nervous tissue. However, most studies of homeotically transformed neural structures have focused on sensory neurons derived from imaginal ectoderm, while relatively few have reported effects on motor systems.

In the present study, extensive homeotic transformation of metathoracic muscles and nerves was observed in flies of the genotype abx pbx bx3/Df(3R)p2. Serial sections revealed that the size, shape and position of transformed metathoracic muscles resembled the normal mesothoracic pattern. Transformed muscles (names adapted from Zalokar 1947, Rev. Suisse Zool. 12:17) were (1) indirect flight muscles including dorso-ventral muscles 1 and 5 and longitudinal dorsal 2 muscles; (2) direct flight muscles including both anterior and posterior pleural muscle groups; and (3) other thoracic muscles including the sternopleural muscle, the longitudinal ventral muscle and leg muscles 1, 2, and 3.

Furthermore, nerves in the transformed segment displayed a pattern of axon diameter and grouping similar to the wildtype mesothoracic nerves. For example, in the normal fly mesothoracic muscle pa4 has no proposed metathoracic homologue, but in the transformed metathorax a corresponding muscle is easily identified. This muscle is innervated by an exceptionally large axon which corresponds in diameter to the motor axon of the normal mesothoracic muscle (King & Tanouye 1984, J. Exp. Biol. 105:231) and which travels in a mixed nerve not seen in the normal metathorax but corresponding in position and composition to the anterior dorsal mesothoracic nerve (ADMN). The pattern of innervation does differ when one observes the pa2 muscle. In the wildtype mesothorax, the pa2 muscle is innervated by the mesothoracic accessory nerve (King & Tanouye 1984), while in the transformed metathorax the pa2 muscle is innervated not by the presumably homologous metathoracic accessory nerve but by the transformed metathoracic homologue of the ADMN. The metathoracic accessory nerve does however show homologous posterior pleural muscle innervations. Therefore, not all axons are identically transformed, and segmental differences may reflect an anterior/posterior transformation gradient.

Finally, preliminary data from pupal bithorax specimens show that a large metathoracic axon, presumably the pa4 motor axon, appears before any muscle differentiation can be noted at the pa4 muscle site. Thus this system, wherein bithorax mutations restructure the metathoracic flight muscles and motor neurons to resemble their mesothoracic homologues, suggests that motor axons may be the architectural precursors which direct muscle transformation. This bithorax system may additionally provide an opportunity for exploring the differentiation of central neurons.

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74.3 THE ONTOGENETIC DEVELOPMENT OF SUPRASPINAL AFFERENTS IN GOLDFISH. S.C. SHARMA* AND M. BERTHOUD. Dept. of Ophthalmology, New York Medical College, Valhalla, New York 10595.

The onset and development of descending pathways to the spinal cord was studied following horseradish peroxidase application to the severed thoracic spinal cord of goldifsh embryos ranging from embryonic Stage 23 (onset of heart pulsation) through hatching (Stage 25-100 hrs. after spawning) and posthatched fries (ranging from 2 to 40 days old). Earliest HRP labeled cells were reliably seen in the inferior reticular formation at Stage 23-24 following HRP application at the cervical level. In addition Mauthner cells were also labeled. Within 24 hours a few retrogradely labeled cells were seen in the medial and rostral reticular formation and the nucleus of the medial longitudinal fascicle. At about the same time a few labeled neurons were seen in the future octaval area, however, no clear distinction could be made between octaval nuclear areas until two weeks after hatching. Thus the earliest supraspinal fiber connections appear to be formed simultaneously. Retrogradely labeled cells were seen in the raphae nucleus and in the Ruber nucleus. A corollary ^{3}H-thymidine labeling study confirmed that some cells in these nuclei had ceased DNA synthesis and were postmitotic at Stage 23. HRP labeled cells, however, were not found in the facial lobe and the nucleus preopticus magnocellularis and nucleus ventromedialis. Facial lobe was not differentiated until Stage 25 and a distinct facial lobe was not present till one month after hatching. Since facial lobe neurons and the preoptic area neurons project to the spinal cord in adult, it is speculated that their projection must occur later in development. In addition, transitory projections from hypothalamic area was observed in recently hatched embryos. This projection, however, was missing in adult animals. It appears that during the first few weeks after hatching the number of retrogradely labeled cells increase in number. This observation is supported by continued ^{3}H-Thymidine labeling of cells in these nuclei.

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74.4 FROG PREY ORIENTING AND BRAIN DEVELOPMENT: NEURONAL PROLIFERATION IN VENTRAL TEGMENTUM. A. Berkowitz* and P. Grobstein. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The anuran nervous system must successively serve two different sets of functions. Tadpoles of most species are lateral-eyed grazing herbivores, while frogs are frontal-eyed visual predators who rapidly orient toward and strike at prey. Both sets of functions might in principal be served by a stable, general purpose nervous system laid down early in life. An alternative is that aspects of nervous system organization are highly specific to particular functions. Consistent with the latter possibility are previous findings that an eye-position related change in retinal projections involves metamorphic neuron production. Visual prey orienting in the frog depends on a descending tectofugal pathway, which recent studies suggest involves significant processing in ventral tegmentum. To determine when ventral tegmental neurons are produced, we injected tritiated thymidine into Rana pipiens tadpoles and analyzed autoradiographic labelling in the serially sectioned postmetamorphic brains.

Tadpoles were injected at Taylor and Kollros stages ranging from V to XX, the latter corresponding to forelimb emergence. Labelling was observed in all brains. Injections at the earliest stages resulted in a reasonably compact, roughly sagittal sheet of labelled cells extending through the lateral ventral tegmental gray. Rostrally, the sheet was located at the medial edge of nMLF. Caudally, it extended through the middle of AV and into lateral AD, PV, and PD. Injections at progressively later stages resulted in progressively more medial sheets. Rostrally, the sheet was at the medial edge of the tegmentum for injections at stage XV and became indistinct with later injections. Caudally, medial portions of AD, PV, and PD were distinctly labelled even in the oldest injected animals. For injections after stage XVII, a second labelling pattern appeared. In each of three brains examined, substantial numbers of labelled cells were seen scattered in the white matter lateral to the tegmental gray, an area nearly devoid of labelling following injections at all earlier stages. Scattered labelled cells in nMLF were also relatively more frequent for late than for intermediate injections.

The dominant labelling pattern observed, progressing from lateral to medial across several nuclei in the ventral tegmentum, suggests orderly production, migration, and settling of neurons, such that, irrespective of nuclear boundaries, older neurons lie laterally and younger ones medially. Lateral tegmental structures, which include those so far implicated in frog orienting, thus may have a general purpose organization of use in both tadpole and frog. The late, lateral labelling pattern raises the possibility that there is also some metamorphic alteration in the organization of lateral structures. Many of the scattered lateral cells labelled by late injections appear cytoarchitecturally to be neurons. Our findings also show substantial metamorphic cell addition medially. This may also relate to the metamorphic onset of orienting, and warrants further investigation.

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74.5 **OCULOMOTOR NUCLEUS OF XENOPUS LAEVIS : THE NEIGHBOR TO NEIGHBOR RELATIONSHIP OF NEURONS IS ALTERED DURING METAMORPHOSIS.**

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Each oculomotor (OM) nucleus contains neurons with axons that cross the midline, and neurons that do not. The crossed projection, as shown by HRP labeling, is to the superior rectus muscle, the uncrossed to the medial and inferior recti, and inferior oblique muscles. From premetamorphosis to adulthood, about 30% of the neurons have a crossed projection. In the OM nerve of Xenopus laevis, half of the axons are lost during metamorphosis. Does metamorphosis modify the spatial distribution of these two sets of motoneurons ?

Pre-metamorphic and metamorphic tadpoles, and juvenile frogs, were injected with 2% fast blue in the retro-ocular region of one side; 24 hours later, the opposite side was injected with 2% rhodamine. Two days after the last injection, the animals were anesthetized and their brains processed to be cut horizontally in the cryostat. Sections were examined with the aid of a computer microscope.

No double-labeled cells were identified. Nevertheless, the two populations of neurons are not segregated strictly within the nucleus; rather there is a mosaic pattern. At each horizontal level, a grid superimposed on computer-generated drawings divided each left and right nucleus into squares whose sides measured 90 μm. In premetamorphic tadpoles (n=8), 21% of the squares contained both fast blue labeled and rhodamine labeled cells; for metamorphic animals (n=4), this value was 27%, and for juvenile frogs (n=4), 42%.

The number of labeled cells per occcupied square ranged from 1 to 14; with metamorphosis, the percentage of those that contained more than 5 cells had increased from 14% to 31%. A cell-by-cell analysis showed that at premetamorphosis, 21% of all cells have a nearest neighbor with a different label, at metamorphosis 25%, and 31% in the juvenile.

In summary, after metamorphosis, neurons are clustered more tightly, and more clusters contain neurons with crossed and with uncrossed axons.

Without discarding other mechanisms, we hypothesize that this intermingling of neurons is enhanced by a continuing migration, beyond metamorphosis, of motoneurons whose axons have already reached their target. Support : SwissNSF 3.158.

74.6 DEVELOPMENT OF THE OPTIC TECTUM IN LATE PREMETAMORPHIC STAGE OF THE BULLFROG. L.C. Towns and N.J. Uray. Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

In a previous report (Towns, et al., Soc. Neurosci. Abstr., Vol. 12 Part 1, 1986, p. 319) the development of the optic tectum during the first 26 weeks of larval growth was described. During the initial period of larval development (2-3 weeks), cells proliferate in the rostrolateral quadrant of the optic tectum then migrate caudally. At later ages (8-14 weeks), an arc of proliferating cells lies rostromedial to caudolateral across the tectum and by 26 weeks the principal zone of proliferation is caudomedial. The present experiment describes tectal development during weeks 26-52 as the larva approaches the prometamorphic period when the hindlimbs begin to grow. Larvae were injected with [^{3}H]-thymidine at 26 weeks, 7, 8, 9, 10, 11, or 12 months and were then killed at 53 weeks of age; the entire brain and spinal cord were processed for standard autoradiography. The current results demonstrate that from week 26 to 52, a time period that corresponds to late premetamorphic stages, the principal zone of cell proliferation remains in the caudomedial sector of the optic tectum where the developing, laminated tectum meets the membranous posterior wall. The posterior wall of the optic ventricle diminishes in size but remains membraneous. However, the region of the optic tectum which lies between the membraneous posterior wall and the already formed anterior region continues to differentiate by at least two mechanisms: ependymally derived cells migrate outward and cells which proliferate in the adjacent thickened area push medially between pia and ependyma.

Scattered cell proliferation is seen in other areas of the tectum, also. The ependyma and subependyma are labeled throughout the tectum at all ages injected. When tadpoles are injected at 26 weeks, the ependymal layers are diffusely and uniformly labeled at 53 weeks. The label becomes increasingly heavy and punctate with later injections until injections made at 52 weeks (one week prior to sacrifice) reveal only discrete, heavily-labeled cells scattered throughout the ependyma. In addition to ependymal labeling, scattered medium-to-heavy labeled cells are seen throughout the previously developed optic tectum, principally in cell layers, at all injected times.

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74.7 THYROXINE-INDUCED CEREBELLAR DEVELOPMENT IN THE PREMETAMORPHIC BULLFROG TADPOLE. N.J. Uray, S.L. Pyatt*, and M.D. Stuart.* Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

Numerous studies have shown that thyroxine treatment of large premetamorphic tadpoles induces premature cerebellar differentiation. This study examines the effect of thyroxine treatment on cerebellar development during the entire premetamorphic period which ranges from early stages of cerebellar differentiation to advanced stages of development. Bullfrog (Rana catesbeiana) tadpoles were raised from eggs and their ages were noted beginning with the time they swam free of their jelly-coat. At various time periods ranging from 3 weeks to 12 months of age, groups of tadpoles were treated with thyroxine (sigma, L-thyroxine sodium pentahydrate) by immersion at the concentration of 1:100M. After 2-4 weeks of thyroxine exposure the tadpoles were fixed, their brains dissected out, made into slides, stained with H and E, and were examined.

Our findings show that thyroxine induces premature development of the cerebellum at all the stages examined, however, the degree of development is not uniform. The overall effect of thyroxine is the promotion of corticogenesis, and evidence of this is apparent even in larvae treated at 3 weeks of age. In successively older age groups, the area of the cerebellar plate which demonstrates a cortical pattern in response to thyroxine treatment increases, however, the ventral part of the cerebellar plate continues to be occupied by amorphous cell masses. Thus, in young tadpoles only part of the cerebellum is competent to respond to thyroxine by forming a cortical pattern prematurely. In older premetamorphic tadpoles starting at 6 months of age, most of the cerebellum is able to form a cortical pattern, and in 12 month old tadpoles, the entire cerebellum is competent to complete cortical formation.

The increase in tissue in young larvae which are capable to form an adult-like cortical pattern in response to thyroxine indicate that cerebellar formation in the frog tadpole may proceed in a modular fashion by the activation of discrete zones of cerebellar tissue capable of undergoing differentiation and maturation. A zone of competent cerebellar tissue which can form a cortical pattern appears to pass through the cerebellar plate as a wave starting at the dorsal free edge and proceeding ventrally. The passage of this zone of competent cerebellar tissue through the cerebellar plate appears to be an age-dependent phenomenon. Supported by KCOM Warner Fund (to N.J.U.).

74.8 MATERNAL AND ZYGOTIC EFFECTS OF THE RECESSIVE MUTANT JERKY (jk) IN XENOPUS LAEVIS ON BRAIN AND ANTERIOR SPINAL CORD DEVELOPMENT AND FUNCTION. R. Tompkins, T. Bui*, B. Levet*, O. Shafey*, Q. Le*, D. Reinschmidt*. Biology Dept., Tulane Univ., New Orleans, LA 70118.

Homozygous jerky (jk/jk) tadpoles of the South African clawed frog, Xenopus laevis, derived from heterozygous crosses develop normally until stage 49. From then until metamorphosis they exhibit episodic behavioral abnormalities. Tail tip movements cease and swimming depends on trunk muscles only, or, in some cases all motility ceases and the animals rest on the bottoms of their containers. Heavy feeding and darkness exacerbate these symptoms whereas starvation and strong light alleviate them. Rearing tadpoles in lithium carbonate solutions suppresses the mutant phenotype. Mutant animals are normal after metamorphosis.

Offspring of jk/jk females mated with any genotype male are normal until stage 32. Subsequently, morphogenetic stasis affecting only the anterior spinal cord and brain occurs until death at stage 41-43. Grafting analysis showed that the posterior portions of maternally affected embryos are viable in combination with normal anterior parts, but that the reciprocal chimerae die of the maternal effect. Normal genotype anterior half/ jk/jk posterior half chimeric tadpoles develop normally. Hence both the maternal effect and the zygotic effect are similarly localized.

Two-dimensional electrophoresis revealed that a peptide component, measuring about 47 kd, of normal eggs and normal tadpole brain is absent from the eggs and tadpole brains of jk/jk animals. The mutant allele is associated with a similar size peptide with an altered isoelectric focusing position. Although injection of whole cytoplasm of normal eggs into fertilized eggs of jk/jk females partially corrects the maternal effect, injections of poly A^+ RNA from normal eggs has no effect on the expression of the maternal effect. These results suggest that the maternal effect on anterior spinal cord and brain development, and the zygotic effect on the function of these same structures, is due to the absence of the peptide associated with the normal allele.

74.9 FORMATION OF INDIVIDUAL MUSCLES IN THE CHICK THIGH. S. Schroeter and K.W. Tosney. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Although individual muscle targets appear to provide specific guidance for the corresponding motoneuron populations, we know little about the processes underlying the development of individual muscles from the large pre-muscle masses in the chick thigh. At the light microscopic level, any two incipient muscles are separated by "cleavage planes," regions of lower cell density that progress in a characteristic proximo-distal or disto-proximal direction. Different phases of cleavage can be seen in serial, 15 µm plastic sections through stage 26-32 thighs. We selected sections showing distinct phases of cleavage and reembedded them for ultrastructural analysis as in Tosney and Landmesser (1986, *J. Histo. Cytot.* 34: 953).

One possible explanation of muscle separation is that myotubes do not differentiate in the incipient cleavage planes. However, we frequently found cells containing myofibrils within the developing cleavage planes. This rules out the possibility that spatially patterned differentiation of muscle cells is responsible for the development of separate muscles.

Our results suggest that differentiating muscle cells are actively removed from the forming cleavage plane. We found macrophages and other cells that appeared to be engulfing isolated myotubes within forming cleavage planes and at the edges of muscles. These were never seen within the body of the muscle. The phagocytosis appeared to be specific: all engulfed cells had myofilaments. In addition, we occasionally saw cells with pycnotic nuclei that may have been dying. Active removal of cells would explain the gradual decrease in the number of differentiated myotubes between muscles that were separating.

We do not know if the phagocytosis is mainly responsible for the decrease in cell density that separates muscles or if it is a secondary mechanism for removing cells that have become isolated as the muscles are separated by other cellular mechanisms. In either case, cell death and phagocytosis of myotubes appears to play an important role in the patterned individualization of these muscles.

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74.10 **CELL LINEAGE DETERMINES THE INCREASE IN THE TOP GRADIENT DURING RETINAL DEVELOPMENT.** M. Schneider and D. Trisler, Sect. Cardiology, Baylor Coll. Med., Houston, Tx 77030 and Lab Biochem. Genet., NHLBI, NIH, Bethesda, Md 20892.

TOP is a cell surface molecule that is distributed topographically in a 35-fold gradient from the dorsal margin to the ventral margin of avian retina (Trisler,et al., PNAS, 78, 2145, 1981). The gradient is present at all developmental ages tested from day 3 postfertilization to adult. However, the magnitude of the gradient increases 20 times during embryonic growth of the retina from a 1.8-fold gradient in d.3 embryos to a 35-fold gradient in d.12 embryos. The retina grows by accretion of rings of cells at the peripheral margin. The TOP gradient increases during development by progeny cells at the dorsal pole of the gradient expressing more TOP than parental cells and those at the ventral pole expressing less than parental cells. Cells dividing along the axis of the gradient showed the greatest magnitude of change in TOP expression during retinal growth. Little or no change in TOP expression occured in cells with growth along the perpendicular axis. Intermediate rates of change were found during cell division at a 45° angle to the gradient axis.

Ablation of cells at the poles of the gradient in 60 hr. embryos altered TOP expression during subsequent retinal development. Cells at the dorsal pole of d.13 embryo retinas 11.5 days after dorsal ablation expressed 50% less TOP than normal and those at the ventral pole after ventral ablation expressed 300% more TOP than normal. Cells in other regions of these retinas expressed normal levels of TOP.

Cells from dorsal, middle, and ventral retina grown separately as monolayer cultures in vitro expressed a level of TOP equivalent to that expected for cells from those positions in vivo. Retinal cells from d.8 embryos grown in vitro 6 days showed the same rate of change of TOP expression as cells in vivo during this period of development. Dorsal cell cultures increased TOP per mg of cell protein; those from middle retina were unchanged; and those from ventral retina decreased TOP expresson. TOP synthesis was blocked by 1 µg/ml Actinomycin D and by 2 µg/ml Cycloheximide. TOP was lost from retina cells grown in the presence of these drugs (TOP turnover $T_{1/2}$ in Cycloheximide was 5 hrs and in Actinomycin D was 6 hrs).

Thus, TOP expression is tightly regulated. The TOP gradient is present early in retinal development. Retina cells are determined by 60 hrs postfertilization for their level of TOP expresson and that of their progeny. The pattern of TOP expression in retina during subsequent development depends on cell lineage.

74.11 A MONOCLONAL ANTIBODY DIRECTED AGAINST THE NERVE GROWTH FACTOR RECEPTOR DELINEATES THE BARREL SUBFIELD IN DEVELOPING RAT SOMATOSENSORY CORTEX. Nigel G. F. Cooper, John B. Schweitzer and Dennis A. Steindler. Dept. of Anatomy and Neurobiology, Dept. of Pathology, Univ. of Tennessee, Memphis, TN 38163.

It has previously been shown that the peroxidase conjugates of various lectins recognize glycosylated molecules in the prospective sides and/or septae (in preference to the hollows) of the barrel subfield of mouse somatosensory cortex during a restricted period of time in early postnatal development (Cooper, N.G.F. and Steindler, D.A., J. Comp. Neurol., 249:157, 1986). It has also been shown that the GFAP-positive immature glia are located predominantly within the presumptive septae of the barrel field during the same postnatal period (Cooper, N.G.F. and Steindler, D.A., Brain Res., 380:341, 1986). Both of these patterns of binding are lost in mature somatosensory cortices. We now report that monoclonal antibody 192 (Mab 192), a well-characterized monoclonal antibody that recognizes only the rat nerve growth factor (NGF) receptor, binds to the sides/septae in preference to the hollows of the developing barrel subfield of the rat during a similar developmental period. Postnatal rats, aged P3 to P21 (day of birth = P1), were anesthetized and perfused with standard fixatives. Following removal of the brains, the hemispheres were flattened, 50 um tangential sections were cut and the sections incubated in 5 to 10 ug/ml solutions of Mab 192. They were then developed using standard avidin-biotin horseradish peroxidase methods. Well-delineated barrel subfields showing both large and small barrels were demonstrated by Mab 192 immunohistochemistry on days P5 thru P7, but barrel fields did not appear to be present after the second postnatal week. The barrels detected with Mab 192 occur before the adult neuronal barrel patterns are established. Since the pattern obtained is the same as that obtained with lectins and monoclonal antibody against GFAP, and since the lectin binding pattern has been localized to glial processes ultrastructurally, it appears that radial glia/immature astrocytes express the NGF receptor during development. Our hypothesis is that a population of central glial cells may express the NGF receptor as a mechanism involved in axonal guidance during development. These observations may also be relevant to previous observations on the distribution of nerve growth factor-inducible glycoproteins which reveal patterns related to fiber tract formation during development. This work was supported by USPHS grants EY-02708, NS-20856 and NSF grant BNS-8603593.

74.12 WGA- AND CON A-BINDING GLYCOPROTEINS AND THE PROCESS OF NEURULATION. L.I. Nelson*, Y. Ersahin*, R.G. Higbee, D.G. McLone, and P.A. Knepper. Div. of Neurosurgery, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

The coordination of cellular interactions, e.g., recognition and adhesion, in the process of neurulation requires a combination of intercellular and extracellular events that are under the direction of the genome. Although a variety of glycosylated adhesive molecules have been implicated in neurulation, neither the precise time of expression nor concomitant changes in other classes of glycoproteins are known.

In the present study, chick embryos were studied at 39, 45, 51, and 68 hours of incubation at 37°C (stages 10, 11, 13, and 16, respectively). In order to determine the overall biochemical changes, embryos were fixed in 100% ethanol for 24 hours at 4°C to stabilize the tissues for microdissection of the neuroepithelium. The neuroepithelium was solubilized in lysis buffer for one- and two-dimensional polyacrylamide gel electrophoresis and Western blots using peroxidase-coupled Concanavalin A (recognizes mannose and glucose) and wheat germ agglutinin (recognizes N-acetyl-glucosamine and sialic acid). Proteins were detected by silver staining. Two-dimensional maps indicated that several proteins varied, i.e., appeared, disappeared, or changed in their coordinates during the time frame of 39 to 68 hours: tail, 5 variances, and head, 9 variances, which represented approximately 7% of all observed proteins.

Western blots revealed numerous differences in glycosylation patterns in the four stages of neurulation. WGA-binding protein profiles were: (1) 22 minor and four major glycoproteins with apparent molecular weights of 8, 20, 66, and 185 Kd; and (2) four WGA-binding proteins changed in the developmental process, i.e., a progressive increase in the glycosylation of 8-, 14-, and 170-Kd proteins and a progressive decrease in the glycosylation of a 66-Kd protein. Con A-binding profiles were: (1) 18 minor and 12 major glycoproteins; and (2) three Con A-binding proteins changed, i.e., a progressive decrease in the glycosylation of 24-, 30-, and 97-Kd proteins.

These results indicate that there are demonstrable changes in WGA- and Con A-glycosylation patterns of proteins during the process of neurulation in the developing chick embryo; and (2) these differences in the type or amount of glycoproteins may be involved in the process of normal or abnormal neurulation.

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74.13 SITES OF SEROTONIN UPTAKE AND BINDING PROTEIN IMMUNOREACTIVITY IN THE CULTURED MOUSE EMBRYO: ROLES IN MORPHOGENESIS? J.M. Lauder, H. Tamir** and T. Sadler* Dept. of Anat., Univ. N.C. Sch. Med., Chapel Hill, NC 27514 and Div. Neurosci., N.Y. State Psychiat. Inst., NY, NY 10032 **.

Serotonin (5-HT) and related compounds have been proposed to influence morphogenesis of early embryos, and 5-HT has been localized in the notochord, neural tube, somites and endoderm of the neurulating chick embryo (Wallace, J.A., Am. J. Anat., 165:261, 1982). To investigate this question in a mammalian animal model, sites of 5-HT uptake and synthesis have been examined in mouse embryos cultured in the presence of 5-HT or precursors. Additionally, serotonin binding protein (SBP) has been localized adjacent to these 5-HT sites and the teratogenicity of 5-HT and related compounds has been established. Mouse embryos 9 or 12 days in gestation (E9 or E12) were grown in whole embryo culture. For immunocytochemistry, embryos were cultured for 3-4 hrs in the presence of: 5-HT (10^{-4}, 10^{-5} or 10^{-7}M) + nialamide (N, 10^{-5}M) and L-cysteine (C, 10^{-3}M), 5-HTP (10^{-5}M) + NC, NC alone, C alone or were left untreated. These treatments were repeated in the presence of the specific 5-HT uptake inhibitor fluoxetine (fluox, 10^{-5}M). Embryos were fixed, embedded in paraffin, sectioned and stained with specific antisera to 5-HT or SBP using the ABC peroxidase method. For studies of malformations, E9 embryos were cultured for 24 or 48 hrs in the presence of these same substances, followed by fixation and preparation for SEM or plastic sections. Sites of 5-HT immunoreactivity (IR) were found in the heart and hindgut of E9 embryos. At E12, such sites were found in the epithelia of the head, face, neck (visceral arches) and oral cavity. Sites were also seen in parts of the otocyst and brain. In all cases except the oral cavity, mesenchyme adjacent to these sites exhibited heavy SBP-IR. Moreover, when embryos were cultured in the presence of 5-HT, NC or fluox, malformations of the visceral arches and forbrain were seen which increased in frequency and severity in a dose-dependent manner. Fluox was the most teratogenic in this regard and also caused heart defects. These results suggest that 5-HT may play a role in morphogenesis in the mouse embryo, a process which may be vulnerable to the influence of psychoactive drugs acting through serotonergic mechanisms during critical periods of development.

74.14 **BIOMETRICAL GENETIC ANALYSIS OF A CEREBELLAR FOLIATION PATTERN.** **P. Cooper*, M. Hahn*, J. Hewitt*+, and R. Benno. Dept. of Biol. Wm. Paterson College of N.J. 07470, and +Dept. of Human Genetics, Med. Coll. of Virginia 23298.**

In this study we sought to describe genetic influences on cerebellar foliation patterns in inbred strains of mice. We used a 4 x 4 diallel cross breeding design with the inbred strains DBA/2J, C57BL/10J, Balb/cJ, and SJL/J. This cross generated 16 genetic groups and we used approximately 5 liters per genetic group. Mice were sacrificed on day 42 ± 2 by CO_2 inhalation and weighed. Their brains were removed and weighed to 0.1 mg accuracy. Whole brains were placed in 4% buffered formalin overnight and then cut along the mid-sagittal plane. The right half of the brain was stained with cresyl violet and viewed under a dissecting scope for cerebellar foliation pattern. Two distinct foliation patterns were noted: Type I (Mouth) in which the 3rd primary branch of the arbor vitae was split into two distinct foliate branches by a sulcus and Type II (No Mouth) in which the 3rd primary branch ended in a single cerebellar folia.

Contrary to earlier studies, we found a lack of consistency existed for the phenotypic expression of cerebellar foliation pattern in the homozygotes (i.e. inbred strains) of the diallel cross. Only one strain (DBA) showed a consistent phenotypic foliation pattern (Type I). The other three inbred strains exhibited both Type I and Type II patterns.

Next we investigated the relationship between foliation pattern and brain weight. We suspected that a relationship would exist since we had previously shown that mice selected for low brain weight [the L-line of the Fuller Brain Weight Selection (BWS)] exhibited the Type I pattern. We compared the brain weights of animals that had Type I or Type II foliation patterns and found a significant difference: Type I animals had smaller brains. A point biserial correlation, however, showed that foliation pattern accounted for only 13% of the variance in brain weight.

We next developed a genetic analysis for discrete data (Mouth/No Mouth) based on Hayman's Analysis of Variance procedure for a diallel cross. The results of that analysis indicate: significant additive genetic effects, a significant directional dominance effect, and a maternal effect. The interpretation of these results within the theory of biometrical genetics indicates an evolutionary history of directional selection pressure for the presence of a cerebellar foliation phenotype which includes a mouth pattern at the third branch of the arbor vitae.

In summary, we have shown that cerebellar foliation pattern in mice is highly dependent upon genotype. Further, the Type I pattern has undergone positive selection pressure. We are currently assessing the degree to which genes and the environment interact to influence cerebellar foliation patterns in mice.

74.15 PRE- AND POST-NATAL ONTOGENY OF 5-HT INNERVATION IN THE RAT SPINAL CORD. N. Rajaofetra*, F. Sandillon*, M. Geffard* and A. Privat* (Spon ENA), Neurobiologie du Développemment, INSERM U.249-CNRS L.P.8402, Institut de Biologie, 34060 Montpellier, France, and IBCN, CNRS, Bordeaux, France.

The development of 5-HT innervation in the spinal cord was studied from embryonic day 14 (E14) to adulthood. Sprague-Dawley rats were fixed by perfusion with 5 % glutaraldehyde in phosphate buffer, and vibratome sections were processed for immuncytochemistry with a 5-HT antiserum. For electron microscopy, the section were flat-embedded in araldite, and thin section were performed. 5-HT neurons growing backwards from raphe nuclei invade the spinal cord at E14, and reach the caudalmost levels by E16-E17. In longitudinal sections, axons are seen by E15, at cervical and upper thoracic levels, to invade the presumptive grey matter from the anterior and lateral funiculi. This occured either by sharp angulation of the axon, or by branching of a collateral. By E16, the anterior horn and the intermediolateral columns are profusely innervated by very thin and varicose fibers, and synapses are seen with EM in these two locations, at E17 and E18, involving 5-HT immunoreactive boutons. After birth, 5-HT innervation of these two areas evolves progressively from a diffuse netwotk to a more restricted pattern, especially at the thoracic level for the intermediolateral column, and at cervical and lumbar levels for the anterior horn. The adult pattern is reached by postnatal day 20 (P20). The growth of axons towards the dorsal horn becomes noticeable by E19, when fibers invade the neck of the horn from the lateral funiculus, and innervation proceeds diffusely until P5. At P7, thin fibers course dorsally and laterally along the border of the grey matter, and ramify profusely in layers I and II. The adult pattern is also reached by P20. This progressive organization, contrasting with early innervation, will be discussed in comparison with the maturation of motor and sensory circuitries of the spinal cord. Supported by INSERM, CNRS, and an IRME fellowship to N.R.

74P0 **THE ZEBRAFISH HINDBRAIN IS SEGMENTALLY ORGANIZED.** Bill Trevarrow, Walter K. Metcalfe, Charles B. Kimmel, and Dan Marks Institute of Neuroscience, University of Oregon, Eugene, OR 97403

The evolutionary origin of the vertebrate head, including the brain, is unknown. According to one theory it arose by specialization of rostral body segments; thus the brain represents a rostral extension of the spinal cord. In the zebrafish the myotomes are overtly segmented, and in the spinal cord identified neurons are serially reiterated at intervals corresponding to the length of a myotome. In accord with the above theory we have shown previously that in the hindbrain there are clusters of interneurons, some of which are individually identified, that are also serially repeated. Early in development the repeat length is the same as a trunk segment.

We have now extended the evidence for head segmentation in the zebrafish. We have identified segmental swellings (neuromeres) of the hindbrain at 17 hours post-fertilization (h) using Nomarski optics with living embryos and by SEM with fixed material. At this stage a single cluster of interneurons within each neuromere is labeled with a monoclonal antibody. By 24 h, more neurons are labeled within these clusters, and smaller segmentally repeated clusters of neurons have formed at the boundaries between the neuromeres. At 48 h, staining with other monoclonal antibodies shows a segmental pattern of a variety of other structures in the hindbrain, including fiber tracts, radial glia, neuropil areas, and other neurons. We propose that the organization of these structures is the result of a segmental plan of hindbrain development.

Does segmentation extend to other structures of the head? In more rostral areas of the brain (the midbrain and forebrain) neurons develop as clusters of cells, but not in an obviously segmental manner. We have not yet identified head somitomeres beside the brain, but the seven branchial arches that underlie it are in register with the rostral seven hindbrain segments. Thus, in both the head and body the segmental pattern includes peripheral structures as well as the CNS. Supported by NS17963.

PROCESS OUTGROWTH II

75.1 PUTATIVE GLIA DURING *DROSOPHILA* NEUROGENESIS. J.R. Fredieu*and A.P. Mahowald*. (SPON: H. Gluck) Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ.Sch. of Med., Cleveland, Ohio 44106.

The role of glia during neural development in lending structural and metabolic support, as well as providing guidance cues for advancing growth cones is well documented in the vertebrate literature. Recently, non-neuronal cells have been implicated in serving as guideposts and substrate pathways for pioneer growth cones in the embryonic grasshopper and *Drosophila* nervous systems. During a screen of monoclonal antibodies (kindly provided by Dr. Seymour Benzer) raised against adult *Drosophila* heads, Mab 5B12 was found to recognize two subsets of cells which do not label with antibodies to horse radish peroxidase: one set is located in the ventral midline just dorsal to the central neuropil; the second set is located lateral to the CNS in each hemi-segment and project laterally in a pattern coincident with the peripheral nervous systems.

Whole embryos (2-21hrs) were dechorionated in 50% chlorox, permeabilized in heptane, and fixed in 10% formalin or 4% paraformaldehyde. Prepared embryos were then incubated in antibody overnight at 4°C, washed in PBS, and incubated in secondary antibody conjugated to FITC, RITC, or HRP.

Cells, positive for Mab 5B12, are detectable in the embryo beginning at about 10-11hrs of development within the ventral midline of each segment dorsal to the neuropil. Later, beginning anteriorly, clusters of cells lateral to the CNS in each hemi-segment stain positive for Mab 5B12. As development proceeds, these lateral cells extend processes into the periphery and elaborate a pattern coincident with the developing PNS. Simultaneous incubations of late embryos in Mab 5B12 and rabbit anti-HRP reveal a close association of the peripheral nervous system with these "glial" processes, in particular, the anterior and posterior fascicles of each segmental nerve.

Dissociated cells from gastrula stage embryos plated on glass coverslips in appropriate media will differentiate into a variety of cell types including neurons. A sub-population of cells in culture is visualized with Mab 5B12. Presently, experiments are underway to investigate lineage relationships, as well as, selective affinities of neuronal cell-types for Mab 5B12 positive cells both of which may reveal a cooperation between neuronal and non-neuronal cells during development.

75.2 THE BRANCHING PATTERN OF A LEECH MOTONEURON DEPENDS UPON ITS INNERVATION OF THE NORMAL OR AN ECTOPICALLY-LOCATED TARGET ORGAN. C.A. Baptista* and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

The bilateral pair of rostral penile evertor (RPE) motoneurons are found in the sixth segmental ganglion (SG6) in the leech *Hirudo medicinalis* (B. Zipser, *J. Neurophysiol.* 42:455, 1979). In the adult, each cell projects its principal process to the contralateral side of SG6 where it bifurcates, one branch exiting the ganglion along the anterior nerve root and the other exiting along the anterior interganglionic connective. The latter branch travels to SG5 and exits along its anterior nerve root. Both root branches then innervate the male genitalia, which are located between SG5 and SG6. Dye-filling of the RPE neurons at early developmental stages reveals, however, the existence of additional extraganglionic projections (e.g., posteriorly towards SG7, beyond SG5 towards SG4, and in the posterior nerve root of SG6). Most of these have almost entirely disappeared by 50d (embryogenesis is completed by 30d), when the adult morphology of the RPE neurons is evident. Thus, several projections disappear over the course of differentiation of these motoneurons, as is the case for several other leech neurons, and those projections that remain are those that do innervate the target organ.

To test for the possible involvement of the target organ in the determination of the adult pattern of axonal projections of the RPE neurons, we first ablated the male genitalia at 10d of development, when branches of the RPE neurons are just beginning to innervate the organ. When examined at 50d, the RPE cells in the experimental animals showed not only the usual projections and those normally lost, but also a profusion of additional ones. These novel projections extended many segments anteriorly or posteriorly and erratically to the body wall and back into the nervous system, and even, in a few cases, appeared to innervate the female genitalia between SG6 and SG7. Ablation of the female genitalia, or prevention of the normal innervation of the male organ via one of the two normal routes, for example, did not elicit the extensive changes in morphology that followed ablation of the normal target.

As a second test, male genitalia were transplanted at 10d of development to ectopic, more posterior locations in hosts that had also had their normal male genitalia ablated. More than half of the RPE cells examined at 50d in these experiments had been able to exit from more posterior ganglia (SG7 or SG8) and innervate the ectopic organ. In these cases, only the unusual projections innervating the target were of large caliber, while the few remaining projections in other directions, including those that would normally innervate the target, were much thinner and appeared to be atrophying or retracting.

These observations suggest to us that the RPE neurons are initially programmed to extend multiple branches and to grow out and explore many pathways in order to find their target. Once some branches encounter the target, an interaction with the target triggers a signal that causes the inhibition of growth and atrophy of other processes of the neuron which have failed to find the target and the maintained growth and increase in diameter of the successful ones. Lacking a target, growth continues in an unchecked manner, but encountering an ectopically located target leads to the retention of novel projections and the loss of the normal ones. (Supported in part by NIH Grant NS-20336.)

75.3 SELECTIVE NEURITE RETRACTION BY SOME LEECH NEURONS CAN BE PREVENTED BY CUTTING GANGLIONIC NERVE ROOTS EARLY IN DEVELOPMENT. W.-Q. Gao* and E.R. Macagno (SPON: D. Hood). Dept. of Biological Sciences, Columbia University, New York, NY 10027.

Our previous studies (*J. Neurobiol.* 18:295, 1987) showed that, from 10 to 18 d of embryogenesis, AP and AE neurons in the leech CNS have lateral projections to the periphery through two contralateral nerve roots as well as longitudinal projections toward adjacent ganglia along the interganglionic connective nerves. While the lateral peripheral projections are maintained throughout the life of the animal, the longitudinal projections gradually atrophy and disappear before the end of embryogenesis (30 days). Ablation of AP or AE neurons in individual segmental ganglia at about 10d of development resulted in the retention of the longitudinal projections of adjacent homologues, suggesting that homologues play a role in the normal loss of longitudinal projections.

To begin to assess whether competition for peripheral targets or a direct inhibitory interaction among homologous neurons might be responsible for the loss of early projections, we cut the nerve roots in midbody ganglia at various times in development, thus severing the direct early projections of neurons to peripheral targets in the same segment. The pattern of projections of these neurons was then observed by injecting them with dye in 30d or older animals.

In those cases in which nerve roots were cut before AP or AE longitudinal projections begin to atrophy (i.e., before 18 days), the affected neurons retained and expanded their longitudinal projections to and sometimes even beyond adjacent ganglia, irrespective of whether they succeeded or failed to re-establish the severed projections along the regenerated nerve roots, while all neighboring homologues displayed normal axonal projection patterns. The rescued longitudinal processes were generally found to branch and project to the periphery in adjacent ganglia, following closely in the nerve roots the projections of the local homologous neuron. Preliminary physiological and dye-fill studies of AE neurons in adult experimental animals show that these abnormal peripheral projections make funcional connections to peripheral targets. In those cases in which nerve roots were cut after the longitudinal processes had atrophied (i.e., after 30d), however, no longitudinal processes were found. The severed nerve root projections were re-established in most of these cases. These observations suggest that atrophied projections do not have the capacity to regenerate, even when the neuron is completely disconnected from its peripheral targets. In control experiments, neither severing longitudinal projections before atrophy nor non-specific damage to the nervous system appeared to affect the final axonal branching patterns of AP or AE neurons.

These results show that neuronal processes which are normally fated to atrophy can be rescued by early disconnection to peripheral targets and suggest that a direct interaction among homologues is not sufficient to explain the loss of initial projections by these leech neurons.

(Supported in part by NIH Grant NS-20336.)

75.4 AGE-DEPENDENT CHANGES IN THE DENDRITIC MORPHOLOGY OF RAT SYMPATHETIC NEURONS IN VITRO. [1]D.A. Bruckenstein*, [2]M.I. Johnson, and [1]D. Higgins. [1]Dept. of Pharm &Ther., State Univ. of N.Y., Buffalo, NY 14214 and [2]Dept. of Anat., Washington Univ., St. Louis, MO 63110

Previous studies have shown that neurons from the embryonic rat superior cervical ganglion fail to form dendrites when they are cultured in serum-free medium in the absence of nonneuronal cells (Trans. Amer. Soc. Neurochem. 17:209). To determine whether there might be developmental changes in the morphological capacities of sympathetic neurons, we compared the ability of embryonic (20-21 day) and postnatal (1-2 months) sympathetic neurons to form dendrites under the same culture conditions. Dendrites were distinguished from axons by light microscopic criteria after the intracellular injection of Lucifer Yellow, by differential immunostaining with an antibody to the nonphosphorylated forms of the M and H neurofilament subunits, and by the autoradiographic localization of RNA within dendrites.

When maintained in the absence of nonneuronal cells, most neurons (>75%) derived from postnatal ganglia were multipolar and extended both axons and dendrites. Most often the dendritic morphology of postnatal neurons was relatively simple with cells commonly having from 2 to 6 short (50-200 micron), relatively unbranched dendrites. In contrast, most (>80%) embryonic neurons were unipolar and had only a single axon. These data indicate that postnatal sympathetic neurons that had well-established arbors in situ have the capacity to regenerate dendrites when grown in dissociated cell culture in the absence of nonneuronal cells; in contrast, embryonic neurons which had only primitive arbors at the time of dissociation are unable to extend dendrites under these conditions.

To determine whether similar changes in regenerative capacity might occur with aging in vitro, ganglia were removed from embryonic rats and grown as explants for 3 weeks in the presence of nonneuronal cells; under these conditions, embryonic neurons within the explant became multipolar. Moreover, most neurons obtained from such aged explants extended dendrites when they were subsequently maintained in dissociated cell culture. Thus, the acquisition of the capacity to form dendrites in dissociated cell culture is not dependent upon normal afferent input or upon contact with the target tissue. (Supported by NS 22126 and GM 07145)

75.5 LONG TERM SURVIVAL OF RAT HIPPOCAMPAL NEURONS AT LOW DENSITY: ADVANTAGES OF LOW OXYGEN. G. J. Brewer*, C. Peterson and C. W. Cotman. Dept. Psychobiology, Univ. California, Irvine, CA 92717.

The study of the development and plasticity of hippocampal circuitry would greatly benefit from methods which allow the long term culture of neurons at low density under precisely defined culture conditions. Defined media supports culture of neurons at high density but not low density; serum-containing medium is inconsistent and associated with massive glial proliferation. We report that isolated hippocampal neurons from embryonic day 18 rats can be cultured for several weeks at densities low enough to allow identification of individual connections when maintained in an environment of reduced oxygen. Neurons develop in vivo at subatmospheric concentrations of oxygen with physiological anti-oxidants in a close-pack environment that reduces diffusion of small molecules. To simulate these conditions, a serum-free medium with added anti-oxidants was studied. Improved survival and neuritogenesis relative to that seen in serum-based cultures suggested a toxic role of oxygen. This chemically defined medium, derived from the formulation of Romijn, omits ascorbate, but includes vitamin E, glutathione, pyruvate, catalase and superoxide dismutase as antioxidants. To directly test the effect of reduced oxygen, cultures incubated in 9% oxygen were compared to those in the normal 19.7% oxygen (95% air). Cultures in low oxygen were superior to those in normal oxygen. Further improvement in neuronal survival was produced by covering the cells with a glass cover slip an hour after plating. Neuronal survival relative to uncovered cultures increased from 40% to 90% at 5 days, with processes on nearly 80% of the cells. Covered cells exhibited a ring of neuronal growth which did not extend to the center or the edge of the cover slip. In 9% oxygen the outer ring of growth was closer to the edge of the cover slip than in normal oxygen. The cover slip did not provide an additional substrate for attachment since removal of the slip left the neurons attached to the original substrate. However, this removal resulted in cell death within 24-48 hours suggesting exposure to a toxic factor. Although variations in glial cell content (<10%), pH, and pCO_2 were unlikely explanations of the high survival, a role for autocrine factors is still suggested by the density dependence of survival below 10,000 cells/cm^2. These results suggest that growth in a diffusion-limited space, reduction of the oxygen concentration to physiological levels and control of toxic oxidation with physiological antioxidants can improve the survival and neuritogenesis of isolated hippocampal neurons in primary culture making these cultures potentially useful for studies in neuronal connectivity.

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75.6 REGENERATING AXONS REESTABLISH NORMAL SECTORAL ORDER IN THE OPTIC BRACHIA OF GOLDFISH. R. Bernhardt and S.S.Easter, Jr. The University of Michigan, Ann Arbor, MI 48109.

The positions of retinal axons in the cross section of the normal optic pathway of goldfish reflect their retinal origins, defined as r, the radial distance of the parent somata from the center of the retina, and θ, the sectoral (or clock face) position (Bernhardt and Easter, J. Comp. Neurol. 254: 493, 1986). We have now examined sectoral order in experimental pathways regenerated after optic nerve crush.

In fish that had survived for 2-4 months after nerve crush HRP was applied to a stab wound in the retina, on either the nasal, temporal, dorsal, or ventral radius. This application labels a narrow sector of ganglion cells, extending from the lesion site to the retinal periphery, and their axons. The axons were traced in serial cross sections of the pathway.

On the retinal side of the crush axons of common θ (a "θ-group") were clustered. On the tectal side of the crush, in the regenerated portion of the nerve, they were scattered, occupying 75-88% of the total cross sectional area. Comparable θ-groups in control nerves remained clustered and occupied approximately 50% of the cross sectional area. Regenerated axons tended to reaggregate at the nerve/tract junction. More downstream in the tract they were often arranged in a criss-cross pattern, indicative of changes in their neighbor-neighbor relationships. In the normal pathway such changes lead to the segregation of axons of dorsal and ventral hemiretinal origins into the ventral and dorsal brachia of the tract, respectively (Scholes, Nature 278: 620, 1979; Springer and Mednick, J. Comp. Neurol. 247: 233, 1986). In agreement with previous studies (Attardi and Sperry, Exp. Neurol. 7: 46, 1963; Stuermer and Easter, J. Comp. Neurol. 223: 57, 1984) we find that the brachial choice is reestablished by regenerating axons. Moreover, we find that within the brachia regenerated axons of nasal and temporal retinal origins occupy different subregions, as in the normal. The visual impression of reordering was confirmed by counting individual labeled axons and calculating their densities as a function of position within the cross sectioned dorsal brachium. While the regenerated order was less precise than normal, the distribution peaks of nasal and temporal axons did not overlap. Regenerated axons exited the brachia and stratum opticum in a normal sequence; temporal ones rostrally, nasal ones more caudally. Regenerating axons have no predecessors to rely on for guidance. Our results suggest that they might have access to θ-specific cues in the non-axonal environment of the tract and its brachia. (Supported by EY-00168 to SSE.)

75.7 **DETECTION OF AREAS OF NEURITE EXCLUSION IN CULTURES OF RAT FETAL CORTEX.** R. D. Todd (SPONSOR: S. Guze) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

The mechanisms by which cortical neurons migrate to their final brain locations and extend neurites to their targets are complex and incompletely understood. Differential cell adhesion has been suggested as one mechanism for cell and neurite guidance. This theory proposes that the direction of cell migration or growth cone elongation can be altered by substrate adhesiveness. Chemotactic factors, both attractive and repellant, have also been proposed as mechanisms for cell and neurite guidance. In this case, cells or growth cones travel along gradients of diffusing molecules. We have detected transiently present structures in primary cell cultures which seem to exclude or inhibit growing neurites and may represent the presence of a differentially adhesive substrate for cell and growth cone motility or the presence of a repellant chemotactic factor.

Frontal cortices from E15 to E23 fetal rats were dissected, triturated, and plated onto poly-lysine coated dishes as single cell suspensions. Within one or two days of plating, rare structures were observed which consisted of a central cell (or cells) surrounded by an area free of neurites and enclosed by a border or corral of neurites. The structures were from 20 to 60 microns in diameter. The central cell or cells excluded trypan blue. From day E15 to about E17.5 there was an exponential increase in the number of these structures from about 5 to about 200 per million viable plated cells. After about day E17.5 there was a dramatic decrease in these structures to less than 5 per million viable cells on day E19. Similar structures were observed on collagen coated dishes and in cultures of hippocampal cells. Preliminary electrophysiological and antibody staining studies suggest these structures contain viable non-neuronal cells. The properties and time course of the presence of these structures are consistent with their involvement during development as negative regulators of cell and growth cone movement.

75.8 GROWTH OF CORTICOSPINAL AXONS THROUGH A GLIAL DEFICIENT ENVIRONMENT. M. A. Pippenger*, T. J. Sims, and S. A. Gilmore. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The major corticospinal tract in the rat, located at the base of the dorsal funiculus, does not grow into the lumbosacral region of the spinal cord until the latter part of the first postnatal week. The growth of these axons into and through the lumbosacral region was studied in normal rats and in rats in which a 5 mm length of the lumbosacral spinal cord was exposed to x-rays on the third postnatal day. The irradiated rat model was used since the exposure to x-rays markedly decreases the number of glia and, thus, alters the milieu through which the developing corticospinal axons must grow.

The developing corticospinal axons were studied by horseradish peroxidase (HRP) tracing techniques. HRP chips, dried from a saline solution containing 50% HRP and 4% Nonidet P-40, were placed on the pial surface overlying the cerebral cortex of two groups of rats on postnatal day 1, 2, 3, 4, 5 and 8. One group consisted of normal rats and the other of rats irradiated (4000 R) over the lumbosacral spinal cord on postnatal day 3. Two days following HRP application the rats were killed by perfusion and the spinal cords were removed, sectioned, and processed by the tetramethyl benzidine (TMB) procedure.

Anterograde filling of growing tips (growth cones) was readily observed on all days studied. That the HRP-filled structures were in fact growth cones was supported by observations made in spinal cords stained by the Golgi method during the first postnatal week. By 3 days postnatally (time of irradiation) growth cones were present in the high thoracic levels but not in the lumbosacral region. Growth cones were not observed in the latter region until day 5, and by day 7 corticospinal growth cones were present at all levels. These observations were consistent for both normal and irradiated spinal cord. Thus, despite the marked, radiation-induced loss of glia (especially astrocytes) from the region to be occupied by the ingrowing corticospinal axons, the development of the tract appeared to be normal with respect to location and to time of arrival of the axons in the lumbosacral region. Supported by NIH grant NS-04761.

75.9 CULTURED SQUID NEURONS EXTEND NEURITIC PROCESSES. T.D. Parsons and R.H. Chow*. Dept. of Physiol., Univ. of Penn., Phila, PA 19104 and Marine Biological Laboratory, Woods Hole, MA 02543.

The squid giant axon arises from the fusion of hundreds of smaller axons. These smaller axons originate from cell bodies located in the giant fiber lobe (GFL) of the stellate ganglion. Using an isolated GFL neuron preparation (Loligo pealei) developed by Llano and Bookman (1986), we have begun to study the extension of processes from these cells. GFL neurons obtained from healthy squid reliably produced neuritic processes in culture. The processes were of two types -- a sheet-like veil or thin branching neurites. The latter type was often associated with bulbous expansions at the termini, at branch points, or at sites of contact among branches from two different cells. On poly-L-lysine substrate, veiling usually occurred by the fourth or fifth day in culture, while the branching processes appeared by one week to ten days. The elaboration of the outgrowths by the GFL neurons was influenced by the culture substrate and incubation temperature. This culture system in which isolated somata develop neurites over several weeks may permit the study of spatial and temporal expression of ionic conductances.

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75.10 SOME GROWTH CHARACTERISTICS OF AXONS IN A RE-GROWING OPTIC TRACT IN NEONATAL HAMSTERS. L.S. Carman*, S. Jhaveri* and G.E. Schneider. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

In Syrian hamsters, retinotectal axons transected on or before postnatal day 3 (P3; P0 = day of birth) will grow across the lesion site and reinnervate their normal tectal target (So et al., '81). However, if their normal tectal target is ablated, they grow across the necrotic tissue, cross the midline, and terminate in the opposite tectum (Schneider, '73). If the eye ipsilateral to the ablated tectum is enucleated, this recrossing projection expands across the entire surface of the undamaged tectum. This study examines growth characteristics of recrossing axons during the period immediately following the lesions.

Combined lesions--ablation of right superior colliculus (rSC) and enucleation of the right eye (rEE)--were made in 22 Syrian hamsters on P1. The left eye was injected with horseradish peroxidase (HRP) either 4 hr or 1-2 days after rSCrEE lesions. In some cases, the left optic tract was transected at the time of HRP injection to ensure that we were observing only recrossing axons in the left SC. Hamsters were perfused 20 hr after eye injections, and brain sections were processed for visualization of the HRP. Age of the animal refers to the time of perfusion.

By P2 (24 hr after rSCrEE), regrowing axons have crossed the necrotic right SC and have reached or crossed the midline. The longest pioneers at this age extend 260 μm (mean for 3 cases) from the midline, and 1260 μm from the center of the rostral edge of the lesion. By P3, pioneers extend 620 μm (mean for 7 cases) into the intact tectum, and by P4 pioneers extend 850 μm (mean for 12 cases). These data indicate that recrossing axons are not growing at a uniform rate. In the initial 24 hr after surgery, they grow at a minimum estimated rate of 25 μm/hr, but may advance at an estimated 60 μm/hr (depending on exact route of single axons). These rates are conservative estimates, since their calculation assumes that axons begin regrowing immediately after injury, and that the rate is uniform from lesion edge to the midline. After P2, growth rate slows to approximately 10 μm/hr. This shift in growth rate occurs by the time the axons cross the tectal midline.

Regrowing axons typically cross the damaged rSC in two fasciculated bundles: a ventral bundle grows below or through the dead tissue, and a dorsal bundle follows a tissue bridge above a fluid-filled cavity. After crossing the midline, axons appear to defasciculate, forming a 'spray' of extending axons. These recrossing axons are oriented mediolaterally and grow within the superficial gray layer; they are not restricted to the optic fiber layer as in normal animals.

Normal retinotectal axons in embryonic hamsters grow at 80 μm/hr and form dense fascicles, extending to the caudal tectal margin (Jhaveri et al., '83). By P0, they initiate a slower, non-fasciculated growth of collateral and terminal branches. Thus, left tectal tissue in the early lesion cases, which normally supports arborizing axons by P2, may provide some signal to alter growth characteristics. (Supported by NIH grants EY 00126, EY 05504, and EY 02621, and by a Poitras predoctoral fellowship.)

75.11 DRG NEURITE EXTENSION IN THE ABSENCE OF EXOGENOUS GROWTH FACTORS. E.D. Pollack and V. Liebig*. Inst. Study Develop. Disabil. and Dept. Biol. Sci., Univ. Ill. at Chicago, Chicago, IL 60608

In contrast to the generally held assumption that neurons of dorsal root ganglia do not extend substantial neurites in vitro in the absence of exogenous NGF or appropriate target tissue products, we find that the DRG of frog tadpoles do not have these requirements when grown in defined medium on a polylysine surface. Whole lumbosacral DRG of larval Rana pipiens at stages V, IX, XI and XV were explanted individually onto poly-DL-lysine coated coverglasses in Sykes-Moore chambers with defined medium lacking NGF. Although nearly 100% of the explants exhibited outgrowth, stage V DRGs had relatively short neurites, while those from stage IX were of greater average length. Quite clearly, DRGs from stage XI tadpoles exhibited maximal outgrowth with extraordinarily elaborate neuritic arrays often by 48 hours. Extensive neuritic outgrowth was a characteristic of 100% of the stage XI explants by 4 days in vitro. Even at two weeks in vitro, the growth cones remained highly active, although they tended to be confined within the dense meshwork of neurites in the outgrowth zone. Individual neurites often exhibited regularly spaced collaterals, or anchors, to either the surface or adjacent neurites giving a "step-ladder" appearance. For each stage, there seems to be a limit to the outgrowth zone, beyond which neurites do not extend, but instead turn back toward the explant or wander tortuously within this zone. Slightly older stage XV DRGs had neuritic growth that was decremental to that of stage XI. In contrast to this NGF-independent growth on polylysine, DRGs placed on collagen (type I) fail to extend neurites unless exogenous NGF is provided or the explant is co-cultured with a developmentally appropriate target tissue. As the stage-responsive DRGs on polylysine are long-lived in the absence of NGF, we suggest that an appropriate substratum can subserve the function of NGF in vitro. It is possible that the extended neurites in these cultures are from an NGF-independent neuron population, while NGF-dependent neurites are not observed. However, we then would have expected to see neurites extended on collagen as well. Similarly, if the explant itself were the source of NGF then outgrowth should occur on collagen also. This finding points to the need for increased attention to the relative roles for growth factors and attachment surfaces in understanding nerve fiber growth phenomena in vitro.

75.12 **DOPAMINE AND NICOTINE INHIBIT GROWTH CONE ACTIVITY AND NEURITE OUTGROWTH IN TWO DIFFERENT MORPHOLOGICAL CLASSES OF CHICK RETINA NEURONS.** **K.L. Lankford, M.I. Fonseca* & W.L. Klein Dept. Neurobiology & Physiology, Northwestern University, Evanston, IL 60201**

Increasing evidence suggests that, in addition to their role in synaptic communication, neurotransmitters may also play a role in the morphological differentiation of neurons (Barnes 1986, Science 234: 1324-1326). In a previous report, we described a dopamine stimulated inhibition of growth cone activity and neurite outgrowth in approximately 25% of cultured embryonic chick retina neurons (Lankford et al 1987 Neurosci. Lett. 75: 169-174). In this report we further characterize the morphological response to dopamine and describe a population of neurons in the retina that responds in a similar manner to cholinergic agonists. Neurons were cultured on glass coverslips, inserted into a Dvorak-Stotler chamber and monitored before, during and after exposure to dopamine (1uM), carbachol (1mM), or nicotine (100 uM) with continuous VEC-DIC microscopy. Morphological characteristics of the neurons and changes in neurite length, filopodial length, and filopodial movement were assessed from photographs of recorded images. Dopamine sensitive neurons were characteristically monopolar or bipolar, with narrow growth cones and short, thin filopodia. A great variability was observed in the magnitudes of the dopamine responses in different cells. Neurite lengths were reduced 7-85%, and total filopodial lengths were reduced 14-70% within 10 minutes after dopamine addition. Nicotine and carbachol sensitive neurons were multipolar and had larger cell bodies, longer neurites, more well defined growth cones, and more robust filopodia than dopamine sensitive neurons. Sensitive neurons responded to nicotine or carbachol with a strong inhibition of filopodial activity, and in some cases, a retraction of filopodia or neurites. Cell morphologies and the detection of a cholinergic response in neurites extending from explants suggest that cholinergic agonist sensitive neurons may be ganglion cells. Preliminary results indicate that nicotine responsive cells are not inhibited by dopamine, and dopamine responsive cells are not inhibited by nicotine. The data presented here suggest that both dopamine and nicotine inhibit neurite outgrowth in different populations of embryonic chick retina neurons.

75.13 AN ULTRASTRUCTURAL STUDY OF AXONAL DIFFERENTIATION IN HIPPOCAMPAL NEURONS IN CULTURE. J.S. Deitch and G.A. Banker (SPON: F.A. Haun). Department of Anatomy, Albany Medical College, Albany, NY 12208

Hippocampal neurons elaborate two morphologically and molecularly distinct classes of processes, axons and dendrites, which arise by a stereotypical sequence of developmental events (Dotti and Banker, Soc. Neuro., this volume). Initially the neurons extend several short processes that are morphologically indistinguishable from each other. Several hours later one of these processes undergoes a rapid increase in growth rate. This process becomes the axon; the remaining short processes subsequently become dendrites. We have examined the ultrastructural changes that occur during the course of these events.

Hippocampal neurons, dissociated from 18-day-old fetal rat brains, were plated onto polylysine-treated Aclar coverslips. After 1-2 days in vitro, shortly after axons first appear, the cultures were fixed in a glutaraldehyde-osmium tetroxide solution and embedded in Maraglas. Individual neurons were selected and photographed by phase-contrast microscopy to identify the axon and presumptive dendrites. The cells were then sectioned parallel to the substrate, so that all of their processes could be examined in a few sections.

In mature hippocampal neurons in culture, ribosomes are restricted to the soma and dendrites. After 1-2 days in vitro polyribosomes were present throughout the somatic cytoplasm and in the short presumptive dendrites, their density decreasing with distance from the soma. Polyribosomes were found along the axon as well, but were present at a much lower density, and found mostly in small clusters near the axonal periphery. The growth cones of the two types of processes also differed in two important respects. For one, the body of axonal growth cones contained an extensive reticulum of elongated membranous elements; this was a minor component of the dendritic growth cones. Furthermore, a number of axonal growth cones contained large, organized bundles of microtubules; often these looped back toward the cell body. Single scattered microtubules were more common in the growth cones of presumptive dendrites.

The greater amount of membrane and distinctive microtubule organization in the axonal growth cone may well be associated with the more rapid growth rate of the axon. The presence of small clusters of polyribosomes in the axon may represent a dilution of ribosome content, without replenishment, during the conversion of one of the short, ribosome-rich processes to the axon.

(This research was supported by NIH grant NS17112.)

75.14 THE DEVELOPMENT OF POLARITY BY HIPPOCAMPAL NEURONS IN CULTURE. C. G. Dotti* and G. A. Banker. Department of Anatomy, Albany Medical College, Albany, NY 12208

During the first week in culture hippocampal neurons establish distinct axonal and dendritic processes that differ in shape, in molecular constituents, and in synaptic polarity. By sequential photography and time-lapse video recording of individual cells, we traced the sequence of morphological changes by which this polarization is established.

Hippocampal cells were obtained from the brains of 18 d fetal rats and plated onto polylysine coated coverslips. The cells first established several short processes, about 15um in length, which were tipped with growth cones. These processes were initially indistinguishable from one another in appearance or behavior, but after several hours one of them began to grow much more rapidly than the others. It became the axon. Significant elongation of the remaining processes began a few days later; they became the cell's dendrites. In a few instances two processes exhibited the rapid growth characteristic of axons, but only one maintained this growth; the other retracted and subsequently became a dendrite.

These results suggest that, when neuronal processes first arise, each may be equally capable of forming either an axon or a dendrite. To test this possibility, we cut the axons of young hippocampal neurons during the first day after the axon developed (n=122). Over 80% of the cells survived for at least a day following surgery. In about 30% of these cases, the original axon regenerated. In the remainder, one of the other processes, which if undisturbed would have become a dendrite, instead became the axon. Frequently the stub of the original axon persisted following the transection; if so, it subsequently became one of the cell's dendrites. In some cases cells were maintained for an additional 5 days and the respecification of the cell's processes was confirmed by differential distribution of Microtubule-Associated Protein 2.

These results show that the processes of hippocampal neurons are not rigidly determined when they first develop, but have the capacity to form either axons or dendrites. The acquisition of axonal characteristics by one neuronal process apparently prevents the others from becoming axons; they subsequently become dendrites.

(Supported by NIH Grant NS17112; C.D. was supported by a Fogarty International Fellowship.)

75.15 **THREE-DIMENSIONAL GROWTH AND DIFFERENTIATION OF SINGLE HIPPOCAMPAL NEURONS IN HYDRATED COLLAGEN LATTICES.** P.W. Coates. Dept. Cell Biol. & Anat., Texas Tech Univ. Sch. Med. Lubbock, TX 79430.

When neurons from cerebral hemispheres, hypothalamus or spinal cord are cultured on three-dimensional substrates that simulate extracellular matrix and consist of hydrated native collagen lattices (3-D HCL), they quickly express - as single cells - features that characterize neurons including morphologically identifiable axons and dendrites that grow into the matrix (Coates, Dev.Br.Res.25:11,1986; SN Abs.12:1504,1986; SN Abs.11:762,1985). To determine whether individual neurons from the hippocampus could also regenerate distinct neuronal features when cultured in such systems, dissociated cells from late gestation fetal rat hippocampi were cultured at low cell density using three different 3-D HCL preparative methods: 1) a "sandwich" in which neurons were plated on a polymerized matrix and covered with another layer of matrix; 2) incorporation of neurons into the matrix before polymerization and 3) plating neurons on a polymerized matrix alone. Within hours, single hippocampal neurons grew axons and dendrites that coursed three-dimensionally within all matrices. However, it was technically difficult to satisfactorily observe, photograph and measure axons and dendrites in the first two methods. Therefore, quantitative and qualitative analysis was performed on single hippocampal neurons grown using the third method. Microscopy revealed many well-differentiated long and short axon neurons. Axons typically grew straight and originated from the soma or a basal dendrite; some were branched and/or displayed varicosities. Growth cones were prominent. Apical and basal dendrites exhibited first order branching. An image analysis system coupled to an inverted phase contrast microscope and microcomputer was used to measure indices of growth and differentiation of single hippocampal neurons. Within 3 days after plating, mean axon length was over 500 um/neuron, mean length of dendrites/neuron was about 200 um and the average total length of all processes combined was 700 um/neuron. Other indices also increased during the test period. These data show that when cultured in 3-D HCL systems, single hippocampal neurons grow rapidly and are capable of expressing an inherent plan for characteristic neuronal morphology that does not require physical contact with other neurons, glia or other non-neuronal cells. (Support from The Salk Institute-Texas Research Foundation and NIH NS20802.)

75.16 CLUSTERS OF DENSE-CORE VESICLES BEHAVE AS AUTONOMOUS BODIES WITHIN THE GROWTH CONES OF APLYSIA NEURONS IN VITRO. M. Chen, D.W. Burmeister, C.H. Bailey and D.J. Goldberg. Ctr. for Neurobiology and Behavior and Depts. of Pharmacology, Anatomy & Cell Biology, and Psychiatry, Columbia U. Coll. of P. & S. and N.Y.S. Psychiat. Inst., N.Y., NY 10032.

Growth cones of the Aplysia neurons B1 and B2, grown in culture and viewed with video enhanced contrast - differential interference contrast microscopy, display a previously undescribed structure. This large, irregular, refractile body (LIRB) is an insular structure that seemingly floats in the cytoplasm of the central, vesicle rich region of the growth cone and is distinct from both lysosomes and lipid droplets. Video observations revealed that LIRBs were present within minutes of the first formation of growth cones from freshly plated neurons and were seen in growth cones of neurons in culture for as long as 6 days. The formation of most LIRBs occured through the agglomeration of smaller refractile structures at the periphery of the growth cone into larger LIRBs, with both vesicles and mitochondria apparently fusing with them. The LIRBs in turn tended to coalesce with both other LIRBs and with a broader band of similar appearing material frequently seen at the proximal end of the growth cone. As the proximal region of the growth cone matured, there was a tendency for the newly forming neurite to "squeeze" the LIRBs and associated materials forward so that individual LIRBs advanced with the growth cone.

Complete electron microscopic reconstructions from serial thin sections of individual identified growth cones demonstrated a one-to-one correspondence of the LIRBs with dense accumulations of membranous organelles. The LIRBs contained predominately dense-core vesicles similar to synaptic vesicles previously identified in these cells, interspersed with mitochondria and occasional profiles of smooth ER. There was no delimiting membrane to the structure, but it was characterized by the absence of the mesh-work of cytoplasmic ground substance seen in the other regions of the growth cone. The same LIRB characteristics were seen within small LIRBs on the periphery of the growth cone and the large mass of LIRB-like material in the central proximal regions of the growth cone. The appearence of this material changed, however, in the region where the growth cone becomes neurite. Here aligned cytoskeletal elements appeared to break up the material, and the intravesiclar distance in the remaining clumps was reduced.

Although the function and significance of the LIRB is as yet unclear, its persistence over time argues that it is not simply a reaction to axotomy, and its ability to segregate many membranous organelles could be important in the overall membrane economy of the growing axon tip.

75.17 **Patterning the Location of Dissociated Cerebellar Cells on Artificial Substrates** D. Kleinfeld* & P.E. Hockberger (SPON: B. Friedman) Dept. of Molec. Biophys., AT&T Bell Laboratories, Murray Hill, NJ 07974.

We present a method for patterning the location of cells cultured on two-dimensional substrates. The method uses a combination of surface chemistry and photolithographic techniques. The adhesive properties of either silicon or silicon dioxide (quartz) surfaces were controlled by covalently linking small organic molecules to the surface with silane coupling agents. Patterns of selected adhesivity were formed using standard photochemical resist materials and lithographic masking techniques.

Dissociated cerebellar cells from perinatal rats were cultured on chemically patterned substrates. Surfaces linked with ethylenediamine, via a propyl spacer group, were used to form regions that promoted cell adhesion. The attachment of cells was inhibited by linking alkane chains, such as tetradecane, to the surface and plating the cells in media containing 5 % to 10 % (v/v) serum.

The gross morphology, immunoreactivity (GFAP and NSE) and electrical activity of cells cultured on patterned substrates was similar to that of cells cultured on conventional substrates, *i.e.*, poly-*D*-lysine coated glass. Neurons and their processes could be confined to grow on lines with a width smaller than 10 μm. This width is comparable with the diameter of the smallest cells in the cerebellum, *i.e.*, granule neurons. The patterned growth of the neurons was maintained up to 10 days *in vitro*. This time is consistent with the development of electrical excitability of the neurons in these cultures.

The results of this work suggest that surface modification techniques may be useful for controlling and studying the guidance of neural processes. These techniques may also be useful for studying the formation of synapses in ordered neural structures.

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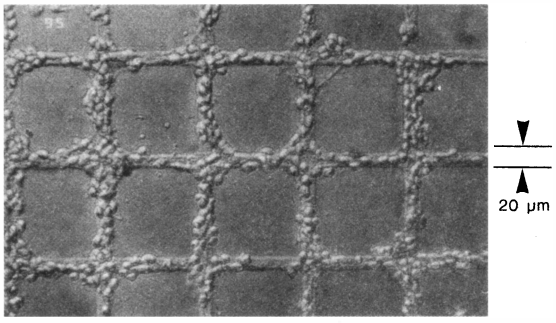

75.18 MODULATION OF NEURITIC EXTENSION OF IDENTIFIED LEECH NEURONS. J.W. MCRORIE III*, B. ZIPSER, DEPT. PHYSIOLOGY, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI 48824

We are studying molecular events underlying the regeneration of individual, identified leech (Hirudo) neurons in dissociated culture. Our probes are monoclonal antibodies to surface and intracellular antigens of leech neurons. Characterizing the neuritic growth patterns of identified neurons in isolated culture, we have made the following observations.

The distance over which neurites extend on the substrate Con A is a characteristic feature for a given class of neurons. A modulatory neuron, the Retzius cell, and a presumptive modulatory neuron, the AP cell, project neurites over a mean distance of 190 microns. In contrast, sensory neurons, the N and P cells, project neurites over a mean distance of 60 microns. Thus, in tissue culture, modulatory neurons project over a 3 fold greater distance than sensory neurons. The short neurites of sensory neurons in culture are an unexpected finding since, in vivo, sensory neurons regenerate vigorously. Comparing the axoneuritic projections of modulatory and sensory neurons in the CNS and periphery suggests that sensory neurons and modulatory neurons are differentially affected by substrate clues. The Retzius and AP cells project neurites three dimensionally throughout the neuropile while N and P cells project predominently into a 2 dimensional plane. Neuritic extension in our culture system may be affected by interactions between the plant lectin Con A and surface glycoconjugates. Specific surface glycoconjugates are increasingly being identified in vertebrate and invertebrate sensory neurons. Thus, individual types of neurons appear to have their own distinct regeneration patterns utilizing substrate clues for neuritic extension.

We observed dramatic differences between AP neurons that were explanted with axonal stumps versus those explanted just as cell bodies in terms of a) primary origin of neuritic projections, b) varicosites resembling typical synaptic endings and c) molecular characteristics as probed with monoclonal antibodies. Thus, the anatomical and molecular characteristics of a neuron can change depending on whether it regenerates from a residual axonal stump or an isolated cell body.

76.1 MATURATIONAL CHANGES IN FETAL LAMB EEG. Y. Zhu*, S. Clare* G. Dwyer* and H.H. Szeto*. (SPON: M. Okamoto). Dept. of Pharmacology, Cornell University Medical College, New York, NY 10021

We have followed the maturation of the EEG of 10 fetal lambs thoughout the third trimester. A minimum of 3 hours recording was obtained from each fetus 1-2 times/week. Continuous EEG analysis was accomplished using a microcomputer-based system that permitted a sampling rate of 1/30 seconds. Power spectral analysis revealed two fundamental waveform patterns in all fetuses. They differed primarily in percent power distribution (% Dist.) and amplitude of selected Hz bands. Major maturational changes in the two patterns are summarized below:

Pattern I

Gastational	% Dist.			Amplitude		
age(days)	1-4Hz	8-13Hz	15-30Hz	1-4Hz	8-13Hz	15-30Hz
120	45	20	20	376	161	165
120-130	50	17	18	887	284	319
130	53	15	17	1286	350	408

Pattern II

Gastational	% Dist.			Amplitude		
age(days)	1-4Hz	8-13Hz	15-30Hz	1-4Hz	8-13Hz	15-30Hz
120	31	24	29	214	159	197
120-130	32	21	29	394	254	346
130	29	19	36	410	271	506

In addition, the frequency and the duration of the fundamental harmonic cycling of these 2 EEG patterns changed with maturation.

76.2 EEG SLEEP CHANGES IN AN ANIMAL MODEL OF DEPRESSION. D. M. Gartrell* and J. M. Weiss (SPON: P.G. Simson). Duke Univ. Sch. of Med., and Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Rats exposed to uncontrollable shock (UCS) have been shown to exhibit both behavioral depression and behavior consistent with anxiety. Studies using behavioral observation have found that these rats have an increase in waking during the light cycle without a compensatory increase in sleep during the dark cycle (Weiss, Simson, Ambrose, Webster, and Hoffman, in: Advances in Behavioral Medicine, vol 1 [Katkin and Manuck, eds.], 1985, 233-275). The study reported used EEG recordings to examine sleep changes produced in rats by UCS.

Seven pairs of Sprague Dawley rats were implanted with skull electrodes for recording hippocampal and cortical EEG, and cervical muscle electrodes for recording EMG. Beginning two days after surgery, one animal of each pair (the experimental rat) was exposed to 3 hours of UCS on two consecutive days. The second animal in each pair was placed in the shock apparatus but no shock was given. Shock sessions occurred just prior to the onset of the 12 hour light cycle. Recordings during the light cycle were made for 6 days. The first recording day corresponded to the first day of UCS. Recordings were visually scored in 30 second epochs as either waking, REM sleep, non-REM sleep, or non-REM sleep interrupted by waking.

Following UCS, experimental rats showed more waking, more interrupted non-REM sleep, longer REM sleep latencies, less REM sleep, and a lower REM sleep percent than control animals. In general, effects were largest immediately following the UCS, and then dissipated across the 6 days of recording. Statistically significant differences were as follows: experimental rats showed more waking on the first ($p<.05$) and third ($p<.001$) days, showed more interrupted non-REM sleep on the third day ($p<.05$), showed longer REM sleep latencies on the first ($p<.005$) and second ($p<.005$) days, showed less REM sleep on the first through fourth days ($p<.005$ on each day), and showed a lower REM sleep percent on the first through fourth days ($p<.01$ on each day). There were no statistically significant differences between the experimental and control animals on the fifth and sixth recording days.

Evidence indicates that locus coeruleus (LC) neurons are hyperactive in rats exposed to UCS. In that REM sleep is not seen when the LC is active, the decrease in REM sleep seen in this study may be a result of this alteration in LC activity. Stimulation of the LC in a human subject was found to produce sleep changes similar to those seen in this study (Kaitin, Bliwise, Gleason, Nino-Murcia, Dement, & Libet, Biol. Psychiat., 21, 1986, 710).

The sleep changes in this study resemble those in humans with anxiety disorders. Thus, there may be an anxious component to the depressive condition produced by uncontrollable shock.

76.3 MACROPHAGE PROCESSING OF BACTERIA; CNS-ACTIVE SUBSTANCES ARE PRODUCED. L. Johannsen*, J. Wecke*[1], J. M. Krueger, Dept. Physiology and Biophysics, University of Tennessee, Memphis, TN 38163 U.S.A., and [1]Robert Koch-Institut of the Federal Health Office, Berlin (West), F.R.G.

Several muramyl peptides (MPs), including a slow wave sleep (SWS)-promoting substance extracted from brain, increase SWS in mammals. MPs are well known constituents of bacterial cell wall peptidoglycan. However, there are no synthetic pathways known in mammals for MPs. This led to the proposal that MPs might be vitamin-like. We report here that macrophages, when fed bacteria, process the bacterial cell walls, release MPs, and the resulting macrophage supernatants have biological activity.

Bone marrow-derived murine macrophages were fed viable *Staphylococcus aureus* that were ^{14}C-marked in their cell wall (*Arch. Microbiol.* 121: 103, 1979). Supernatants of the macrophages were collected after 3, 12, 48, and 96 h of digestion, pooled, and freeze-dried. The supernatant was processed by gel filtration. Fractions containing ^{14}C in the molecular weight ranges between 5,000 D and the salt volume were pooled and dried. After suspension in artificial CSF, the samples were heated to 60°C for 30 min to eliminate biological activity of lymphokines. Fifty ul were injected into a lateral ventricle of male rabbits, and EEG, brain temperature, and movement were recorded for 6 h during daytime. Rectal temperatures were taken immediately before and after the experiment.

The infusion of the macrophage supernatant resulted in enhanced SWS and body temperature and decreased REM sleep (Table). The time course of these effects (not shown) was similar to those observed after MP administration, *i.e.*, an hour delay before long-lasting (6-10 h) effects are observed. This time course is different from that elicited by prostaglandins or lymphokines.

Table	Control	Experiment
% SWS	51 ± 2	72 ± 5
% REMS	4.5 ± 0.6	0.9 ± 0.2
% AWAKE	45 ± 2	28 ± 5
Temp. Diff. (6h-0h)	0.0 ± 0.1	1.8 ± 0.1

(all means ± std. err., n = 8)

Present results provide a possible mechanism for our recent findings that infection of rabbits with *S. aureus* also enhances SWS. The processing of bacterial cells walls into low molecular weight products by macrophages is a daily occurrence, as well as an early event in the initiation and amplification of the immune response. That the resulting products enhance SWS suggests a role for MPs in sleep regulation and an early involvement of the CNS in the immune response. Supported by ONR N00014-85-K-0773 and DFG JO 149/1-1.

76.4 GROWTH HORMONE-RELEASING FACTOR INCREASES BOTH NON-REM SLEEP AND REM SLEEP IN RABBITS. F. Obal, Jr.*, L. Johannsen*, A. B. Cady*, and J. M. Krueger (SPON: L. Share), Department of Physiology and Biophysics, University of Tennessee, Memphis TN 38163.

The well known association between non-REM sleep (NREMS) following sleep onset and growth hormone (GH) release may indicate that (i) NREMS promotes GH secretion or (ii) the regulation of GH release and sleep onset involve common mechanisms. In support of the latter assumption, recent findings suggest that intracerebroventricular (icv) administration of the GH-releasing factor, GRF, may induce sedation and increase EEG slow wave activity and both NREMS and REMS in the rat (*Neuroendocrinology* 42: 467, 1986; *Clin. Neuropharmacol.* 9(S4): 459, 1986; *Neuropharmacology* 26: 75, 1987). The aim of the present experiments was to study the sleep effects of GRF. Rabbits were implanted with EEG electrodes, brain thermistors, and a guide cannula for icv injections. Sleep-wake activity was recorded for 6 h following icv infusions of either GRF (Human GRF1-40) or artificial CSF (20 ul) as control during the light cycle.

DOSE		POSTINJECTION HOUR 1			TOTAL RECORDING TIME		
nmol/kg	N	Wakefulness	NREMS	REMS	Wakefulness	NREMS	REMS
0.01	7	-13.7 ± 4.4*	+15.5 ± 3.7*	-1.8 ± 0.9	-5.3 ± 2.5*	+4.6 ± 2.4	+0.6 ± 0.5
0.1	8	-21.4 ± 4.7*	+19.9 ± 1.6*	+1.5 ± 1.0	-9.3 ± 2.1*	+5.5 ± 1.6*	+3.8 ± 1.1*
1.0	6	-29.5 ± 6.2*	+24.2 ± 6.0*	+5.3 ± 2.7	-17.8 ± 3.6*	+12.0 ± 3.5*	+5.8 ± 0.8*

*Wilcoxon test, two-tailed

Icv infusions of GRF elicited a dose-dependent suppression of wakefulness and increases in both NREMS and REMS. The effect on NREMS was most pronounced in postinjection hour 1. The 6-h recording time showed significant increases in REMS in response to both 0.1 and 1.0 nmol/kg GRF. Brain temperature was not affected by GRF.

The results clearly show that exogenous GRF has a definite NREMS-inducing effect. The increases in REMS might be secondary, possibly due to GH secretion (*Neuroendocrinology* 18: 1, 1975; *Horm. Behav.* 6: 189, 1975; *Biol. Psychiat.* 15: 613, 1980). We conclude that GRF is a putative endogenous sleep-promoting substance. (Supported by NIH NS-25378.)

76.5 EFFECTS OF STAPHYLOCOCCUS AUREUS INFECTION ON SLOW WAVE SLEEP IN RABBITS. L. Toth* and J. M. Krueger (SPON: T.W. Gardiner), Animal Resource Division and Department of Physiology and Biophysics, University of Tennessee, Memphis, TN 38163.

Increased body temperature and altered immune system activity are well known features of infectious disease. Several compounds associated with infectious processes, including muramyl peptides and interleukin 1, are pyrogens and immunomodulators. Another action of these agents is the potentiation of slow wave sleep (SWS) (Fed. Proc. 45:2552, 1986). Subjective reports of increased "sleepiness" often accompany states of illness; however, the effects of infectious disease on sleep have not been evaluated systematically. To examine the effects of an infectious disease on sleep, EEG was monitored in 14 adult male New Zealand white rabbits during a 24-hr control period and for 48 hr following intravenous inoculation with 10^7 to 10^8 colony-forming units of Staph. aureus. Despite variability in the time course and the magnitude of responses, every animal tested showed enhanced SWS following inoculation. The time spent in SWS increased by about 50% during a 6-16 hr period post-inoculation (PI) and then returned to control levels. The enhancement of SWS was associated with increases in both the amplitude of EEG slow waves (from 6-8 hrs PI) and the duration of individual bouts of sleep (6-16 hrs PI). After 18 hrs PI, both the amplitude of EEG slow waves and SWS bout duration dropped below control levels, eventually returning to baseline by 48 hrs PI. The inoculation also produced several changes that typically are associated with infectious disease. Rectal temperatures increased by 1-2°C within 6 hrs PI and remained elevated for 48 hrs. The febrile effects of the inoculation could thus be dissociated temporally from the somnogenic effects. Hematologic analysis revealed a severe lymphopenia in all animals and a marked neutrophilia in most animals tested. The lymphopenia generally persisted for 48 hrs PI. The neutrophil response was more variable in terms of both magnitude and duration. Those animals that failed to develop a strong neutrophil response tended to show a shorter duration of enhanced SWS and a more severe clinical progression than did animals with marked neutrophilia. Postmortem blood cultures were positive in 10 of the 14 animals tested.

These data thus demonstrate that inoculation of rabbits with Staph. aureus results not only in the febrile and hematologic manifestations of infectious disease, but also in a pronounced increase in SWS. The time courses of these effects differed substantially, suggesting that they result from separate mechanisms. We speculate that changes in SWS represent an adaptive response to infectious disease. Supported by ONR-N00014-85-K-0773 and NIH-NS25378.

76.6 **PROTEIN SYNTHESIS IN THE RAT BRAIN IS LINKED TO SLOW WAVE SLEEP BUT NOT TO REM.** P. Ramm & C. T. Smith*. Dept. of Psychology, Brock University, St. Catharines, Ont. and Dept. of Psychology, Trent University, Peterborough, Ont. Canada.

The restorative hypothesis of sleep function proposes that SWS and/or REM sleep are linked to restoration of cerebral proteins. We have used [14C]leucine autoradiography (Smith, C. et al., J. Neurosci. 4:2489-2496, 1984) to test the restorative hypothesis.

Twenty-seven rats received recording electrodes, and cannulae in the femoral artery and vein. Four days after surgery, the freely-moving animals were REM-deprived for 24-72 hr. They were then returned to their home cages, where they received intravenous injections of L-[1-14C]leucine. During the next 45 min, EEG and EMG recordings were made and arterial blood samples were taken for the measurement of plasma levels of [14C]leucine and of free leucine. The animals slept quite normally during the leucine incubation period, and many showed elevated levels of REM.

Following the incubation period, the brains were extracted, cut, and autoradiographs were prepared. Rates of regional cerebral protein synthesis were calculated by computer densitometry.

Correlations were obtained between regional rates of protein synthesis, and the proportion of injected L-[1-14C]leucine cleared from plasma during wakefulness, SWS and REM. Effectively, the correlations show the relation between the rate of protein synthesis, and the amount of time spent in wakefulness, SWS or REM.

Correlations Between Cerebral Protein Synthesis and State

Wake	SWS	REM	Total Sleep
-.14	.54*	-.31	.15

* ($p < 0.01$, two-tailed)

Higher rates of (global) cerebral protein synthesis are associated with higher SWS times. We did not observe a link between REM and cerebral protein synthesis. This global pattern was replicated regionally. Most brain regions exhibited moderate positive correlations (up to .68 in the iterpeduncular nucleus) with SWS, and non-significant negative correlations with REM. These data suggest a restorative function linked specifically to SWS. They also converge with previous metabolic findings (Ramm, P. & Frost, B.J., Brain Res 365:112-124, 1986) to suggest that fundamental life processes in cerebral tissues are differentially linked to SWS and REM. (Supported by the Natural Sciences and Engineering Research Council of Canada).

76.7 **UNIT ACTIVITY DURING CATAPLEXY: FIRST RECORDINGS OF SINGLE NEURON ACTIVITY IN THE NARCOLEPTIC ANIMAL.** J. M. Siegel, R. Nienhuis, R. Paul, H. Fahringer, T. Kilduff, and W. C. Dement. Neurobiol. Res., VAMC Sepulveda, CA 91343, Dept. of Psychiatry, UCLA School of Medicine, Los Angeles CA 90024, Stanford University School of Medicine, Stanford, CA.

The narcoleptic dog provides an opportunity to investigate, at the neuronal level, the pathology producing cataplexy, a sudden loss of muscle tone occurring in both canine and human narcoleptics. Cataplexy has been thought to be an manifestation of a hyper-excitable REM sleep atonia mechanism activated during waking states. Studies in the cat indicate that the medial medulla forms the final common pathway for generating the atonia of REM sleep. Receptor binding studies have demonstrated abnormalities in the medial reticular formation of the narcoleptic dog. Accordingly, we have begun to record unit activity in the medial medulla of these animals in both normal REM sleep and cataplexy.

Five narcoleptic doberman/labrador dogs between 3 months and 6 years of age served as subjects. Cataplectic attacks were elicited by play, by providing novel food or by injecting physostigmine. The unit recording technique was similar to that used in previous studies of medullary unit activity in the unrestrained cat (Brain Res. 179:49-60, 1979).

Units were recorded in the rostral nucleus gigantocellularis and magnocellularis of the medulla. As in the normal cat, most medullary units in the narcoleptic dog had high discharge rates in REM sleep. However, despite their high discharge rates during REM sleep with its associated muscle atonia, most cells decreased discharge rate during cataplectic attacks. 94% of the cells encountered (n=61) decreased or ceased discharge during cataplexy. REM sleep discharge in these cells tended to be phasic, with burst discharge especially during periods of rapid eye movement, and short pauses at other times. A second cell type comprising less than 6% of the cells encountered (n=4), had increased or maximal discharge during cataplexy and discharged at high rates during REM sleep. In contrast to the first group, discharge in the second group tended to be continuous in REM sleep.

We hypothesize that most medial medullary cells are not contributing to the atonia of REM sleep and cataplexy. REM sleep discharge in these cells is related to the motor activation of this state. We further hypothesize that the cells in our second group, which are active in relation to cataplexy, form the final common brainstem pathway generating atonia in REM sleep. Increased excitability of these cells in narcoleptics, leading to waking discharge, is responsible for cataplexy.
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76.8 **LOCALIZATION OF NEURONS AND TRANSMITTERS INVOLVED IN THE PONTO-MEDULLARY INHIBITORY SYSTEM.** Y. Y. Lai and J. M. Siegel, Sepulveda VAMC, Sepulveda CA 91343 and Dept of Psychiatry, UCLA School of Medicine, Los Angeles CA, 90024.

Injection of cholinergic agonists into the dorsolateral pontine tegmentum produces a loss of muscle tone, often in conjunction with other signs of REM sleep. A number of studies have indicated that the medial medullary region, which receives projections from the dorsolateral pontine area, forms the final common brainstem pathway for triggering atonia. However, since atonia has been elicited only by electrical stimulation of the medial medulla, it remains possible that this effect is due to activation of fibers of passage and not cell bodies. Moreover, the localization of the neurons involved and the neurotransmitters responsible for activating them is unknown. Chemical stimulation of the medial medulla has not been reported to produce atonia.

To answer these questions we have performed a series of studies in 18 adult decerebrate cats. Tracheostomy, cannulation of both femoral artery and vein, and decerebration were performed under halothane-oxygen anesthesia. Electromyograph activity was recorded from neck and forelimb muscles. The inhibitory areas in pontine and medullary reticular formation (PRF and MMRF) were identified by electrical stimulation. Once the inhibitory area was located, the stimulating electrode was removed and 0.5 µl of each pharmacological agent was injected through a 26-gauge Hamilton 1µl microsyringe. Chemicals were dissolved in Ringer solution in the concentration of 0.2M of L-glutamate (Glut), 0.2M of L-glutamic acid diethyl ester (GDEE), 2 µg/µl of r-D-glutamylglycine (DGG), 200 µg/µl of acetylcholine (Ach), 8 µg/µl of carbachol (Carb), and 2 µg/µl of atropine.

Microinjection of Glut into PRF (5/5) and rMMRF (14/17) but not the caudal MMRF (cMMRF; 1/11) inhibitory areas produced bilateral inhibition of the muscle tone. This muscle atonia induced by Glut injection could be blocked or attenuated by GDEE in PRF and GDEE and DGG in rMMRF. However, Ach injection in PRF (4/4) and cMMRF (11/13) but not the rMMRF (1/11) was found to produce bilateral muscle atonia. Atropine blocked this Ach effect. Carb injection in all three areas produces a long period of muscle atonia with a short latency (≤3 min) in PRF and cMMRF and a long latency (≥20 min) in rMMRF, suggesting that the effect of rMMRF injection resulted from Carb diffusion to either PRF or cMMRF. We conclude that at least three neurochemically distinct regions in the brainstem can trigger muscle atonia: 1) A dorsolateral pontine region sensitive to Ach and glutamate. 2) A rostral medullary region sensitive to glutamate but insensitive to Ach. 3) A caudal medullary region sensitive to Ach, but insensitive to glutamate.
(Supported by the Medical Research Service of the V.A. and USPHS grant NS14610.)

76.9 CARBACHOL EXCITATION OF MPRF NEURONS IN VITRO: A POSSIBLE MODEL FOR MECHANISMS OF DESYNCHRONIZED SLEEP GENERATION. R.W. Greene and R.W. McCarley. Neuroscience Lab., Dept. of Psychiatry, Harvard Med. Sch./Brockton VAMC, Brockton, MA 02401

A behavioral state characterized by all of the phenomena of desynchronized sleep is elicited by localized injections of ugm amounts of cholinergic agonists in the medial pontine reticular formation (mPRF). However, the mechanisms by which ACh agonists produce these effects have remained unclear since the in vivo iontophoretic applications of ACh and extracellular recordings previously used have intrinsic limitations. They can not: (1) determine if effects are pre- or post-synaptic; (2) assure the application of physiological concentrations; (3) determine effects on resistance; or (4) determine the duration of effects. We thus turned to intracellular recordings in the in vitro mPRF slice preparation.

Coronal slices, 500uM thick, from young rats (7-11 days old) were cut with an ultrasonically vibrating blade and then totally submerged in a modified Haas chamber. Standard intracellular recording techniques were used with glass pipettes containing 2M KCl; neurons had stable membrane potentials more negative than -58mV and > 5mV action potential overshoot. Bath-applied carbachol (0.25-1.0um) produced a depolarization of 16 ± 6mV S.D. amplitude in 6 of the 7 mPRF neurons tested with an increase of input resistance observed in 5 neurons (mean increase 21 ± 18% S.D.). One neuron had a transient hyperpolarization preceeding the depolarization.

Of particular interest as a mechanism for desynchronized sleep effects was the long time course of the carbachol action. The time from onset of carbachol-induced depolarization to peak effect was 2-4 minutes, and recovery time following removal of carbachol ranged from 7 to 26 minutes, despite our set-up's having a complete exchange of perfusion media every 30 seconds. That this long-duration depolarization was not dependent on synaptic activity was demonstrated by its presence with tetrodotoxin added to the perfusion medium. In 4 of 4 neurons tested atropine (0.25 - 1uM) blocked these carbachol effects, suggesting the involvement of muscarinic receptors. The cholinergic excitatory effects, depolarization and increased membrane resistance, and their time course are consistant with a neurotransmitter role for ACh in generation of naturally-occuring desynchronized sleep episodes.

76.10 BRAINSTEM MECHANISMS OF ALERTING. W.A. Ball*, A.R. Morrison, R.J. Ross, D.R. Levitt, P.J. Gresch*, and G.L. Mann*. Sleep Res. Lab., Vet. Sch., Univ. of Pa., Phila., Pa. 19104-6045.

Ponto-geniculo-occipital waves (PGO) are spontaneous, macropotential waves typical of lateral geniculate (LGN) recordings in paradoxical sleep (PS) of cats. We earlier showed that PGO can also be elicited by repeated tones in both slow wave sleep (SWS) and PS. PS was characterized by a lack of habituation to the tones; SWS was not (Morrison,A.R. and Bowker,R.M., Acta Neurobiol. Exp.,35: 821-840,1975). This indicated that PS is a state of "hyperalertness" defined in part by an absence of habituation of the elicited waves. We report here a more rigorous, quantitative analysis of this phenomenon that has suggested a degree of habituation of elicited PGO in PS but at a rate slower than in SWS.

Four cats had standard electroencephalogram (EEG), LGN, electrooculogram (EOG), and electromyogram (EMG) electrodes implanted. After 12 days, they were presented tones (1000 Hz, 90 msec, square wave at 90 db) in SWS and PS. A session consisted of up to 32 tones (interstimulus interval= 5 sec) in a sleep state with polygraph and FM tape recording of PGO. Cats were tested twice a day on 2 consecutive days in one sleep state, then 2 additional days in the other. Sham trials consisted of stimulus time marks without tones.

Within 200 msec after the stimulus, tones were more likely than sham stimuli to elicit PGO in both sleep states ($p<.01$). Overall, tones in PS elicited PGO more readily than tones in SWS ($p<.01$). A rapid decrease in the probability of eliciting a PGO wave in SWS accounted for the difference: by trial 8 the proportion of trials with waves at least 40% as large as the first elicited wave had fallen from .68 to .22. The pattern in PS differed markedly: the probability remained constant at about .5 across 16 trials, then showed a decline to .29 by the end. On all but the first 4 trials, the probability of eliciting a PGO was greater in PS.

The data replicate our earlier results that overall, PGO are elicited more frequently in PS than SWS. Close analysis of the pattern across trials reveals that the probability of eliciting PGO in PS did decline, albeit much more slowly than in SWS. The frequency of spontaneous PGO did not decline, however, across the experimental session during either tone or sham trials. This suggests the possibility of true,if slow, habituation in PS rather than a decrease in the underlying responsiveness of the PGO system (e.g., during a change of state to SWS or PS).

This work was supported by grants NS 13110 and MH 14654.

76.11 ACTION SITES FOR SLEEP AND EEG EFFECTS OF MORPHINE IN CATS. J. de Andrés and I. Corpas*. Depto. Morfología. F. Medicina. UAM. Madrid 28029. Spain.

Sleep deprivation and some types of EEG/behavioral dissociation are basic phenomena observed across species after morphine and other opiates administration. These features are easily noticeable in the cat, since under low single doses of morphine sulfate (MS), this animal presents a dose dependent suppression of both Non-Rem and Rem sleep, and during the insomnia period it exhibits a peculiar wakefulness associated with a high voltage-slow frequency burst pattern in the concomitant EEG (De Andres et al. Pharmacol. Biochem. and Behav. 21:923-928. 1984). To learn about the CNS sites (prosencephalon vs. brain stem) involved in the above mentioned MS actions, we have used cats with a mesencephalic transection to study: a) the MS effects on the EEG of the isolated forebrain, and b) the MS effects on Rem sleep of the decerebrate cat .

Under general anaesthesia (Nembutal 35 mg/Kg.) intercollicular transections were performed in 11 adult cats. Electrodes were implanted in motorsensory and visual cortices to record EEG of the isolated forebrains. Also, to characterize Rem sleep of the decerebrate cats, two electrodes were stereotaxically placed in the pons (PGO activity), as well as, the standard ones to record EOG and EMG. Experiments elapsed one week from surgery . Single doses of MS (.5, 2.0 and 3.0 mg/kg, via i.p.) were administered 7 days apart. EEG of the isolated forebrains and polygraphic records of the decerebrate cats were taken during the next 24-72 hrs after MS. Pearson's correlation coeficient was used to compare MS dose with the onset latency of Rem sleep in the decerebrate cats. Two-way ANOVAs were used to compare the amount of synchronized and desynchronized activity in the forebrains as well as the amount of Rem in the decerebrate cats.

In the cases that a complete transection was achieved with little damage of the mesencephalon (transections at prepontine level in 7 cats), all MS doses were followed by a long lasting continous desynchronized EEG, full mydriasis was associated with the EEG desynchronization. These effects were independent of the behavior exhibited by the descerebrate cats . Naloxone blocked the EEG and pupil effects of MS, since at least for one hour after the antagonist, the isolated forebrain EEG was fully synchronized with myotic pupils. Decerebrate cats presented a dose dependent decrease in the amount of Rem during the first 24 hrs, reaching to an initial suppression that -depending on the MS dose- could last a few hours. However, these effects were moderate in comparison with the Rem suppressión seen previously with the same doses in intacts cats. Likewise, decerebrate cats did not manifest a strong behavioral activation which resembled the behavioral wakefulness exhibited by intacts cats under MS. Moreover, present results showed that Rem suppression in decerebrate cats had always a shorter duration that the MS effects seen in the isolated forebrains.

In summary, our results indicate that: 1) Prosencephalic mechanisms are seemingly not involved in the generation of the electroencephalographic slow burst activity produced by morphine . 2) Rem sleep suppression is only partially originated in the lower brain stem, suggesting that the prosencephalon and/or mesencephalon generate/s a strong Rem sleep inhibition under morphine, and 3) Behavioral wakefulness under the drug might essentially depend on prosencephalic and/or mesencephalic structures.

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76.12 EFFECT OF INSULIN INJECTION ON ELECTROENCEPHALOGRAPHIC (EEG) SLEEP IN THE RAT. M.J. Bakalian* and J.D. Fernstrom (SPON: M.H. Fernstrom). Departments of Psychiatry and Behavioral Neuroscience and the Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15213.

Brain serotonin (5HT) neurons are involved in sleep regulation. In particular, recent studies suggest that stimulating 5HT receptors suppresses rapid-eye-movement (REM) sleep, while producing minimal effects on non-REM sleep. Insulin injection reportedly suppresses REM sleep (Sangiah et al. Life Sci 31:763,1982). Since insulin injection stimulates brain tryptophan uptake and 5HT synthesis (see Fernstrom J.D., Physiol Rev 63:484,1983), it may suppress REM sleep by indirectly stimulating brain 5HT synthesis and release. However, the insulin dose employed by Sangiah et al. (1 IU/kg) reduced blood sugar levels substantially. Thus, one cannot conclude if insulin suppressed REM sleep via an increase in 5HT release or via a non-specific effect of hypoglycemia. We have begun to examine this issue, to ascertain which mechanism accounts for the reduction in REM sleep.

Male Sprague-Dawley rats (300 g) underwent stereotactic placement of cortical, dorsal hippocampal and nuchal electrodes (Vivaldi et al. EEG clin Neurophysiol 58:253, 1984). Following recovery, they were placed in special recording chambers with an internal, 12:12 light:dark cycle. EEG electrodes were connected to a Grass Model 78 polygraph, which was output both to pen chart recorders and to an Apple II+ microcomputer. The Apple detects and stores waveband data used in the scoring of sleep-waking states. For these studies, rats (n=8-11 at each dose) were fasted 12-hr before insulin (Iletin U-100, Lilly; 0.25, 0.5, or 1.0 IU/kg) or vehicle injection at light onset. EEG recordings were made for 6-hr thereafter.

First, fasting did not alter the normal occurrence of REM and NREM sleep (comparing fasted and non-fasted rats receiving vehicle). And second, insulin injection into fasted rats caused a statistically-significant (ANOVA), dose-related increase in latency to REM sleep (a doubling [low dose] to quadrupling [high dose]) and significant reductions in total REM sleep time (16-75%) and the number of REM episodes (40-60%). A small, statistically-significant reduction in NREM sleep time also occurred at the 1 IU/kg dose, but no effect on any NREM sleep measure was observed at the lower doses. Our results therefore affirm the finding that insulin injection suppresses REM sleep, but contrasts with the earlier data in showing a small NREM sleep suppression, rather than the reported increase. Studies now in progress will ascertain if these effects are altered when glucose is administered with insulin, to prevent insulin-induced hypoglycemia. If they are not, the results will suggest that the effects of insulin on REM sleep may be mediated specifically by an enhancement of 5HT synthesis and release by brain neurons.

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76.13 FEVER ALTERS CHARACTERISTICS OF SLEEP IN RATS. M.Price*, S.Kent* & E. Satinoff. Dept. of Psychology and Program in Neural and Behavioral Biology, Univ. of Illinois, Champaign, IL 61820

Fever alters both the amount and organization of sleep. We recorded stages of sleep and waking and body temperature (Tb) in 6 male Long-Evans rats (b.w. 350-450 g). Rats were kept on a 12:12 light/dark cycle with an ambient temperature (Ta) of 23 $\pm$ 1^{o}C with food and water always available. The rats were placed in the sleep-recording chamber (Ta 23^{o}C) and left there overnight. At the start of the next day's light cycle, chamber Ta was set to either 20 or 30^{o}C, and 1 hr later the rats were injected subcutaneously with 5 ml saline. Sleep and Tb were then computer-recorded for 24 hr at the given Ta. 24 hr after the saline injection, the rats were injected with 30% brewer's yeast in saline, after which recording was continued for another 30 hr.

At both Ta's, peak febrile responses of 1.5-2.2^{o}C occurred within 8 hr post-injection, after which there was a 0.5-1.0^{o}C decline over 4-5 hr to a level that was maintained for at least a day and a half. Normal circadian Tb rhythmicity was thus temporarily abolished: Tb in the dark after the daytime injection was close to normal nighttime Tb, whereas Tb's in the next light period were elevated relative to normal daytime Tb's and, in fact, were within 0.5^{o}C of normal nighttime high Tb's. The difference between the fever and saline conditions at the time of the circadian trough Tb at Ta 30^{o}C was 0.6^{o}C higher than that at 20^{o}C.

At both Ta's fever increased slow-wave-sleep (SWS) and decreased waking. The largest changes occurred at night: SWS increased from 15-35% compared to daytime increases of 3-5%. The percentage of REM sleep to total sleep time changed in the opposite direction: the ratio decreased 20-50% during the day and increased 50-200% during the night.

These results indicate that febrile Tb is not simply the same as the normal Tb rhythm, only elevated, but rather that it is primarily an elevated Tb during the day. The results further show that SWS and REM sleep are both affected by fever, but the circadian changes in each are different.

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76.14 EVIDENCE AGAINST SEROTONIN-MEDIATED SLEEP INDUCTION FOLLOWING ADMINISTRATION OF L-TRYPTOPHAN. D.W. Preussler and M.E. Trulson. Dept. Anat., Coll. Med., Texas A&M Univ., College Station, TX77843.

Self-administration of large doses of the essential amino acid L-tryptophan has become widespread for the induction of sleep. Numerous clinical studies in human as well as animal research support the hypothesis that large doses of L-tryptophan decrease sleep latency. Since a major metabolic route of L-tryptophan in the brain is its conversion to the monoamine neurotransmitter, serotonin (5HT), it has been assumed that L-tryptophan-induced sleep is attributable to 5HT produced by administration of tryptophan. This hypothesis is supported by numerous studies that have implicated the serotonin in the sleep/waking cycle. We directly tested the hypothesis that L-tryptophan induces sleep by producing excess 5HT. Cats received bilateral injections of 5,7-dihydroxytryptamine (5,7-DHT) or saline into the lateral ventricles and were implanted with macroelectrodes for recording the electroencephalogram (EEG), neck electromyogram (EMG), and eye movement potentials (EOG). After recovery from surgery and adaptation to the recording chamber, the animals were given L-tryptophan at a dose of 50 mg/kg by oral administration. The latency to sleep onset was monitored. The results showed that L-tryptophan produced a significant decrease in sleep latency in both 5,7-DHT and control groups. Furthermore, there was no significant difference in the latency to sleep onset between control and experimental groups of animals. At the completion of the study the brains were removed and assayed for 5HT and its major metabolite, 5-hydroxyindolacetic acid (5HIAA) using high pressure liquid chromatography and electrochemical detection. Animals that had received 5,7-DHT exhibited a depletion of 5HT and 5HIAA between 82 and 97%. An additional group of animals received L-tryptophan (50 mg/kg) and were sacrificed for assay 1 hour later. The animals that had received 5,7-DHT showed no significant increase in 5HT or 5HIAA above the levels found in 5,7-DHT animals that received no treatment. On the other hand, the normal animals that had received L-tryptophan showed large increases in brain 5HT and 5HIAA. These data demonstrate that L-tryptophan is effective in reducing sleep latency, but that the effect does not appear to be mediated by 5HT, since L-tryptophan was just as effective in inducing sleep in animals that had destruction of brain 5HT neurons by 5,7-DHT as in control animals. Furthermore, neurochemical data showed that these chemically-treated animals had no increase in brain 5HT or 5HIAA even though sleep latency was decreased by the same magnitude as in the control group. Thus, L-tryptophan appears to induce sleep through some mechanism other than producing an increase in brain 5HT.

76.15 GAMMA-HYDROXYBUTYRATE IN MONKEYS - SEDATIVE OR CONVULSANT? R.K. Nakamura, M. Myslobodsky, R. Coppola, J. Johannesen-Conway*, Y. Salu*, and A.F. Mirsky. National Institute of Mental Health, Bethesda, MD 20892.

The evaluation of drug activity can be more informative if physiological measures are combined with well controlled behavioral observations. Here we report on such a combination used to evaluate gamma-hydroxybutyrate (GHB).

GHB has been reported to produce a state in monkeys resembling petit mal status. This implies that an animal would produce erroneous responses immediately prior to, and discontinue any cognitive effort concurrently with, an episode of GHB-induced generalized 3 cps wave-spike bursts in the EEG. This prediction was not confirmed in the present study. Rhesus macaques (Macaca mulatta) were trained to perform in a go/no-go visual discrimination task. Thereafter bipolar transcortical electrodes were implanted in the hemisphere contralateral to the preferred hand so that the short electrode of the pair penetrated the dura by 0.5 mm; the longer one was located in the depth of the cortex or in the white matter 2 mm away from the cortical surface.

All monkeys were unable to complete the task involving lever-pressing for water-reward when administered GHB (125 or 250 mg/kg, esophageal intubation) and exhibited signs of reduced postural control and somnolence punctuated by episodes of arousal under stimulation. EEG showed local and generalized hypersynchronous activity. While occasional wave-spike bursts did occur, they were poorly regulated often 'focal' (ie. developed only in isolated areas), and had a frequency of 1.5-2.0 cps. At the same time, there were pronounced changes in event-related potentials to visual stimuli with a large increase in the amplitude of the slow components during continued accurate performance. In this state, animals could easily be roused by sensory stimuli. All of them reacted with a characteristic aversive-aggressive display when confronted by a direct gaze.

Overall, these effects are interpreted to be more consistent with GHB as a potent hyponotic rather than convulsant agent.

76.16 INCREASED REM SLEEP IN RATS SELECTIVELY BRED FOR CHOLINERGIC HYPERACTIVITY. J.C.Gillin, P.J. Shiromani, D.Levy,* C.A.Goodrich,* A. Norman & D.Overstreet. Psychiatry, SDVAMC & UCSD, La Jolla, CA 92093, Univ Cincinnati College Med (AN), & School Biol Sci, Flinders Univ South Australia, Bedford Park, South Australia (DO)

Overstreet and colleagues (1) have developed a strain of rats which show a central cholinergic hyperactivity. The Flinders Sensitive Line (FSL) of rats show a greater sensitivity to cholinergic agonists and have increased number of muscarinic receptors in the striatum and hippocampus compared to controls. Given the strong link between cholinergic mechanisms and REM sleep (REMS) (2,3), we hypothesize that the FSL rats should have increased REMS.

14 male rats were used. Eight rats were of the FSL strain while six rats were Flinders Resistant Line (FRL). The latter group was derived by breeding successive generations of rats least sensitive to DFP and it has been established that this group is not different from a randomly bred stock. Two weeks after recovery from surgery (Nembutal), a two day adaptation period was used and then a continuous 48 hr sleep recording was obtained. The sleep records were scored for awake, drowsy, slow wave sleep and REM sleep. The 48 hr sleep records were averaged to yield a 24 hr sleep record. The identity of the rats (i.e FSL or FRL) was revealed after the EEG records were scored and data entered into the computer. 3H-QNB was used as the ligand in the receptor assay.

There were no significant differences between the two groups in total sleep time, waking, drowsy or slow wave sleep. Both groups demonstrated a similar circadian rhythm in REMS. However, the FSL rats had significantly more REMS during the 1500 and 1800 hr time periods ($P<.05$). The increased REMS seen in the FSL rats occurred because they entered into REMS significantly more often from drowsy than from slow wave sleep. This suggests that in the FSL rats the latency to REMS onset is considerably faster compared to the control rats. Preliminary data suggest that the FSL rats have increased number of muscarinic receptors in the brainstem (FSL B max=28.9 picomole/gm, Kd=246.7, n=3; FRL=24.3, 220, n=5).

The alterations in REM sleep are consistent with the amassed evidence that increased central cholinergic activity produces a corresponding selective increase in REM sleep. We suggest that these rats may provide a means to study depression and narcolepsy where a central cholinergic hyperactivity is hypothesized to contribute to the mood, sleep, and REM sleep disturbance.

(1) Overstreet, D.H. Biol Psychiat, 21:49,1986. (2) Hobson J.A. etal. Behav Brain Sci, 9:371, 1986. (3) Shiromani, P. etal. Ann Rev Pharmacol Toxicol, 27:137, 1987. Supported by American Narcolepsy Assoc, VAMC Research Service and NIMH-38738

76.17 Regional Cerebral Metabolic Rates After Sleep Deprivation: Hoang-Ly, J.C. Wu, M.S. Buchsbaum, J.C. Gillin, Psychiatry Dept., Univ. Calif. Irvine, Irvine, CA 92717 (Sponsor: R. Jevning)

Introduction: Sleep deprivation (SD) has been used as an experimental probe to infer what role sleep plays in behavioral and neurochemical activities and functions. Positron emission tomography (PET) scans were done to assess changes in local cerebral glucose utilization after SD.

Methods: Four normal controls (4 males, x = 25.3 + 6 yr) were studied. The subjects were scanned twice. The subjects were in a normal waking state on the first occasion and had slept the night before. The subjects were sleep-deprived for the entire night on the second occasion.

The subjects performed the Continuous Performance Test during the thirty-minute uptake after injection of 4 to 5 mCi of 18-fluorodeoxyglucose for both scans. Nine slice images were obtained on the CTI NeuroEcat IV scanner with an in-plane resolution of 7.6 mm. Glucose metabolic rates for the scan were derived according to the model of Sokoloff.

Results: Metabolic rates for the caudate are presented relative to the whole slice metabolism. Normal controls showed a significant decrease (21%) in relative caudate metabolic rates after sleep deprivation on the left side only using paired t-tests ($p < .05$, 1 tailed).

Patients also showed a decrease in frontal/occipital ratio after sleep deprivation (before SD = 1.10, after SD = 1.04, T = 1.78, DF = 3, $p < .05$, 1 tailed).

(This study was supported in part by NIMH, MH 30914, and by Upjohn Pharmaceutical)

76.18 ROLE OF α-1-ADRENOCEPTORS IN REM SLEEP AND CATAPLEXY. E.J.M. Mignot*, C. Guilleminault, S.S. Bowersox and W.C. Dement. Sleep Disorders Center, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The purpose of this study was to investigate the possible involvement of central alpha-1 adrenergic receptors in the cataplexy manifested by genetically narcoleptic Doberman pinschers. Treatment of narcoleptic dogs with prazosin, a selective alpha-1 adrenergic receptor blocker, was found to exacerbate cataplexy, while treatment with the alpha-1 agonist methoxamine ameliorated the cataplexy. Subsequent studies demonstrated that the beneficial effects of amphetamine in narcolepsy are mediated indirectly through alpha-1 stimulation. The possibility that the observed effects were due to peripheral alpha-1 cardiovascular involvement was excluded. The effects of prazosin in combination with atropine and with methylatropine were also examined. Results described suggest that the cholinergic neurons implicated in cataplexy and REM sleep are directly or indirectly controlled by alpha-1 adrenergic receptors. Binding studies using ^{3}H-prazosin reveal an increase in alpha-1 receptor binding apparently limited to the amygdala. Little is known about the physiological role of central alpha-1 adrenergic receptors; the results reported strongly implicate these receptors in canine narcolepsy, an autosomal recessive disorder of REM sleep.

76.19 GENDER DIFFERENCES IN THE ULTRADIAN CYCYLICITY OF SLEEP. P. F. Bright* and W. Fishbein. (Spon: P. Sajovic) Psychobiology Lab, CUNY, The City College and Graduate School, New York 10031.

Many sex-related variations in nonreproductive behaviors have been described in humans and animals (Beatty, W.W., Horm. Behav., 12, 112, 1979; Halpern, D., Sex Diff. Cog. Abil., Erlbaum, NJ,1986) yet to our knowledge no quantified sex differences in sleep cycle rhythmicity have been reported.

As part of a large multivariate experiment in CF-1 mice designed to study the effects of prenatal stress on various parameters including sleep-wake cycles, we observed highly significant gender differences in the amounts of SWS and REMS displayed. This report centers on the sleep cycle rhythmicity of 6 male and 6 female control subjects. Each subject was born from a mother that had a normal pregnancy, free of the stress treatment imposed on the experimental dams. Within 12 hours postnatally, each subject was randomly cross-fostered to a normal lactating mother and reared with five other litter mates until weaning at 22 days of age. None of the twelve subjects is a sibling or was reared with any other subject. From day 22 to 50 the animals were housed (5-7 per cage), separated by sex, with other control mice which were employed in other aspects of the larger experiment.

On day 61, after surgical implantation of chronic indwelling EEG and EMG electrodes, adaptation to the commutator cables and recording environment, sleep-wakefulness cycles were recorded continuously over a 48 hr. period; throughout the recording session food and water was available ad libitum and as a result the animals were completely undisturbed.

The time spent asleep (or awake) over 24 hrs. was indistinguishable in the two sexes. Males average 9.71 hrs/24 hrs. asleep, females 9.63 hrs. Despite this invariance the distribution of time spent in REMS and SWS was quite different.

Over all 24 hours females spend significantly more time in REMS than males ($F(1,10)=5.90$ $p < .035$) and this differencce was most distinguishable during the day cycle ($F(1,10)=5.36$ $p < .043$). Furthermore the difference is totally accounted for by a greater number of REMS episodes in the females; 2.19/hr, males 1.60/hr ($F(1,10)=14.59$ $p < .003$), whereas the average duration of the REM episodes was precisely the same, 2.35 min vs 2.35 min.

The significantly greater amount of REMS in females measured over 24 hours was not reflected in a reciprocally greater amount of SWS in males ($F(1,10)=1.38$).

Our findings are the first to indicate that the regulation of sleep cycles is sexually dimorphic. The data point to the Y chromosome as a mediator of sleep-cycle control through the sexually dimorphic preoptic and suprachiasmatic nuclei of the basal forebrain by gonadotrophic influences.

76.20 APOMORPHINE-STIMULATED GROWTH HORMONE RELEASE IN HUMAN NARCOLEPSY. T. Baker, R. Hitzemann, J. Hirschowitz*, K. Gujavarty*. Dept. Psychiatry and Behav. Sci., SUNY Stony Brook, NY 11794

Narcolepsy is a lifelong neurological disorder that occurs in humans, dogs, and horses. It is characterized by excessive daytime sleepiness and abnormal tendency for REM sleep or dissociated REM sleep phenomena (cataplexy, hallucinations, sleep paralysis). Neurochemical studies of narcoleptic dogs, including measurement of monoamines and their metabolites in CSF and brain tissue, have indicated abnormal turnover of dopamine (Mefford, I. et al., Science, 220:629, 1983; Faull, K. et al., Brain Res., 242:137,1982).

The apomorphine-stimulated growth hormone test (AGHT) can be used to assess the functional sensitivity of dopamine receptors in vivo. Administration of low doses of the dopamine agonist apomorphine elicits the release of neuroendocrine hormones within 1-2 hours. Growth hormone (GH) is released via stimulation of the hypothalamic postsynaptic D2 receptors. In the present study, we studied D2 receptor sensitivity in narcoleptic humans to test the hypothesis of impaired dopamine turnover in this disorder.

Narcoleptic volunteers (N=25) and normal controls (N=12) were given 10μg/kg apomorphine by i.m. injection at 10 a.m. after an overnight fast. Blood samples were collected via indwelling i.v. catheter at 10 min. intervals during baseline and from 40-90 min. post-injection. GH concentration was determined by radioimmunoassay.

Narcoleptic subjects were divided into two groups; those not using therapeutic medications, and those who were long-term CNS stimulant users, either pemoline (a dopamine agonist) or methylphenidate (more general monoamine agonist properties). Chronic use of CNS stimulant compounds was associated with blunted GH response, as measured by peak GH release or integrated total GH release. Males showed greater peak GH release than females in both medicated (M) and non-medicated (NM) groups: peak GH (ng/ml) NM males (N=6), 32.8 $\pm$ 6.2 vs. M males (N=6), 22.6 $\pm$ 5.9; NM females (N=7), 17.2 $\pm$ 10.2 vs. M females (N=6), 6.8 $\pm$ 4.3. Age was a significant factor influencing apomorphine-stimulated GH release in all groups, with older subjects showing blunted peak and total release. Finally, non-medicated narcoleptic subjects showed a non-significant trend toward elevated GH release, as compared with normal controls (22.6 $\pm$ 11.9 vs. 18.8 $\pm$ 4.5).

These data demonstrate that long-term use of the CNS stimulants pemoline and methylphenidate may cause a blunted GH release on the AGHT. One interpretation of these results is that chronic use of monoamine agonists by narcoleptic subjects leads to down-regulation of hypothalamic D2 receptors. The data suggesting a trend for narcoleptic subjects to have elevated GH release, compared to normal controls, suggests that D_2 receptors may be up-regulated. This finding is consistent with the hypothesis of chronically impaired dopamine release in human narcolepsy syndrome.

76.21 SLEEP STATES IN NEONATES MONITORED AT HOME. E.B. Thoman and M. K. Pugsley*. Biobehavioral Sciences, U. of CT, Storrs, CT 06268.

Using an automated procedure for sleep recording, the sleep of 10 male and 10 female fullterm infants was monitored for 24-hour periods in the home once a week during the first to fifth weeks of life. A pressure sensitive mattress, placed in the infant's crib, was used for recording analog signals from respiration and body movements. The motility signals were recorded on a single channel of a small Oxford 24-hour recorder which was placed under the infant's crib. The system was put in the home one day, then retrieved the following day.

In the laboratory, the recorded signals on the data tapes were demodualted and digitized. They were then computer scored for states using a pattern recognition program which employs a template matching process. For each 30-second epoch, the state was scored as either: Active Sleep (AS), Quiet Sleep (QS), Active-Quiet Transitional Sleep (AQ), Sleep-Wake Transition (TR), or Wakefulness (WA). The states were measured as percent of time spent in the crib during the 24-hour period. Other measures included: the amount of time spent in each of the states, the number of state changes per hour of sleep, the length of Active Sleep bouts, and the length of Quiet Sleep bouts.

Reliability of each state was established by assessing the measures of each state for individual differences over the five weeks. This was accomplished by using a two factor repeated measures analysis of variance for each variable. Individual differences were tested by dividing the Mean Square for Subjects by the Mean Square for Subjects by Weeks.

Significant individual differences were found for AS, QS and AQ. Significant developmental trends over these early weeks were found for the mean length of AS, and QS bouts. The amount of time spent in AQ, TR, and WA, and the number of state changes per hour were found to be remarkably constant over the five weeks. These results were found despite the variations which typically occur in the home week to week.

Validity of state measures obtained by this automated recording procedure was assessed by comparing the data from this study with other studies of infants of the same age, using different observational techniques. First, the mean state distribution over weeks was compared with that from a study in which direct behavioral observations were used (Thoman & Davis, Dev. Psychobiol., in press). A 7-hour daytime period from each study was compared. The time spent in AS, QS, and AQ did not differ. The amounts of time in Wa and TR did differ significantly. Secondly, the 24-hour data were compared with 8-hour nighttime recordings using a time lapse video procedure (Anders & Keener, Sleep, 8:173, 1985). The state distributions were very similar for the two studies. These comparisons establish the validity of the state monitoring and computer scoring procedure.

76.22 PROPORTIONAL JERK: A NEW PARAMETER OF MOTION APPLIED TO OCULAR ACTIVITY IN INFANTS DURING 'SLEEP'. E. Aserinsky. Department of Physiology, Marshall University Schl. of Med., Huntington, WV 25704

The third derivative (jerk) of motion has essentially never been studied in regard to biomedical function. Enthusiasm for its use would be dampened by anticipation that it might simply parallel velocity changes. As a remedy, the concept of jerk has been modified (Aserinsky, E. Psychophysiol., 23:340, 1986) and termed 'proportional jerk' (PJ). PJ is a ratio without units, and is proportional to the change in the rate of acceleration relative to av velocity. The usefulness of PJ is that it can be measured without knowledge of the absolute amplitude or velocity of a mvt, values which in the case of the EOG fluctuate with the corneoretinal potential. Also, PJ can be an index of the 'smoothness' of a mvt, and thus may provide insight into the mechanism of neural control. In the aforementioned paper, the REMs of sleeping adults had an av PJ of 0.101 which was signif. ($p<.05$) higher than the 0.082 found for spontaneous waking saccades under closed lids. This suggested that neural control was not the same for waking and sleeping (dreaming) mvts.

A PJ analysis similar to the one above is presently reported for eight infants, age 12 wks, in order to ascertain whether, as in adults, oculomotor characteristics of so-called REMs are distinguishable from waking saccades. This is of interest because the crucial consideration for designation of a 'REM state' in these young infants is that the lids are shut rather than open while fast eye mvts are present. The typical adult pattern in which the REM state follows non-REM sleep is not seen in these infants. Is it possible that instead of being a REM state the infants are awake with the eyes shut? The EOG potentials from 8 infants were obtained from records of a previous study (Lynch, J.A. & Aserinsky, E., Behav. Brain Research, 20:175 1986) and dichotomized into saccades occurring in the conventionally defined REM state and into saccades appearing with the lids open.The eye mvts were limited to conjugate mvts which showed no vertical vector. All such mvts in one 'REM pd' for each subject were submitted to PJ analysis on an Apple computer, and were compared with all pure horizontal mvts occurring in eyes-open (waking) pds comparable in length to the 'REM pds'. The results revealed that the mean (+S.E.) PJ for all the subjects in the 'REM state' was $0.073 \pm .005$ (101 eye mvts) which was not signif. different from the value of $0.078 \pm .003$ (166 eye mvts) for eye mvts in the waking state. Since small mvts tend to have higher PJ values than large mvts, the data were reanalyzed for mvts restricted to the 9^{o}-12^{o} range; these PJ values were $0.068 \pm .004$ for REMs, and $0.071 \pm .003$ for waking saccades----again an insignificant difference.

PJ appears to be sensitive to differences in motion which are not revealed by velocity or simple third derivative measures. While the latter parameters tend to be higher for mvts with the eyes open versus eyes closed, this is not true for PJ.

76.23 **NEUROPSYCHOLOGICAL AND EVOKED POTENTIAL MEASURES OF COGNITIVE FUNCTION IN SLEEP APNEA.** **V.L. Rothenberger*, J.A. Walsleben*, and N.K. Squires* (SPON: M.A.Nathan). Dept. Psy., State Univ. New York, Stony Brook, 11794.**

Sleep apnea is a syndrome characterized by repeated episodes of cessation of respiration during the night, which may result in severe hypoxia and disrupted sleep. Daytime symptoms of sleepiness and cognitive deficits are common. Treatment with continuous positive nasal air pressure (nCPAP) produces rapid and dramatic relief of the daytime symptoms. This study was undertaken to document the subjective complaints and to determine whether the daytime symptoms are the result of brain dysfunction as a consequence of the repeated hypoxic episodes, or whether they reflect sleepiness produced by repeated disruptions of sleep. Eight subjects with sleep apnea were given a battery of neuropsychological and evoked potential tests. Testing was performed on the evening prior to treatment with nCPAP, on the night following the initiation of treatment, and four months after continued nightly treatment.

Clinical evaluation of sleep parameters performed before the initiation of treatment indicated that sleep was profoundly disturbed prior to treatment. Neuropsychological testing indicated deficits in measures of attention and memory. Recordings of the auditory brainstem evoked responses (ABRs) showed slowed conduction in the auditory pathway, and prolonged latencies of the long-latency components of the auditory evoked response (N2, P3) indicated delays in the speed of cognitive decision processes.

Tests performed after the initiation of nCPAP treatment indicated that the subjects' sleep patterns had returned almost to normal values. Also, the latency of the P3 component normalized. There was no change in neuropsychological performance or in the ABRs. At the four-month follow-up test essentially the same pattern was observed; the ABRs still indicated slowed conduction in the auditory pathway while the long-latency evoked potentials remained in the normal range.

Overall the data confirm that sleep apnea produces pervasive psychological deficits. The ABR results indicate that there may be permanent changes in brain function, perhaps as a result of the repeated hypoxic episodes. Other abnormalities, particularly the excessive sleepiness and the reduced speed of cognitive processes are more transient and may involve disruption of brain metabolism that resolves rapidly after treatment with nCPAP. Also, the data indicate that cognitive evoked potentials are sensitive to brain dysfunction that does not involve structural damage and that these potentials may provide a sensitive index of treatment efficacy.

77.1 COMPARISON OF TETRAHYDROAMINOACRIDINE AND PHYSOSTIGMINE AFTER ACUTE AND CHRONIC ADMINISTRATION IN RATS. K.A. Sherman and E. Messamore*, Dept. of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

Tetrahydro-9-aminoacridine (tacrine, THA) reportedly results in marked improvement in patients with Alzheimer-type dementia (Summers et al., NEJM 315:1241, 1986). This contrasts with the relatively circumscribed and variable response to physostigmine (PHYSO) (Sherman et al., In: CNS Disorders of Aging, 1987, in press). THA is a reversible inhibitor of brain and red blood cell acetylcholinesterase (IC_{50}: 1 μM) with only slightly less potency than PHYSO in vitro (IC_{50}: 0.3-0.5 μM). To characterize the pharmacological properties of THA after in vivo administration, we examined the effects on behavior (maintenance of splay posture and inhibition of forward locomotion in an open field) and brain cholinergic mechanisms following acute and chronic s.c. injection of THA. These results were compared with previous findings after PHYSO. Inhibition of forward walk occurred by 1 hr and hindlimb splay posture was affected by 2 hr after acute THA treatment; both effects lasted for more than 4 hr after 15 mg/kg THA·HCl s.c. By contrast, effects of a maximal dose of PHYSO sulphate (0.88 mg/kg, s.c.) occurred within 15 min, but lasted less than 2 hr. During the first two weeks of repeated administration of THA (10 mg/kg, s.c., b.i.d.), tolerance developed to the effects on splay posture and forward locomotion. Increasing the dose of THA to 15 mg/kg partially reinstated the locomotor deficit, but not the effect on splay. Tolerance to the locomotor deficit occurred within 3 d of continued treatment with the higher dose. Acute administration of THA (10 mg/kg, s.c.) resulted in inhibition of in vitro high affinity choline uptake (HACU) in P_2 fractions from hippocampus (-31 ± 4% of control) and cortex (-20 ± 4%), but did not affect striatal HACU. After 48-54 d of THA administration, the effects on HACU were less in hippocampus (-19 ± 3%) and cortex (-12 ± 3%); striatal HACU was unaffected. The inhibition of hippocampal HACU after acute PHYSO (0.88 mg/kg) was greater (-44 ± 5%) and this effect was not altered with repeated administration (-45 ± 5%). Although the inhibition of acetylcholinesterase (52-58%) was comparable in the various brain regions, acute PHYSO resulted in elevation of HACU in cortex (+20 ± 11%), whereas repeated administration resulted in reduced cortical HACU (-20 ± 11%) (acute vs. chronic PHYSO: $p<.05$). Neither acute nor chronic PHYSO affected striatal HACU. Thus, THA has a longer duration of action and produces less presynaptic compensatory inhibition after acute treatment; tolerance to THA is more profound behaviorally and neurochemically. These differences in pharmacological characteristics may contribute to a different spectrum of clinical activity. (Supported in part by SIU-CRC.)

77.2 DIFFERENTIAL EFFECTS OF THREE CHOLINESTERASE INHIBITORS ON BRAIN ACETYLCHOLINE. M. Hallak* and E. Giacobini (SPON: M. Downen). Department of Pharmacology, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, IL 62708 USA

The effect of three drugs producing inhibition of cholinesterase (ChE), physostigmine (PHY), metrifonate (MTF) and tetrahydroaminoacridine (THA) on the activity of ChE and on levels of acetylcholine (ACh) and choline (Ch) on the brain of the rat were compared. Both single-dose and multiple-dose regimens were studied. Following a first i.m. dose of the inhibitor (PHY, .3 mg/kg; MTF, 80 mg/kg and THA, 20 mg/kg) ACh levels increased 70%, 45% and 70%, respectively. A second and subsequent administration of PHY and MTF produced the same increase in ACh. On the contrary, THA failed to raise ACh levels inspite of persisting 40% ChE inhibition. Toxic symptoms were more severe with THA. In addition, THA (1 mM) inhibited K^+-stimulated ACh release from striatal slices. ^{3}H-Ch pools were also lower following THA treatment. Cholineacetyltransferase activity was not inhibited by any of the three inhibitors at a wide range of concentrations. The results of this study reveal some major differences among the effects of these substances in brain and between the effect of single and repeated administrations of the drugs on CNS levels of ACh. These findings have potential clinical implications for the symptomatic therapy of Alzheimer patients, suggesting new strategies and modes of administration of ChE inhibitors in order to achieve optimal effects at minimal-toxic levels. (Supported by National Institute of Aging #AG05416-01A1)

77.3 ACETYLCHOLINESTERASE INHIBITION: A FUTURE THERAPY FOR ALZHEIMER DISEASE? E. Giacobini, M. Hallak* and R. Becker. Departments of Pharmacology and Psychiatry, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, IL 62708 USA

A decline in memory and cognitive function during physiological aging and in senile dementia of Alzheimer type have been related to a decline in cholinergic function. One approach to improve cholinergic activity in brain is to increase acetylcholine (ACh) levels by inhibiting the enzyme acetylcholinesterase (AChE). We compared the effects of various types of cholinesterase (ChE) inhibitors [physostigmine (Phy), metrifonate (MTF) and tetrahydroaminoacridine (THA)] on experimental animals and humans (Hallak, M. and Giacobini, E., Neuropharmacology, 26:5, 1987). The effect of various routes of administration of ChE inhibitors on plasma and brain ChE activity, as well as on ACh levels in brain was studied (Hallak, M. and Giacobini, E., Neurochem. Res., 11:1037-1048, 1986; Mattio, T. et al., Neuropharmacology, 25:1167-1177, 1986). We studied the relationship between ChE inhibition in plasma, red blood cells (RBC) and brain. A linear correlation was found between the percent of ChE activity in brain and the percent of ChE activity in plasma or RBC following i.m. administration of Phy. With MTF i.m. after 60 min, brain, plasma and RBC ChE are also inhibited in the same proportion with a linear relation between ChE activity in plasma vs. ChE in brain. Thus, in the rat, the general pattern of ChE inhibition in plasma is similar to that in brain. If this relation is true for humans as well, plasma ChE activity could be utilized as an index of Phy and MTF effects in the CNS. In addition, based on our results in the rat, it seems possible to correlate directly Phy concentration in plasma with the ChE inhibition in brain. These conclusions have clinical implications suggesting new strategies and modes of administration of ChE inhibitors in order to achieve maximal therapeutical effects at nontoxic levels (Giacobini, E. and Hallak, M., In: Senile Dementias: Early Detection, pp. 337-341, 1986). (Supported by National Institute of Aging #AG05416-01A1)

77.4 PROGRESS IN THE SEARCH FOR PROTECTANTS AGAINST SOMAN TOXICITY G.H. Sterling, P.H. Doukas*, K.J. O'Neill* and J.J. O'Neill
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Soman is an organophosphonate cholinesterase inhibitor. It is, therefore, generally assumed that the acute toxicity following exposure is the result of excessive accumulation of acetylcholine (ACh). Standard therapy with atropine, artificial respiration and oxime is ineffective due to the rapid "aging" of soman-inactivated cholinesterase rendering the enzyme insensitive to reactivation. In this study, compounds known to affect presynaptic disposition of ACh were evaluated as potential enhancers of the standard treatment regimen. Studies in our lab with rats demonstrated in vivo protection afforded by compounds exhibiting in vitro activity as inhibitors of high affinity choline uptake (HAChU) and choline acetyltransferase, (ChAT). N-allyl-3-quinuclidinol (NAQ), a potent inhibitor of HAChU developed in this lab and N-hydroxyethyl-naphthylvinylpyridine (NHENVP), a quaternary ChAT inhibitor, significantly reduced the mortality following an LD-90 dose of soman in the presence of atropine and 2-PAM. We have recently begun to evaluate in vitro inhibitors of ACh synthesis and release for protective efficacy in a guinea pig model. Animals were given Soman (S.C.) followed one minute later by atropine (16 mg/kg, I.M.). Compounds to be evaluated were administered I.M. at 2 min to 2 hrs prior to the soman. Atropine alone, significantly increased the LD-50 of soman from 28 ug/kg to 46 ug/kg. NAQ enhanced the protective effect of atropine; when given at 100 umol/kg, I.M., the compound reduced the overall percent mortality of soman (50 ug/kg) from an LD-80 (in the presence of atropine alone) to an LD-10. NAQ was most effective when administered 30-60 min prior to soman. NHENVP (3-10 umol/kg, I.M.) also enhanced the protective effect of atropine, reducing overall mortality to an LD-20. In contrast to NAQ, NHENVP was most effective when administered 2-30 min prior to soman. At doses of soman producing an LD-80 at 1 hr post-soman, in the presence of atropine, both NAQ and NHENVP prevented deaths at that time point.

The results confirm our previous findings in rats. It is suggested that compounds that disrupt presynaptic ACh synthesis in vitro may prove effective in treating organophosphate poisoning. The data provide insight for the design of protectants against soman toxicity. Further studies are underway to determine if the protective effects of these compounds correlate with their ability to reduce ACh levels in vivo. (This work was funded by USAMRDC - DAMD17-86-C-6243).

77.5 RELEASE OF CHOLINESTERASE FROM RAT CAUDATE NUCLEUS. P. DeSarno*, E. Giacobini and M. Downen (SPON: J. Couch). Department of Pharmacology, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, IL 62708 USA

It has been suggested that cholinesterase (ChE) may be released from neuronal tissue following high K^+ (Greenfield, S. et al., Nature, 284:355-357, 1980; Greenfield, S. et al., J. Comp. Neurol., 214:87-92, 1983) or drug stimulation (Bareggi, S.R. and Giacobini, E., Neurosci. Res., 3:335-339, 1978; Scarsella, G. et al., J. Neurosci. Res., 4:19-24, 1979; Greenfield, S. and Shaw, S.G., Neurosci., 7:2883-2893, 1982). We have studied the release of ChE from CNS using rat caudate slices following: a) electrical stimulation (5, 10, 100 Hz, 10 min); or b) high K^+ (50 mM, 10 min; 105 mM, 60 min) by means of continuous superfusion or tissue incubation. ChE release was monitored, following equilibration (15-75 min) in oxygenated Krebs solution, for a period of up to 120 min. ChE molecular forms were also analyzed in the perfusate and in the caudate. Acetylcholine release was also measured under these conditions. In non-stimulated controls, spontaneous release of ChE was measured starting 15 min after dissection. This release showed a continuous decrease in time up to 3 hrs. Cholinesterase activity increased significantly following 5 Hz electrical stimulation and 105 mM K^+ stimulation. K^+ (105 mM) evoked ChE release was more long-lasting than electrically evoked release (5 Hz). Our results demonstrate that electrical stimulation (5 Hz) but not 50 mM K^+ stimulation increase the spontaneous release of ChE from caudate slices. In addition, prolonged depolarization with 105 mM potassium which produces a complete but reversible depletion of acetylcholine from synaptic vesicles (Gennaro, J.F. et al., J. Physiol., 280:237-247, 1978) also increased the spontaneous release of ChE. In conclusion, these results suggest that ChE can be released from the CNS following neuronal depolarization and that this release is different from acetylcholine release. The demonstration of a normal release of ChE might help us to interpret changes in CSF ChE activity under normal and pathological conditions in humans (Giacobini, E., Neurobiol. of Aging, 7:392-396, 1986). (Supported by National Institute of Aging #AG05416-01A1).

77.6 INCREASED CHOLINE ACETYLTRANSFERASE ACTIVITY IN A HUMAN CHOLINERGIC NEUROBLASTOMA CELL LINE IS CONTROLLED BY POST-TRANSLATIONAL MECHANISMS. D. Casper and P. Davies*. Departments of Neuroscience and Pathology, Albert Einstein College of Medicine, The Bronx, New York, 10461.

Human neuroblastoma clone MC-IXC, originally isolated by Dr. J. Biedler at the Memorial Sloan-Kettering Cancer Center, Rye, New York, was used to investigate the mechanism of regulation of the enzyme choline acetyltransferase (ChAT). We have previously shown that retinoic acid, sodium butyrate, and high cell density all increase the specific activity of ChAT several fold from its basal levels in these cells. This increase can be expressed with respect to either protein content or cell number, as they are directly related under inducing or control conditions.

We now report that inhibitors of either messenger RNA or protein synthesis, namely α-amanitin, emetine and cycloheximide do not block the induction of ChAT activity by either retinoic acid or sodium butyrate. Cycloheximide concentrations of 1 μg/ml for 24 or 48 hours effectively block both protein synthesis and cell division, as demonstrated by rate of uptake of labelled precursors and incorporation into the appropriate macromolecules. The agent also appears to increase the half-life of the enzyme, as specific activity increases upon incubation with cycloheximide in the absence of other inducing agents. Using cycloheximide, no decrease in specific activity is measurable over 48 hours, suggesting a low rate of turnover of this enzyme, consistent with its low abundance in all cell types which contain it. Cycloheximide also potentiates the activation of ChAT by 0.5mM sodium butyrate, suggesting decreased synthesis of an inhibitor, perhaps with protease activity. Similarly, naphthylvinyl pyridinium derivatives, which specifically inhibit ChAT activity, have been used in a similar fashion in culture to demonstrate the lack of measureable new ChAT synthesis for up to 48 hours.

Kinetics experiments provide further evidence for a binary process of activation or stabilization of ChAT molecules. Lineweaver Burke analysis for retinoic acid-activated ChAT demonstrates an increase in the Vmax of ChAT of approximately 2.2 fold with respect to both acetyl CoA and choline that can account for the concommitant increase of the same magnitude in ChAT activity without changes in the Km for either substrate.

Combined with the data obtained using mRNA and protein synthesis inhibitors, it appears that ChAT activity in this system is regulated by either the activation of existing ChAT molecules or by the stabilization of those previously active.

77.7 N-TERMINAL SEQUENCE OF CHICK BRAIN CHOLINE ACETYLTRANSFERASE. J.L. Dahl, D.B. Bloom*, M.L. Epstein†, and C.D. Johnson‡. Depts. of Pharmacology, Anatomy†, and Zoology‡, University of Wisconsin, Madison, WI 53706.

Choline acetyltransferase was purified to apparent homogeneity from chick brain and optic lobes and the amino terminal amino acid sequence determined for 13 cycles by automated amino acid sequencing procedures. The purification procedure involved polyethylene glycol precipitation, followed by chromatography on CM-Sephadex and a monoclonal antibody affinity column. Purification could also be accomplished by these chromatographic steps after the enzyme was partially purified by acid and $(NH_4)_2SO_4$ precipitation. When subjected to SDS-polyacrylamide gel electrophoresis, the purified enzyme ran as a single band with an apparent molecular weight of 68,000 daltons. In preparation for amino acid sequence determination, immunoaffinity-purified ChAT was concentrated in a Centricon microconcentrator whose membrane had been washed with 25% acetic acid and then with water. After an initial concentration step, the protein was washed with three successive 500 µl portions of 0.1 M NH_4HCO_3, after which the NH_4HCO_3 was removed by lyophilization. The amino-terminal sequence of the protein was determined with an average repetitive yield of 91%. Sequence analysis was performed on an Applied Biosystems 470A Protein Sequencer using no vacuum programs and methanolic HCl conversion. Resulting phenylthiohydantoin derivatives of amino acids were identified and quantitated by high performance liquid chromatography on an IBM Instruments LC/9533 using an IBM C-18 column. The first 13 residues are: pro-asp-leu-glu-lys-asp-met-gln-lys-lys-glu-lys-asp.

77.8 EXPRESSION OF THE DROSOPHILA CHOLINE ACETYLTRANSFERASE GENE IN ESCHERICHIA COLI. H. Sugihara*, V. Andrisani* and P.M. Salvaterra (SPON: J.K. Ono). Division of Neurosciences, Beckman Research Institute/City of Hope, Duarte, CA 91010.

The character of a neuron can be defined by the specific neurotransmitter which it uses to stimulate its target cells. Choline acetyltransferase is the specific enzyme for synthesis of the neurotransmitter acetylcholine. To investigate gene regulation of the cholinergic neurotransmitter phenotype, we have isolated a cDNA clone for this enzyme (Itoh, et al., Proc. Natl. Acad. Sci. USA 83:4081-4085).

RNA analysis of Drosophila head poly $(A)^+$ RNA using the cDNA insert as a probe showed the choline acetyltransferase mRNA to be $\approx$4.7K nucleotides long. The isolated cDNA consists of a coding region that is 2190 nucleotides long, followed by a 3'-noncoding region 284 nucleotides in length. This cDNA clone is thus not full length. To isolate longer Drosophila choline acetyltransferase clones, we have screened several Drosophila head cDNA libraries using this cDNA insert as a probe. We have identified several positive clones with inserts up to 3.3 Kbases and are currently characterizing them in detail.

The coding region of our original cDNA clone spans 728 amino acids which is longer than that required for synthesis of the Mr 67K protein that is estimated for purified Drosophila choline acetyltransferase. Using this insert we constructed plasmids which express choline acetyltransferase activity in E. Coli host JM105 by subcloning into the expression vector pkk223-2 (Pharmacia). This plasmid contains a trp-lac promoter, and one construct we have isolated expresses choline acetyltransferase activity after addition of isopropyl-β-D-thiogalactoside (IPTG). The coding region of our cDNA clone thus has enough information to produce active enzyme.

Supported by NIH-NINCDS.

77.9 EFFECT OF DIISOPROPYL PHOSPHOROFLUORIDATE (DFP) ON CA3 AND CA1 RESPONSES IN RAT HIPPOCAMPUS. D. Lapadula*, L.S. Jones, M. Abou-Donia, and D.V. Lewis. Depts. of Pharmacology and Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27710.

Previous work by others has shown that DFP does not have a marked effect on hippocampal CA1 neurons (Williamson and Sarvey, Neurosci. Abs. 10:167.12). As an extension of our own studies on the role of [Ca2+]i in mediating the neurotoxic effects of DFP, we have examined the effects of DFP on the CA3 and CA1 subfields of rat hippocampus and found that it is a potent epileptogenic agent for CA3 neurons.

625 μ slices from adult, male Sprague-Dawley rats were prepared in the normal fashion. A monopolar stimulating electrode was placed in s. radiatum between the two recording electrodes, which were placed in s. pyramidale of CA1 and CA3 so that the effects of DFP could be monitored in both regions simultaneously. The slices were continuously perfused with artificial cerebrospinal fluid (ACSF); DFP was tested at various concentrations (1-500 μM) by adding it hyperosmotically to the ACSF bathing the slice.

Results showed that while 1 μM DFP had little or no effect on the responses from either region, 10 μM DFP produced spontaneous bursting in CA3 and CA1 within 10 minutes of being washed onto the slice. The bursts were frequent, complex, and apparently irreversible with washing. 5 μM atropine delayed, but did not reliably block the appearance of these bursts, and neither 5 nor 10 μM atropine could reverse the bursting, although the frequency did decrease with time. The effects on CA1 were less marked, in that the bursts were smaller than those in CA3 and appeared to be slightly delayed. The latter observation may indicate that the epileptic activity in CA1 is a downstream reflection of the activity in CA3, rather than of intrinsic hyperexcitability in CA1. At high concentrations of DFP (above 25 μM), the DFP suppressed triggered responses in both regions, though spontaneous bursting of CA3 continued.

The greater epileptogenic effect of DFP on CA3 than CA1 is in agreement with studies using convulsant drugs such as penicillin or bicuculline, and further emphasizes the dissimilarities between CA3 and CA1. Additional studies on the effects of DFP on CA3 neurons may provide information on the mechanisms of epileptogenesis generally, and the convulsant properties of organophosphates specifically.

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77.10 **ACTIONS OF PYRIDOSTIGMINE AND CARBACHOL ON DORSOLATERAL SEPTAL NEURONS STUDIED INTRACELLULARLY FROM RAT SEPTUM *IN VITRO*.** Linda A. Wong*, Hiroshi Hasuo* and Joel P. Gallagher (SPON: O.S.Steinsland). Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550.

Previous anatomical and *in vivo* studies have suggested that dorsolateral septal neurons are cholinoceptive. However, little is known about the direct membrane actions of cholinergic agents on these neurons. Our current findings with pyridostigmine, a reversible cholinesterase inhibitor, and carbachol, a mixed cholinergic agonist, indicate that both pyridostigmine and carbachol activate nicotinic receptors, directly, to alter membrane properties and synaptic responses of dorsolateral septal neurons. Conventional intracellular techniques were used when recording from dorsolateral septal neurons in a rat septal slice preparation. Drugs were applied by superfusion. Carbachol (1 to 100 μM) caused a concentration dependent membrane hyperpolarization of dorsolateral septal neurons which was associated with a decrease in membrane input resistance and suppression of spontaneous firing activity. Atropine (2 μM) decreased but did not block the response to carbachol. Mecamylamine (20 μM), however, completely blocked the effect of carbachol, indicating that nicotinic receptors might be involved. Superfusion of pyridostigmine (1 μM) also produced effects similar to carbachol, i.e., membrane hyperpolarization accompanied by a decrease in membrane input resistance and suppression of spontaneous firing. In both cases, spontaneous activity returned with enhanced firing and bursts of action potentials after the wash out of drugs. When the superfusion solution was altered by changing to a low calcium/high magnesium composition, the hyperpolarizing effect of pyridostigmine persisted. Mecamylamine (10 μM), but not atropine (10 μM), was able to block the hyperpolarizing action of the drug. These results suggest that pyridostigmine was acting directly on a postsynaptic nicotinic receptor. In addition, pyridostigmine affected the synaptic response evoked by focal stimulation of the dorsolateral septum. In particular, the late hyperpolarizing potential was depressed.

Our findings indicate that: (1) cholinoceptive neurons are present in the dorsolateral septal nucleus; (2) cholinergic effects of carbachol and pyridostigmine are mediated through nicotinic receptors; and (3) synaptic transmission at dorsolateral septal neurons is affected by cholinergic agents. (Supported by DAMD-17-86-C-6032).

77.11 EFFECTS OF CARBACHOL ON PYRAMIDAL NEURONS IN THE IN VITRO HIPPOCAMPAL SLICE. L.S. Leung and C.Y. Yim. Depts. of Clinical Neurological Sciences and Physiology, Univ. of Western Ontario, London, Canada. N6A 5A5

Effects of perfusion of carbachol (CCH) at 10^{-3} to 10^{-5} M on pyramidal neurons in the in vitro rat hippocampal slices were examined in a series of 40 experiments. Stable intracellular recordings (characterized by resting membrane potential of > 60 mV with overshooting action potentials) were made from more than 30 pyramidal neurons in the CA1 and CA3 layers. In all neurons recorded, CCH perfusion produced 5-10 mV membrane depolarization which was in 80% of the neurons accompanied by a clear increase in membrane resistance. Excitatory postsynaptic potentials (EPSPs) following stimulation of the stratum oriens or stratum radiatum were attenuated by CCH. The attenuation was not entirely due to membrane depolarization since restoring the membrane potential to the control value by passing hyperpolarizing currents failed to fully restore the attenuated EPSP. Inhibitory postsynaptic potentials (IPSPs) following stratum radiatum or stratum oriens stimulation were were similarly attenuated by CCH. It was observed, however, that restoration of the somatic membrane potential to the normal resting potential by current injection consistently resulted in a partial reversal of the IPSP compared to the control. Modeling of the pyramidal cells as contiguous cable compartments indicates that IPSP reversal does not result from conductance changes only at the soma when the somatic membrane potential is restored. Other possibilities, including a shift in the IPSP equilibrium potential by CCH, are being considered. Extracellular field potential recordings indicated that both the population EPSP and population spike were attenuated while the threshold and latency of the evoked population spike were increased by CCH. These results suggest that CCH may produce selective depolarization of the soma, and that presynaptic inhibition of both excitatory and inhibitory afferents seems probable.

(supported by MRC and PSI)

77.12 ELECTROGRAPHIC CHANGES FOLLOWING LESIONS OF RODENT NUCLEUS BASALIS. P.G. RAY* and W.J. Jackson. Department of Physiology and Endocrinology, Medical College of Georgia, Augusta, GA 30912.

Changes in central cholinergic activity have been closely related to changes in the EEG. We are currently investigating EEG frequency changes which follow damage to the major source of cortical cholinergic innervation in the rat, the nucleus basalis magnocellularis.

Male Sprague-Dawley rats were infused bilaterally with 4ug ibotenic acid in .8ul phosphate-buffered saline. Screw electrodes were implanted in the skull over frontal, parietal and visual cortices. Sham-operated animals served as controls. Following a 14-day recovery period, each animal was familiarized with the recording chamber during five daily sessions. During the final recording session, the animal was allowed to sleep and was aroused by the presentation of an auditory stimulus lasting 8 seconds. All EEG data during the session were recorded by polygraph and stored on magnetic tape. The animals were sacrificed and choline acetyltransferase (ChAT) activity was determined in each cortical region.

The EEG data were analyzed by determining the spectral power from 1-31 Hz at 1 Hz increments using a Fast Fourier Transform routine. Three 4s epochs of EEG representing the sleep period immediately prior to arousal and two 4s epochs during stimulus presentation were analyzed. 1-12 Hz activity was expressed as a percent of the total power (1-31 Hz) in each epoch and averaged to determine a final value of percent slow wave activity (%SWA) present during sleep or arousal.

In the frontal region of lesioned animals, the level of ChAT activity ranged from 0-45% below that of controls. A significant negative relationship was shown to exist between the level of ChAT activity and the %SWA present during sleep ($r = -.5489$, $p < .005$) and during the aroused state ($r = -.5074$, $p < .05$). This finding is in accord with the suggested role of acetylcholine in influencing EEG background rhythms. However, this ChAT activity-EEG frequency relationship was apparent only in the frontal cortex. In the parietal area, where comparable ChAT declines were seen, no similar correlations were found. Also with regard to frontal EEG activity, the lesioned animals, as a group, showed a greater response to the stimulus, as indicated by a larger decline in SWA on arousal than that shown by controls (Mann-Whitney U Test, $p < .05$). There was a similar tendency seen in the parietal EEG but the difference there did not reach statistical significance. This enhanced response to the stimulus may be related to attentional disturbances, as a result of changes in the cortical signal-to-noise ratio (Warburton, D.M. From: Inhibition and Learning, Boakes, R.A. and Halliday, M.S. (eds.), 431-460, 1972). ChAT activity levels declined no more than 25% in the visual cortex; no correlative relationships were seen.

77.13 CHARACTERIZATION OF LATERAL DORSAL TEGMENTAL NEURONS, IN VIVO, BY ANTIDROMIC AND ORTHODROMIC ACTIVATION. S.J. Grant, Dept. Psychology, Univ. of Delaware, Newark, DE 19716, G.R. Christoph, Neuroscience Section, E.I. Dupont de Nemours and Co., Inc., Wilmington, DE 19898, A.Y. Deutch, Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The lateral dorsal tegmental nucleus (LDT) lies rostral and medial to the locus coeruleus (LC) in the pontine tegmentum. Anatomical studies have shown that the LDT consists of cholinergic and peptidergic neurons which project primarily to limbic structures. Although the anatomy of the LDT is now well characterized, the present studies provide the first description of LDT neuronal activity.

Extracellular single unit activity was recorded from chloral hydrate anesthetized rats. After localizing the LC in vivo, recordings were made in a region extending up to 0.6 mm medial and 1.5 mm rostral to the LC. This region represents the boundaries of the Ch6 cholinergic cell group in the LDT. Recording sites were histologically verified by depositing a small amount of dye from the tip of the recording pipette at the end of each successful penetration.

To date, 74 neurons have been recorded from the LDT in 12 rats, and 37% responded to single pulse electrical stimulation of known projection areas. Nearly all LDT neurons had large, initially positive action potentials, and most LDT neurons responsive to stimulation (70%) were not exhibit spontaneously active. LDT neurons were antidromically activated from the antero-ventral thalamus (AV), dorsal bundle (DB), and ventral tegmental area (VTA). These neurons had slow conduction velocities (0.23-0.63 m/s) indicative of fine, non-myelinated axons. LDT neurons were also orthodromically activated from the medial pre-frontal cortex (MPFC), AV, VTA, and lateral habenula (HAB). Stimulation of all of these sites evoked a marked excitation of LDT neurons except for HAB which was inhibitory. In addition, some LC neurons located in the rostral A6 region also were orthodromically activated by MPFC stimulation. Afferent projections to the LDT and LC from the MPFC and VTA were confirmed using anterograde tracing techniques (PhAL).

It is likely that some of these recordings correspond to Ch6 cholinergic neurons since dye spots were located within clusters of NADPH diaphorase positive cells (a histochemical marker for brainstem cholinergic neurons).

REGULATION OF AUTONOMIC FUNCTION I

78.1 NEUROPEPTIDE ACTIONS AND INTERACTIONS WITH NON-CHOLINERGIC POTENTIALS EVOKED BY URETERIC NERVE STIMULATION IN THE GUINEA PIG INFERIOR MESENTERIC GANGLION (IMG) IN VITRO. A. Dray, R. Amann and M. Hankins. Sandoz Institute for Medical Research, London, England.

The ureter is densely innervated with sensory nerve fibers (Sikri et al 1982, J. Anat. 133, 425) some of which project via the i.m.g. Ureteric fibers contain and release a number of neuropeptides including calcitonin-gene related peptide (CGRP), substance P(SP), neurokinin A (NKA) and neuropeptide K (NPK), a 36 amino acid peptide containing the NKA sequence at its C-terminal end. These peptides are possible neurotransmitters mediating non-cholinergic depolarization in the i.m.g. evoked by ureter distension or ureteric nerve stimulation (Amann et al 1987, J. Physiol. press). To examine this we have compared the effects of these peptides on i.m.g. neurones and their interactions with the ureteric nerve evoked slow-e.p.s.p. Adult guinea pigs were killed by cervical dislocation. The i.m.g. with attached nerve trunks and both ureters, was removed to a recording chamber and superfused with oxygenated Tyrode solution (37°C, flow 5ml/min) containing atropine and hexamethonium. One ureteric nerve branch (UN) was stimulated with a bipolar platinum electrode and intracellular recordings were made from principal ganglion cells using a 3 M KCl-filled micropipette. Drugs were administered in the superfusate. Repetitive stimulation of UN evoked a s-e.p.s.p. in 45% of randomly sampled cells. Membrane conductance was usually unchanged though in some cells a small increase occured. The amplitude and duration of the s-e.p.s.p. was related to the stimulus intensity and the membrane potential. The s-e.p.s.p. was abolished by TTX (1μM), by low calcium (0.2mM) high magnesium (12mM) perfusion or following desensitization of C-fibers with capsaicin (2μM). Most neurons (80%) were depolarized by SP (1μM) with either an increase or decrease in membrane conductance. NKA (1μM) and NPK (1μM) depolarized a similar proportion of cells (50 and 54% respectively). Membrane conductance was increased or decreased by NKA but consistently decreased by NPK. However NPK also hyperpolarized some cells (9%) and occasionally a sequence of hyperpolarization followed by depolarisation was observed. The similar actions of NKA and NPK suggest that in the majority of cases the activity of NPK was mediated by its C-terminal sequence. CGRP (1-10μM) was generally ineffective though some cells were depolarized (13%)or hyperpolarized (6%). The UN evoked s-e.p.s.p. was attenuated or abolished in the presence of supramaximal concentrations (2μM) of SP, NKA or NKP but unaffected by CGRP. These observations suggest that the UN evoked s-e.p.s.p. is produced by primary afferent C-fiber activation and that SP, NKA or NPK but not CGRP may be mediators of this potential in the i.m.g.

78.2 EFFECTS OF THE INTRATHECAL ADMINISTRATION OF VIP, SUBSTANCE P AND CGRP ON THE MICTURITION REFLEX OF THE CAT. M. Kawatani, C. Takeshige*, and W.C. de Groat. Dept. Physiology, Medical School, Showa Univ., Tokyo, Japan and Dept. Pharmacology, Medical School, and Center for Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15261.

Previous studies revealed that a number of neuropeptides including vasoactive intestinal polypeptide (VIP), substance P and calcitonin gene related peptide (CGRP) are present in afferent neurons innervating the urinary bladder of the cat and rat. The present experiments were undertaken to determine whether the intrathecal administration (IT) of these peptides alters the central reflex pathways controlling bladder function in the cat.

In 25 chloralose anesthetized cats bladder activity was monitored by recording intravesical pressure via a urethral cannula. Pelvic and hypogastric nerves (HGN) and postganglionic nerves on the surface of the bladder were isolated for electrical stimulation or recording. Drugs were injected IT via a polyethylene tube inserted through the dura at the level of the sacral or middle lumbar spinal segments. In some cats an outflow cannula was also inserted IT to allow for superfusion of the cord with artificial CSF. Some animals were spinalized at the T_{12} segment 1-3 months prior to the experiment.

The administration of low doses of VIP (0.1-1 ug) elicited small increases (10-20%) in the frequency and/or amplitude of spontaneous bladder contractions in 50% of the cats. Larger doses of VIP (2-50 ug) markedly or completely depressed bladder activity and reflex firing on bladder postganglionic nerves. The threshold inhibitory dose of VIP ranged from 2-10 ug. The magnitude and duration of the depression were dose dependent, large doses producing inhibition lasting 1-1.5 hrs. VIP did not inhibit the micturition reflex when applied to middle lumbar segments. VIP did not affect reflex firing on the hypogastric nerves. The inhibitory effect of VIP was more prominent in chronic spinal cats in comparison to normal cats. VIP-inhibition was antagonized by the prior IT administration of VIP antiserum (1:500 dilution, 1 ul/min for 30 min).

CGRP (0.5-20 ug) also elicited a prolonged (20-30 min), dose dependent decrease in the amplitude and frequency of bladder contractions, whereas substance P (0.1-10 ug, IT) increased the frequency of bladder contractions for 10-20 min.

These data raise the possibility that neuropeptides which are contained in bladder afferent pathways may have a modulatory influence on the sacral reflex mechanisms controlling micturition.

78.3 EFFECT OF INTRATHECAL SUBSTANCE P ON MICTURITION CONTRACTIONS IN URETHANE ANESTHETIZED RATS. B. Mallory and W.C. de Groat. Dept. of Pharmacology, and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Evidence from previous studies suggests that substance P (SP) may be involved as a neurotransmitter in the reflex pathways controlling the urinary bladder. The present studies were undertaken to further explore the role of SP in the control of micturition.

Isometric micturition contractions were recorded in female Wistar rats anesthetized with urethane (1.2g/kg). The urinary bladder was cannulated transurethrally with PE 60 tubing and infused with saline to produce rhythmic bladder contractions. An L-2 laminectomy was performed and injections of SP or a substance P analog (pGlu5,MePhe8,Sar9)-SP 5-11 (SPA) dissolved in sterile water (1-5 ul) were made under the dura using a microsyringe and 30g needle.

Control injections of water had no effect on bladder contractions or baseline bladder pressure whereas SP (10-100ug) or SPA (25ug) elicited facilitatory or inhibitory effects. The onset of these effects was always within 5 min of the injection. The facilitatory effect of SP or SPA was noted in 8/11 rats. In the presence of ongoing bladder contractions, facilitation was characterized by a 50% or greater increase in the frequency of bladder contractions. The duration of facilitation was 2-60 min and was dose dependent. If the bladder volume was maintained below the micturition threshold so that the bladder was quiescent, the peptides initiated bladder contractions or increased baseline bladder pressure.

In two rats, a trimodal response was seen with an initial early excitation consisting of one or several bladder contractions within 3 min of injection followed by a period of complete inhibition of bladder contractions for 1.5-20 min which in turn was followed by a prolonged (20-90 min) facilitation of contractions.

In four rats the predominate effect of SP or SPA was a complete inhibition of bladder contractions however the first effect noted immediately after injection of SP (10-60ug) was an increase in baseline bladder pressure of 5-20 cm H_2O lasting 0.5-5 min followed by a prolonged inhibition (10-60 min) of bladder contractions and subsequent recovery. In one rat a short lasting (2-5 min) inhibitory response to SP or SPA was seen.

These data raise the possibility that SP may be involved in inhibitory as well as facilitatory mechanisms in the lumbosacral reflex pathways to the urinary bladder. The facilitatory effect of SP is most prominent which is consistent with the view that SP may be a transmitter in the afferent limb of the micturition reflex.

78.4 THE EFFECTS OF CAPSAICIN ON MICTURITION AND ASSOCIATED REFLEXES IN THE RAT. C.L. Cheng* and W.C. de Groat (Spon: A.M. Booth). National Defense Medical Center, Taipei, Taiwan, Rep. of China, Dept. of Pharmacology, and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261

Recent studies have revealed that the urinary bladder (UB) receives an innervation from two types of afferent fibers (myelinated and unmyelinated) which may subserve different functions. The role of these two types of afferents in the initiation of micturition and pseudo-pain responses (ie., arterial pressor reflexes) was examined in the present experiments by studying the effects of capsaicin (CAPS), a neurotoxin, which acts selectively on small diameter afferents.

Experiments were conducted on urethane anesthetized female Wistar rats, in which blood pressure (BP) and bladder pressure (BLDP) were recorded through carotid artery and urethral catheters. Spontaneous bladder contractions (ranging from 40-60 cm H_2O intravesical pressure) and associated increases in BP (11-28 mm Hg) were elicited by distension of the UB under constant volume conditions. Inhibitory reflexes to the bladder elicited by mechanical stimulation of the uterine cervix were also tested for their sensitivity to CAPS.

Small doses of CAPS (5-10 mg/kg, s.c.) produced a rapid onset (20-30 min) suppression of the arterial pressor responses accompanying each contraction of the UB but did not change bladder activity or inhibitory bladder reflexes to cervical stimulation. Large doses of CAPS (30-70 mg/kg, s.c.) abolished bladder activity for 18-24 hrs.

Chronic CAPS treatment (125 mg/kg, s.c. in divided doses on 2 consecutive days) 4 days prior to the experiment also markedly suppressed the arterial pressor responses associated with bladder contractions (4.6 $\pm$ 1.2 mm Hg in CAPS-pretreated rats versus 23.4 $\pm$ 9.2 mm Hg, vehicle controls) but did not significantly depress the amplitude of rhythmic contractions of the UB. However, these doses of CAPS shifted the volume threshold for inducing micturition (0.47$\pm$ 0.1 ml, in controls versus 0.62 $\pm$ 0.1 ml in CAPS-pretreated). CAPS also suppressed the eye wipe test and the bladder inhibition elicited by cervical stimulation.

These data indicate that afferent pathways from the UB which trigger arterial pressor responses and those from the uterine cervix which mediate bladder inhibition are very sensitive to CAPS and therefore are likely to be unmyelinated, whereas those from the UB which trigger micturition appear to be CAPS insensitive larger diameter myelinated axons. It is concluded that the nociceptive information from the UB is carried by a population of afferents distinct from those which initiate the micturition reflex.

78.5 CARDIOVASCULAR EFFECTS OF CHRONIC CERVICAL SPINAL TRANSECTION (ST) IN THE RAT: A MODEL OF AUTONOMIC HYPERREFLEXIA. J. W. Osborn, Jr., R. F. Taylor, and L. P. Schramm. Dept. of Biomed. Eng., The Johns Hopkins Sch. Med., Baltimore, MD. 21205

We have developed a chronic spinal rat model with a complete spinal transection at C8. We describe results of preliminary investigations of the determinants of mean arterial pressure (MAP) and of cardiovascular responses to distension of the urinary bladder after ST.

Male Sprague-Dawley rats were instrumented with chronic arterial and venous catheters for MAP measurement and intravenous drug administration respectively. A gastric cannula was also implanted for long-term administration of water and nutrients as described below. The rats were placed in a temperature controlled caging unit and allowed two days for recovery. On the second recovery day, food and water were removed and a continuous intragastric infusion of a liquid diet was started and maintained at a constant rate for the duration of the protocol. The study began the next day. Daily measurements of MAP were obtained two days before and 8 days after ST. A bladder cannula was implanted at the time of ST to facilitate bladder emptying. Body temperature was maintained at pre-ST levels by ventilating the cage with warmed humidified air.

For the two control days, MAP averaged 99 $\pm$ 4 (mean $\pm$ SE) and 100 $\pm$ 4 mmHg. MAP fell to 74 $\pm$ 3 mmHg the first day after ST and ranged between 79 $\pm$ 3 and 87 $\pm$ 4 mmHg (mean = 83 $\pm$2) for the duration of the study. One week after ST, pharmacological blockade of sympathetic or vasopressin (AVP) vasoconstrictor actions had no significant effect on MAP. In contrast, angiotensin II (AII) blockade resulted in a 27 $\pm$ 4 mmHg fall of MAP.

Small stepwise increases of bladder pressure from zero to 20 mmHg increased MAP 15 $\pm$ 3 mmHg and did not significantly alter heart rate (HR). The same stimulus following atropine administration resulted in a small increase of HR (+12 $\pm$ 8 bpm) and an enhanced pressor response (+22 $\pm$ 3 mmHg). Alpha adrenergic blockade, in the presence of atropine, significantly attenuated the pressor response (+5 $\pm$ 4 mmHg) whereas the HR response was not changed (+12 $\pm$ 6 bpm).

These data suggest that 1 week after ST, there was minimal sympathetic vasoconstrictor activity, and chronic hypotension was only partially corrected by other mechanisms. MAP was supported by circulating AII whereas AVP vasoconstrictor activity was minimal. Sympathetic activity was reflexly increased at spinal levels in ST rats by modest elevations of urinary bladder pressure. Although supraspinal control of sympathetic activity was absent after ST, pressor responses to bladder inflation were buffered by baroreflex control of cardiac vagal activity.
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78.6 PROJECTIONS FROM CERVICAL SPINAL CORD TO LOWER THORACIC CORD IN THE RAT. J.A. Eberhart and L.P. Schramm. Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Previous reports from this laboratory suggest that neurons in cervical spinal cord play a role in the regulation of sympathetic activity. Although propriospinal connections between the cervical and lumbosacral enlargements have been thoroughly studied, cervical projections to thoracic spinal cord have attracted less attention. The purpose of the present study was to identify long, descending, propriospinal projections from cervical spinal cord that might mediate the observed sympathomodulatory effects.

Each of 25 rats received a unilateral pressure injection (10-50 nl) of 4% Fluoro-Gold (Fluorochrome, Inc.) at T9. Injections were made with a glass micro-pipette (15-25 μ tip) in either the dorsal horn or the intermediate gray matter. Survival time was either 5 or 20 days. Frozen horizontal sections of spinal cord were cut at 40 μ, and alternate sections were counterstained with thionin. Retrogradely labeled cells in unstained sections were plotted on projection drawings of adjacent, counterstained sections.

Larger (30-50 nl) injections (which labeled both dorsal horns, much of the intermediate gray matter, and part of the ventral horns in several segments of thoracic cord) resulted in many retrogradely labeled cervical neurons. In all cervical segments, labeled cells were located in the more lateral portions of the dorsal horns, and they were very numerous near the central canal. Labeled neurons were also found in the dorsolateral funiculi, particularly in the region of the lateral cervical and lateral spinal nuclei. There were also a few cells in the ventral horns. Smaller (10-20 nl) injections (labeling only one dorsal horn) produced many fewer retrogradely labeled cervical neurons scattered in the dorsolateral funiculi, dorsal horns, and near the central canal.

These results demonstrate that neurons in the dorsal horn, intermediate zone, and dorsolateral funiculus of the cervical spinal cord project directly to the lower thoracic cord. Some of the dorsolateral cervical neurons, in particular, may represent descending systems regulating sympathetic preganglionic neurons.

This work was supported by NIH grant HL16315 (LPS) and by the Hopkins/Dana Sabbatical Program (JAE).

78.7 CERVICAL SPINAL CORD NEURONAL RESPONSES TO GREATER SPLANCHNIC NERVE STIMULATION. E.W. Akeyson*, R. Sarrafizadeh* and L.P. Schramm. Depts. of Neuroscience and Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Visceral afferents are known to influence the firing of thoracic spinal neurons, and the response properties of such thoracic viscero-receptive neurons have been well studied. This afferent information can also influence spinal circuits at other spinal levels. Therefore, we have recorded responses of neurons in spinal segments C2-C4 to electrical stimulation of the left greater splanchnic nerve (GSN) in chloralose-anesthetized, artificially-ventilated rats.

A small fraction of cervical spinal neurons at C2-C4 responded to ipsilateral GSN stimulation. They were located in the marginal zones of laminae II through VII and in laminae IX. Most (91%) exhibited spontaneous activity with high average rates (mean= 21.9 ± 4.0 Hz). The majority of responsive neurons were inhibited by GSN stimulation (59%), but excitations (35%) or excitation/inhibition combinations (6%) were also observed. Inhibitions elicited by GSN stimulation were tightly locked to the stimulus, and they often lasted in excess of 100ms (range = 23-335ms). Excitations were characterized by one or two short duration bursts (mean= 48.6 ± 16.7 ms) of typically 2-8 spikes/ burst.

The majority of neurons received a convergent cutaneous mechanoreceptor input. Typical cutaneous receptive fields included the posterior aspect of the head, the neck, the shoulder, and the upper forelimb, but they rarely extended caudally to the thorax. A very small number of responsive neurons exhibited receptive fields which encompassed the entire side of the body from neck to lower limb.

Several neurons were tested extensively with electrical stimulation of the skin. In all cases, neurons inhibited by GSN stimulation were also inhibited by cutaneous stimulation, and vice versa. Neurons with cutaneous receptive fields restricted to the head, neck and shoulder were not affected by high intensity electrical stimulation (10mA) to the skin of the abdomen and lower limb.

These results indicate that there exists a small population of neurons in the gray matter of upper cervical spinal segments whose activity is modified by abdominal visceral afferents. Their responses to GSN stimulation are varied, and, therefore, they may constitute multiple visceral processing populations. Many neurons receive a cutaneous input from cervical dermatomes combined with a visceral input from abdominal "viscerotomes" while failing to exhibit cutaneous input from thoracic dermatomes. Supported by NIH grants HL16315 and 5-T32-GM07309-12.

78.8 MUSCARINIC AGONISTS POTENTIATE NICOTINE-STIMULATED CATECHOLAMINE SECRETION FROM ISOLATED-PERFUSED GUINEA PIG ADRENAL GLANDS. M.A. Oleshansky, Y. Nakazato*, R.M. Smejkal* and P.K. Chiang, Divisions of Neuropsychiatry and Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Several lines of evidence suggest that muscarinic and nicotinic receptors are involved in acetylcholine (ACh)-stimulated catecholamine secretion from adrenal medulla. It was originally noted that pilocarpine stimulated catecholamine secretion from perfused cat adrenal glands. Additionally, atropine was noted by several groups to decrease the ACh-stimulated release of catecholamines from the adrenals of various species. Subsequently, both muscarinic and nicotinic receptors have been identified in bovine chromaffin cells. Recent studies have demonstrated that muscarinic agonists potentiate nicotine-stimulated catecholamine release from isolated-perfused bovine adrenals and dispersed chromaffin cells. To further characterize the role of muscarinic receptor stimulation in ACh-stimulated catecholamine secretion, we examined the responses of selective cholinergic receptor agonists and antagonists on the isolated-perfused guinea pig adrenal preparation.

Both adrenal glands were perfused with Locke solution through a polyethylene cannula inserted into the lower aorta and the perfusates were collected from a cannula in the caudal vena cava. Drugs dissolved in Locke solution were perfused for one or two minutes. Norepinephrine (NE) and epinephrine (EPI) were assayed by a conventional HPLC method.

ACh stimulated NE and EPI secretion in a dose-dependent manner over the range of 10^{-6} to 10^{-3} M. Atropine (10^{-5} M) shifted the dose response curve to ACh to the right while hexamethonium (5 x 10^{-4} M) tended to decrease the response to higher concentrations of ACh with little effect on catecholamine secretion at the lower concentrations of ACh. ACh-stimulated catecholamine release was completely blocked by a combination of the two drugs. Nicotine or the muscarinic agonists, oxotremorine or pilocarpine, stimulated catecholamine release. One min pretreatment with oxotremorine (10^{-5} M), which only weakly stimulated catecholamine secretion, increased nicotine (5 x 10^{-5} M)-stimulated release of NE and EPI two fold. Application of nicotine (5 x 10^{-5} M) for one min every fifteen min produced progressively smaller increases of catecholamines secretion upon repeated exposure. Muscarinic agonists were able to reverse the tachyphylaxis seen upon repeated exposure to nicotine.

These experiments demonstrate that ACh-stimulated catecholamine secretion from isolated-perfused guinea pig adrenal glands involves both nicotinic and muscarinic receptor activation. Our findings support the recent work with dispersed bovine chromaffin cells which demonstrates that muscarinic agonists potentiate nicotine-stimulated catecholamine release. The dual regulation of ACh-stimulated catecholamine secretion through muscarinic and nicotinic receptors may be involved in both the potentiation and prolongation of the actions of ACh on the adrenal medulla under conditions of stress.

78.9 CATECHOLAMINE SECRETION FROM THE FELINE ADRENAL MEDULLA AS A FUNCTION OF TIME FOLLOWING HIGH SPINAL TRANSECTION. S.L. Stoddard, D.M. Gaumann*, G.M. Tyce and T.L. Yaksh*. Dept. of Anat., Indiana Univ. Sch. of Med., Fort Wayne, IN 46805 and Dept. of Neurosurg. Res., Mayo Clinic, Rochester, MN 55905.

Paraplegic patients with cord transections above the sympathetic outflow frequently exhibit the syndrome of autonomic hyperreflexia in response to a range of visceral and somatic stimuli. Although the manifestations of this syndrome are generally attributed to the release of norepinephrine (NE) from the sympathetic nerve terminals, the role of the adrenal medulla in this situation has not been investigated. Therefore, the purpose of this study was to determine the output of catecholamines from the adrenal medulla in response to both visceral and somatic stimuli following acute (1-4 days) and chronic (14-35 days) transection of the spinal cord at T2-T3. For the experiments, cats were anesthetized with halothane and rendered decerebrate. After decerebration, the halothane was removed. Blood pressure was continuously monitored through a femoral cannula and blood samples were withdrawn directly from the left adrenolumbar vein. Catecholamine levels were determined by HPLC. Bladder distention (60 mm Hg) and ipsilateral sciatic stimulation (50 Hz; 40V; 0.5 sec pulse width; 1 sec on, 1 sec off) were used as visceral and somatic stimuli, respectively. Neither stimulus increased mean arterial blood pressure (MAP) in acutely-transected cats, while both stimuli elicited increases in MAP > 30 mm Hg in chronically-transected cats. Furthermore, the mean increases in NE, epinephrine (EPI) and dopamine (DA) were notably greater in the chronically-transected animals (Table 1). These data suggest that, 1)there is a reorganization of adrenal medullary spinal control mechanisms that occurs by 2 weeks following high cord transection, and 2)autonomic hyperreflexia may involve significant adrenal medullary activation, which has not previously been described. [Supported by NS-15641 and AM-34988(TLY)].

TABLE 1: Mean changes in Catecholamine Levels (ng/ml)

	Bladder Distention		Sciatic Stimulation	
	Acute	Chronic	Acute	Chronic
NE	1.01	22.89	0.25	27.45*
EPI	2.79	16.74*	0.40	19.83*
DA	0.16	-0.73	-0.14	8.62

(*p ≤ 0.1 as compared to acute transection; n = 4, each group)

78.10 FREQUENCY DEPENDENT ADRENAL RELEASE OF CATECHOLAMINES AND NEUROPEPTIDES DURING SPLANCHNIC NERVE STIMULATION IN CATS. D. Gaumann[1]*, T. Yaksh[2]*, G. Tyce[3], D. Lucas[4]* and D. Roddy[4]* (SPON: R. Weinshilboum). Departments of [1]Anesthesiology, [2]Neurosurgery Research, [3]Physiology and [4]Gastroenterology, Mayo Clinic, Rochester, MN 55905.

Stimulation of the left major splanchnic nerve (SN) was performed in 6 cats to evaluate the frequency-dependent release of norepinephrine (NE), epinephrine (EPI), dopamine (DA), met-enkephalin (ME), neuropeptide Y (NPY) and neurotensin (NT) from the left adrenal. Experiments were performed under 1 MAC of halothane anesthesia and baseline samples S1 were taken 1 hr after the termination of the surgical procedure from the adrenal vein (AD) and the femoral artery (FA). Samples S2 were taken during SN stimulation at 5 Hz (1 msec stimulus, 30 volts, train rate 0.5 sec), and samples S3 under SN stimulation of 50 Hz. Plasma levels of catecholamines (CA) and neuropeptides (NP) under baseline conditions with a MABP of 111±14 mm Hg (mean ± SE) are shown in Table 1. EPI, NE and NPY levels in the AD were consistently higher than in the FA, which points to their selective adrenal release under baseline conditions. SN stimulation at 5 Hz evoked a pronounced increase in MABP (+ 33±11 mm Hg from baseline) and a selective release of NE, EPI and ME from the adrenal. SN stimulation at 50 Hz evoked a further increase in MABP (+ 54±12 mm Hg from baseline) and AD CA and NP levels. The increase in NE, EPI and DA was most pronounced with approximately the same ratio of release as under 5 Hz stimulation. Only at high frequencies was there a selective release of NT and NPY from the adrenal. The proportional release of ME from the adrenal, compared to NE and EPI, was less pronounced under 50 Hz than under 5 Hz SN stimulation. Though CA and NP are co-stored in adrenal chromaffin cells, their extracellular movement following low and high frequency SN stimulation, appears differentially evoked. (Supported by NIH grant NS-16541 (TY), 1986 Burroughs Wellcome Research Fellowship (DG).)

Table 1: Plasma Levels at Baseline (mean ± SE)

Source	NE (ng/ml)	EPI (ng/ml)	DA (ng/ml)	NPY (ng/ml)	ME (pg/ml)	NT (pg/ml)
AD (n=6)	6.8 ± 3.8	39 ± 25	0.2 ± 0.3	1.5 ± 0.5	49 ± 15	39 ± 5
FA (n=4)	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.9 ± 0.1	46 ± 12	42 ± 8

Table 2: Increase in Plasma Levels During Splanchnic Nerve Stimulation (Ratio of Baseline, mean ± SE)

Source	NE (ng/ml)	EPI (ng/ml)	DA (ng/ml)	NPY (ng/ml)	ME (pg/ml)	NT (pg/ml)
AD (5 Hz)	6.5 ± 2.8	4.0 ± 1.3	0.8 ± 0.2	1.3 ± 0.4	3.1 ± 1.0	1.8 ± 0.3
FA (5 Hz)	2.0 ± 0.6	2.5 ± 1.6	1.0 ± 0.4	1.0 ± 0.1	1.0 ± 0.3	1.0 ± 0.1
AD (50 Hz)	124 ± 75	85 ± 44	23 ± 13	4.8 ± 1.2	21 ± 11	5.7 ± 1.5
FA (50 Hz)	7.0 ± 1.7	13 ± 6	3.5 ± 2.1	3.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2

78.11 SPINAL AND PERIPHERAL PHARMACOLOGY OF THE URINARY BLADDER IN UNANESTHETIZED RATS. P.A. Durant* and T.L. Yaksh* (SPON: P.R. Wilson). Neurosurgical Research Unit, Mayo Clinic, Rochester, MN 55905.

Using a chronic unanesthetized rat model (Am. J. Physiol. 251: 1177, 1986) we studied the spinal and peripheral pharmacology of the cholinergic, adrenergic, serotonergic and ganglionic systems by examining the dose dependent effects of receptor selective agonists after intrathecal (IT) and intraperitoneal (IP) injections. A polyethylene-100 tubing was chronically implanted through the vertex of the bladder. A polyethylene-10 tubing was inserted in the IT space through the cisternal membrane down to the lumbar level. Both catheters were tunneled subcutaneously to exit at the level of the neck and the scalp, respectively. To examine the volume-evoked micturition reflex (VEMR), the unanesthetized rat was placed in a restraining cage over a strain gauge mounted collecting device for measurement of urine volume. The bladder catheter was connected to a pump for continuous saline infusion (250 µl/min) and to a transducer for monitoring bladder pressures. The following parameters of the VEMR were routinely measured: baseline pressure, bladder opening pressure, peak pressure (PP) and volume of urine expressed per bladder contraction (V/C). The importance of using unanesthetized animals was demonstrated by the inhibition of the VEMR in rats anesthetized with pentobarbital, ketamine, chloralose and halothane. Carbachol (IP), serotonin (IP), apomorphine (IP and IT), dopamine (IP and IT), norepinephrine (IP and IT), methoxamine (IP) and ST-91, an α_2-agonist (IP and IT) evoked an increase in contraction rate (e.g. a reduction in V/C). In all cases, the VEMR during these periods of increased frequency was such that the volume expressed was equal to the volume infused (e.g. there was no residual volume). Atropine (IP), hexamethonium (IP), norepinephrine (IP), isoproterenol (IP and IT) and phentolamine (IP) induced significant drops in PP, suggesting relaxation of the bladder outlet and/or bladder body. Carbachol (IT), atropine (IT), serotonin (IT), methysergide (IP and IT), hexamethonium (IT), cis-flupentixol (IP and IT), methoxamine (IT), terbutaline (IP), phentolamine (IT), yohimbine (IP and IT) and propranolol (IP and IT) produced virtually no effects on micturition at the highest doses examined. The lack of effect of IT injected antagonists suggests that spinal cholinergic, serotonergic, dopaminergic and adrenergic systems are not routinely active during the VEMR. Those systems might be activated in special circumstances, such as the voluntary act of retaining urine. The present study represents the initial efforts to characterize the pharmacology of spinal and peripheral receptor populations regulating the VEMR in the unanesthetized, intact rat. (NIH grant NS-19650 and Mayo Foundation.)

78.12 ELECTROPHYSIOLOGICAL INVESTIGATION OF SEXUAL REFLEXES EVOKED BY STIMULATION OF THE DORSAL NERVE OF THE PENIS IN THE RAT AND CAT. W.D. Steers*, B. Mallory, and W.C. de Groat. Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

The dorsal nerve of the penis (DNP) serves as the somatic afferent limb for multiple sexual reflexes mediated by autonomic efferents. These reflex pathways provide a model for studying somatovisceral integration and the parasympathetic and sympathetic events associated with sexual function.

Male Wistar rats and cats were anesthetised with urethane and chloralose, respectively, prior to exposure of the DNP, pelvic (PV), hypogastric (HG) and penile (PN) nerves for stimulation and recording. Animals were paralyzed with pancuronium and artificially ventilated.

Electrical stimulation of the DNP evoked responses on PN, PV and HG. PN responses in the rat consisted of an early response (ER) with a latency of 47±14 ms and a late response (LR) at 100±18 ms. The ER was eliminated by PV crushing and stimulation frequencies $\geq$3 Hz, while the LR was reduced but not eliminated by these two maneuvers. The LR and not the ER was consistently abolished after ganglionic blocking agents. Only the ER was present after chronic spinal cord transection at T8. These data suggest that the ER was a central reflex originating in the lumbosacral cord. The LR has not been completely defined but may be a supraspinal reflex.

PV stimulation elicited axonal volleys (0.3-5 ms) and occasional ganglionic responses on PN. HG stimulation also elicited responses (11±2 ms) which were abolished by ganglionic blockade. DNP stimulation produced 13±4 ms and 44±16 ms responses on HG; the latter were eliminated by crushing HG centrally.

Preliminary studies in the cat have shown the nerves from the pelvic plexus caudad to the prostate (PN) could be activated by DNP, PV and HG stimulation. DNP stimulation produced long latency discharges (200-300 ms) on these nerves. Spontaneous neural activity on some PN was either facilitated or inhibited by electrical stimulation of the DNP or tactile stimulation of the penis. Stimulation of PV and some PN produced an increase in intracavernous pressure and penile erection suggesting that the DNP reflex may be involved in penile vasodilatation. PV and HG stimulation evoked responses sensitive to ganglionic blockade.

These results combined with previous axonal tracing studies reveal that nerves projecting to the penis from the pelvic plexus contain both parasympathetic and sympathetic pathways whose distal ganglia are distributed randomly. The central reflex mechanisms involve both spinal and supraspinal centers. Studies are underway to determine the sexual functions corresponding to these reflexes.

78.13 AUTONOMIC INNERVATION OF REPRODUCTIVE ORGANS IN THE RAT: ANALYSIS OF THE COMPOSITION OF THE CAVERNOUS NERVE. W. G. Dail, D. Trujillo* , G. Walton* and D. de la Rosa*. Department of Anatomy, University of New Mexico, School of Medicine, Albuquerque, NM 87131.

Previous studies have shown that the cavernous nerve, a presumed postganglionic nerve pathway from the pelvic plexus to penile erectile tissue, also contains a large number of ganglion cells as well as afferent nerve fibers. The present study uses retrograde tracers to analyze the target organs of the neuronal perikarya in the trunk of the cavernous nerve and to localize other neurons whose fibers traverse this nerve. The dyes fluorogold or true blue were placed in the penile crura, penile bulb, bulbourethral gland or on the cut end of the cavernous nerve. Neurons to the penile crura were found in the major pelvic ganglion (195±8.1) and in the more proximal end of the cavernous nerve (106±11.9). In contrast, neurons which innervate the bulbourethral gland are distributed evenly along the length of the cavernous nerve (217±20.5) with a smaller number in the major pelvic ganglion (66±11.8). Dye in the penile bulb filled neurons in the major pelvic ganglion (201±17.3) and in neuronal clusters of the cavernous nerve (531±22.3). There was no evidence of double labeled neurons when multiple target organs were injected with different dyes. Dye placed on the cut end of the cavernous nerve filled neurons in dorsal root ganglia L_6-S_1, sympathetic chain ganglia and preganglionic autonomic neurons at spinal cord levels L_1-L_2 and L_6-S_1. Preganglionic neurons in the L_1-L_2 spinal cord segments were located in the dorsal commissural nucleus while those at the L_6-S_1 segments were found mainly in the intermediolateral gray matter. The presence of adrenergic fibers was confirmed by catecholamine histofluorescence of chronically-ligated cavernous nerves.

Separate populations of neuronal perikarya along the cavernous nerve innervate the penile crura, penile bulb and the bulbourethral glands. Neurons in the cavernous nerve are innervated mainly from preganglionic neurons of the sacral parasympathetic nucleus but also receive preganglionic innervation from the sympathetic hypogastric nerve. There is a topography of the cavernous nerve in that neurons to the bulbourethral gland are located more distally along the nerve than neurons which innervate the penile crura. The relatively large number of neurons labeled after injection of the penile bulb may represent innervation to penile smooth muscle and to glands associated with the urethra. The field of innervation of sensory fibers and sympathetic fibers in the cavernous nerve is not known. Supported by NIH RO1NS1983-04 and NIH RR08139-10.

78.14 THE AUTONOMIC INNERVATION OF THE PENIS AND CLITORIS OF THE RAT. Sookja K. Chung and Kevin E. McKenna, Dept. of Physiology, Northwestern Univ. School of Medicine, Chicago, IL 60611

The present study was motivated by 3 questions. First, what are the sex differences between the autonomic innervation of the penis and clitoris (P/C)? Second, is there a viscerotopic organization in the distinction of preganglionic neurons innervating a given organ? Third, what are the relative contributions of sympathetic (SYMP) and parasympathetic (PSYMP) innervation of the P/C ? The P/C in the rat is innervated by the pelvic ganglion, a mixed sympathetic - parasympathetic ganglion, via the cavernous nerve as demonstrated by gross dissections. Electrical stimulation of this nerve in the male induces vasodilation of the penis, indicating that it may play a role in erection.

Injection of tracer material into the P/C labelled ganglion cells in the pelvic ganglion proper but also cells distributed throughout the length of the cavernous nerve. This finding suggested that it may be possible to label some of the preganglionic neurons (PGNs) innervating these ganglion cells by applying tracer to the cavernous nerve close to the ganglion. Mature male and female rats were anesthetized with halothane and the pelvic ganglion was exposed. The cavernous nerve was identified and cut close to the ganglion. The central cut end was incubated for 1 hour in HRP, fluorogold, or fast blue. Following survival times of 2 to 6 days, the rats were reanesthetized and perfused with buffer and fixative. The pelvic ganglion, spinal cord, and dorsal root ganglia (DRG) were removed and sectioned with cryostat. HRP material was processed with theTMB technique. Examination of the pelvic ganglia revealed that tracer application was confined to cavernous nerve and did not diffuse into the ganglion.

In both sexes, labelled neurons were found in the lateral horn in segments L6-S2, the dorsal commissure of T13-L2, and the DRG of L6 and S1. The L6-S2 neurons were distributed in a tight column at the gray-white matter border. Location and morphology indicated that these were clearly PSYMP PGNs. Comparison of the distribution of these neurons and PSYMP PGNs labelled from pelvic nerve indicate that P/C PGNs are cospatial with non-P/C PGNs in this nucleus.

The labelled neurons in the T13-L2 segments were distributed in a more diffuse column in the dorsal commissure. Only a few cells were seen in the lateral horn. The distribution and morphology of these cells indicate clearly that they are SYMP PGNs. These neurons were also cospatial with non-P/C PGNs labelled from the hypogastric nerve.

Labelled DRG neurons and terminal fibers in the spinal cord were seen in L6-S1 segments, indicating that the cavernous nerve afferents travel in the pelvic nerve.

The distribution and morphology of labelled neurons were similar in male and female rats. However, there were clearly fewer SYMP and PSYMP PGNs in the female, demonstrating a strong sexual dimorphism in the innervation of the P/C.

This study suggests that both SYMP and PSYMP PGNs may be involved in erection of the P/C. Further, there appears to be little viscerotopy of PGNs innervating the P/C and that male and female innervation shows a strong quantitative difference.

78.15 SEXUAL REFLEXES EVOKED IN ACUTELY SPINALIZED, ANESTHETIZED RATS. Kevin E. McKenna and Sookja K. Chung, Dept. of Physiology, Northwestern Univ. School of Medicine, Chicago, Il 60611

The spinal mechanisms involved in control of sexual function remain largely unknown because of the lack of a suitable model in which invasive procedures can be used while reliably eliciting sexual reflexes. The present study demonstrates the utility of the acutely spinalized, urethane anesthetized male rat as a suitable model for studying spinal sexual reflexes. These reflexes appear comparable in many respects to activity seen in rats in copula.

Mature male rats (300-400g) were anesthetized with urethane (1.0g/kg S.C.) and spinalized at spinal segment T5-T9. EMG electrodes were placed in the ischiocavernosus muscle (IC), bulbospongiosus muscle (BC) while the dorsal nerve of the penis (DNP), motor branch of the pudendal nerve (MPN), and cavernous nerve (CN) were prepared for stimulating and recording. Intracavernous pressure (ICP) was recorded with a needle placed in the penis and connected to a pressure transducer. Similar to previous reports using unanesthetized, chronically spinalized rats (Hart, 1967; Sachs, 1983), penile reflexes (erections, cups, flips) could be elicited in this preparation by pulling back the preputial sheath and maintaining pressure on the base of the penis. However, in our hands, this technique was inconsistent in producing penile reflexes and often required long periods of time between trials. We determined that the most consistent, reliable and repeatable stimulus for penile reflexes is mechanical or chemical stimulation of the mucosa of the urethral bulb. Inserting a fine (0.6mm O.D.) catheter into the urethral bulb and manipulating it a few mm. invariably produced penile reflexes. These reflexes consisted of bursts of activity in MPN motoneuron axons and CN vasodilator fibers. These bursts produced either spikes of pressure or staircase increases in ICP.

The penile reflexes consisted of strong bursts of activity of BC muscles and often simultaneous activation of the IC. These bursts lasted from 500-900 msec. They were associated with increases of ICP of 50 to over 400 mmHg which led to full erection, with cups forming in strong reflexes. The bursts occurred with intervals of 1 to 2 seconds. The number of bursts in a sequence was related to the strength and duration of urethral stimulation, but always persisted long after manipulation had ceased. For example, a 5sec manipulation led to a series of bursts lasting approximately 20sec and a 45sec manipulation evoked a sequence of bursts lasting almost 2.5 min. Strong responses resembled ejaculatory events and would often expell the urethral catheter and secretory (bulbourethral?) fluid.

The bursts of BC and IC activity were preceded by 100-200msec by bursts of activity in the CN, which provides vasodilator input to the penis. Short latency (16-20 msec) reflexes could be elicited in this nerve by stimulation of the glans or DNP.

Injection of normal saline into the urethral bulb caused very little response, although hypertonic saline elicited sustained contraction of the BC muscle and sustained erections. The effects of various components of seminal fluid are being examined to test the hypothesis that ejaculation may be induced by chemical stimulation of the urethral mucosa by seminal secretions.

78.16 VAGINOCERVICAL PROBING ELEVATES BLOOD PRESSURE AND INDUCES ANALGESIA BY SEPARATE MECHANISMS. J.M. Catelli, A.F. Sved and B.R. Komisaruk. Inst. Animal Beh., Rutgers Univ., Newark, NJ 07102.

Previous studies have shown that vaginocervical stimulation (VS) produces profound analgesia. Since increases in blood pressure (BP) have been shown to increase pain thresholds, the present studies examined if the analgesic response to VS was secondary to cardiovascular effects of VS. VS, applied as a calibrated mechanical probe force against the cervix, increased BP >50 mmHg and increased heart rate (HR) >80 bpm in conscious, restrained rats. This response began immediately with the onset of stimulation and reached its maximal level within 10 seconds. The magnitude of the response was proportional to the force of the VS applied over a range of 50-600 grams (g). Administration of propranolol (1 mg/kg iv) attenuated the HR response to VS, whereas administration of chlorisondamine (5 mg/kg iv) abolished both the increase in BP and HR in response to VS. These results indicate that VS stimulates the sympathetic nervous system and that the tachycardia resulted from vagal withdrawal as well as sympathetic activation. The analgesic effect of VS, as measured by tail flick latency, was unaffected by any of the drug treatments that abolished the BP and HR elevating effects of VS. Furthermore, elevation of BP by administration of phenylephrine (5 ug/kg iv), to a level comparable to that produced by VS, did not produce analgesia. These results demonstrate that VS produces elevations in BP and HR which are due primarily to an increase in sympathetic activity. In addition, the increase in HR in response to VS appears to be partly due to a decrease in parasympathetic activity. The analgesia produced by VS is independent of the elevation in BP produced by VS.

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78.17 AFFERENT FIBERS SUPPLYING INTERNAL REPRODUCTIVE ORGANS IN THE FEMALE RAT. K.J. Berkley[1], A. Robbins*[,1,2] and Y. Sato*[,2]. [1]Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306; [2]Dept. of Physiology, Tokyo Metropolitan Inst. of Gerontology, Tokyo 173 Japan.

The purpose of this three-part study was to survey the sensory supply of female reproductive organs in the rat. In the first part, injections of horseradish peroxidase into large areas of the uterus produced labeled ganglion cells in the L1 through S1 dorsal root ganglia, with most located in L2 and L6. This result indicates that uterine afferent fibers in the rat travel to the central nervous system through both the hypogastric and pelvic nerves. In the second part, responses of single units to mechanical stimulation of the uterus and vagina were studied in the distal ends of the cut hypogastric and pelvic nerves. In the hypogastric n., receptive fields were most often located on the uterine body particularly over the cervix. Effective stimuli included steady pressure, stretching, squeezing and probing, often at intensities which produced transient ischemia around the probe. In the pelvic n., receptive fields were most often located in the vaginal canal adjacent to the cervix. Effective stimuli were much less intense, rarely producing ischemia. In the third part, responses of multiple units in both nerves to algesic and hypoxic chemical stimulation were studied in an in vitro preparation of the isolated uterus. Fibers in both nerves responded in a dose-dependent manner to the algesic chemicals bradykinin, 5 hydroxytyptamine (5HT) and potassium chloride, with differences in the configuration of the responses primarily to 5HT. Fibers in the pelvic n. were more sensitive than those in the hypogastric n. to hypoxic chemicals (sodium cyanide; CO_2 dissolved in buffer). Only high doses of these hypoxic chemicals activated hypogastric n. fibers, whereas pelvic n. fibers responded to lower doses in a dose-dependent manner.

These results indicate that the hypogastric and pelvic nerves contain afferent fibers supplying female reproductive organs in the rat and that there are significant functional differences between them in the information they each convey.

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78.18 ENHANCEMENT OF TRANSMISSION AND BLOCKADE OF LTP IN BULBOSPINAL PATHWAYS TO SYMPATHETIC PREGANGLIONIC NEURONS (SPGNs) BY PHORBOL ESTERS. Scott C. Steffensen*and Donald N. Franz, Department of Pharmacology and Toxicology, Univ. of Utah School of Medicine, Salt Lake City, Utah 84132.

A recently developed intraspinal microinjection technic (Soc. Neurosci. Abstr. 12:537, 1986) was used to examine the effects of phorbol esters on intraspinal transmission to SPGNs to test the possibility that the excitability of SPGNs is regulated in part by the phosphatidyl inositol system and, more specifically, protein kinase C. The effects of phorbol esters on LTP of intraspinal transmission, which typically reaches 200% of control and subsides within 30-40 min, was also determined.

Sympathetic discharges, recorded from T2 and T3 preganglionic rami and analyzed by signal averaging, were evoked at 0.1 Hz by microelectrode stimulation of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized spinal cats. Phorbol 12,13-diacetate or phorbol 12,13-dibutyrate, dissolved in artificial CSF (1 ug/ul), were pressure injected through micropipettes into the SPGN neuropil near the middle of the T2 or T3 segment. Dye injections in 1-2 ul of vehicle were confined to a 10-15 ul volume along the longitudinal axis of the SPGN neuropil of the injected segment. Microinjections of 1 ug of either phorbol ester into one segment markedly enhanced intraspinal transmission through that segment to 200-240% of control levels within 10 min. Transmission returned to control levels by 1.5-2 hr, but subsequent injections were ineffective for more than 3 hr. During this time, the ability of the intraspinal pathway to generate LTP following a brief tetanus (50 Hz, 10 sec) was completely lost in the pre-injected segment. However, neither transmission nor generation of LTP through the adjacent, uninjected segment were affected.

The marked enhancement of transmission to SPGNs by phorbol esters is likely mediated by activation of protein kinase C which may increase SPGN excitability by phosphorylation of membrane proteins. The results also imply regulation by the phosphatidyl inositol second messenger system. The ability of phorbol esters to prevent both subsequent responsiveness and generation of LTP also suggests a role for protein kinase C in the generation of LTP as has been proposed for LTP in the hippocampus (Akers et al., Science 231:587, 1986; Malenka et al., Nature 321:175, 1986).

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78.19 INTRASPINAL MICROINJECTION OF PHORBOL ESTERS BLOCKS GENERATION OF CYCLIC AMP IN SYMPATHETIC PREGANGLIONIC NEURONS (SPGNs). Donald N. Franz and Scott C. Steffensen*, (SPON: S.A. Turkanis), Dept. of Pharmacology and Toxicology, Univ. of Utah School of Medicine, Salt Lake City, Utah 84132.

Previous studies showed that i.v. phosphodiesterase (PDE) inhibitors or microinjection of two cyclic AMP analogs, forskolin (Soc. Neurosci. Abstr. 12:537, 1986), or phorbol esters into the SPGN neuropil markedly enhanced intraspinal transmission to SPGNs. It thus appears that both cyclic AMP and phosphatidyl inositol second messenger systems are involved in regulating SPGN excitability. The present study tested the possibility that activation of protein kinase C by phorbol esters might influence the cyclic AMP system in SPGNs as has been shown in several other, non-neuronal cell types.

Sympathetic discharges, recorded from T2 or T3 preganglionic rami and analyzed by signal averaging, were evoked at 0.1 Hz by microelectrode stimulation of descending excitatory pathways in the dorsolateral funiculus of unanesthetized spinal cats. Drugs, dissolved in artificial CSF, were pressure injected through micropipettes into the SPGN neuropil of either the T2 or T3 segment while recording evoked sympathetic discharges from both segments. Microinjection of 1 ug of phorbol 12,13-dibutyrate into one segment, which produced a typical phase of enhancement to about 200% of control, completely prevented the typical enhancement produced by subsequent microinjection of the PDE inhibitor, RO 20-1724 (2 ug) or the adenylate cyclase activator, forskolin (1 ug), into that segment. Enhancement of transmission by i.v. IBMX (1 mg/kg) was also prevented through the phorbol-injected segment but not through the adjacent, uninjected segment. However, microinjection of 1 ug of dibutyryl cyclic AMP into a phorbol-pretreated segment produced the typical prolonged enhancement seen in the absence of phorbol ester.

These results demonstrate that transient activation of protein kinase C by phorbol esters can block receptor-coupled generation of cyclic AMP in SPGNs. Since the phorbol esters block the effects of both PDE inhibitors and forskolin but not that of dibutyryl cyclic AMP, the inhibitory effect of phorbol esters appears to be mediated directly upon adenylate cyclase, perhaps through protein kinase C-induced phosphorylation of the enzyme.

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78.20 PARAVENTRICULAR NUCLEUS STIMULATION: EFFECT ON RENAL NERVE ACTIVITY. C.L. Riphagen and Q.J. Pittman. Neuroscience Research Group. Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1

The paraventricular nucleus (PVN) has projections, some of which are vasopressinergic, which descend to the lower thoracic intermediolateral cell column region from which the efferent renal innervation arises. Previously we have reported that intrathecal administration of arginine vasopressin (AVP) to this spinal region can alter kidney function (Riphagen, C.L. & Pittman, Q.J., Brain Res. 336: 346, 1985) and increase renal nerve activity (Riphagen, C.L. & Pittman, Q.J., Proc. West. Pharm. Soc., (in press). In this study we tested the hypothesis that electrical stimulation of the PVN could alter renal nerve activity and examined the role of AVP in the response using an intrathecally administered AVP antagonist.

Seven male Sprague-Dawley rats were anaesthetized with Inactin (0.12g/kg, i.p.). A monopolar stimulating electrode was implanted stereotaxically with the tip directed towards the left PVN. Intrathecal administration of vehicle or the AVP antagonist was made via a cannula threaded caudally in the subarachnoid space from the atlanto-occipital region to T10-12. The left kidney was exposed via a paravertebral incision and a renal nerve was sectioned as it entered the hilus. Multifibre renal nerve activity (RNA) in each animal was recorded conventionally in response to electrical stimulation of the PVN after perfusion of the intrathecal space with vehicle and later with the AVP antagonist $d(CH_2)5Tyr(Me)AVP$. The position of each electrode tip was examined histologically.

In 4 of the animals, the electrode tip was found to be positioned within the PVN. In these animals, electrical stimulation of the PVN (2-9V) resulted in a biphasic excitation of RNA (with the peaks of excitation occurring at between 100-150 msec and 150-250 msec after stimulation) followed by a period of inhibition (at 250-450 msec) prior to recovery. In 3 of these animals that were treated with AVP antagonist, the second excitatory peak was abolished. In 3 different animals in which the electrode tip lay just outside the PVN, a monophasic excitatory response to PVN stimulation, with a latency of 100-150 msec, was observed. Treatment with the AVP antagonist attenuated the excitatory peak in two of the animals but increased the magnitude of the response in the third.

The results of this study suggest that electrical stimulation of the PVN can alter RNA, producing a biphasic excitatory response. A descending AVP pathway may be involved in the second excitatory peak.

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78.21 ELECTRICAL AND GLUTAMATE STIMULATION OF THE PARAVENTRICULAR NUCLEUS ELICITS BRADYCARDIA IN PENTOBARBITAL-ANESTHETIZED RATS. D.N.Darlington, J.Shinsako* and M.F.Dallman*. Dept. of Physiology, University of California, San Francisco, CA 94143 USA.

We examined the role of the hypothalamic paraventricular nucleus (PVN) for regulation of heart rate, arterial blood pressure and adrenocorticotropin (ACTH) secretion before and after microinjection (glass pipet, 50-80um OD) of glutamate or electrical stimulation of the PVN. Experiments were performed one hour after placement of femoral artery and vein cannulas. 50nl of 0.5M sodium-glutamate (pH7.4) injected over 2 minutes into the PVN elicited a decrease in heart rate (-80±6 bpm, n=7) while injection of sodium-acetate (50nl, 0.5M, pH7.4) had no effect (0±8 bpm, n=6). Bradycardia was elicited when glutamate was injected into the posterior part of the nucleus, but not when injected into the anterior part, above, or lateral to the PVN. The decrease in heart rate usually began 60 to 90 seconds after the onset of the 2 minute injection. Arterial pressure also decreased in response to every injection that elicited a bradycardia. Plasma ACTH did not change 5, 10 or 20 minutes after injection of glutamate or acetate (50 or 100nl) into the posterior PVN. To confirm the findings that chemical stimulation of the PVN elicits a bradycardia, bipolar concentric electrodes were placed in the PVN. Heart rate decreased as a function of current strength (range tested-50 to 500uA, 50Hz, 0.5msec for 10 sec) plateauing at 100uA. 20uA did not elicit significant changes in heart rate. Arterial blood pressure demonstated a biphasic response with increasing stimulus strength, decreasing at strengths less than 100uA and becoming variable with greater current intensity. The greatest decrease in heart rate also occured at 50 Hertz (range tested-5 to 300, 50uA, 0.5msec for 10 sec). A mapping study (50Hz, 50uA) demonstrated that the greatest decrease in heart rate was elicited from areas in and around the PVN.

We conclude that excitation of cell bodies in the PVN leads to a decrease in heart rate and blood pressure, however glutamate excitation of PVN neurons does not elicit ACTH release. Supported in part by KD2872 and HL29714.

78.22 CARDIOVASCULAR RESPONSES TO TIBIAL NERVE STIMULATION ARE NOT ABOLISHED BY FOCAL COOLING OR BY ANESTHETIC BLOCKADE OF THE VENTROLATERAL MEDULLA IN ANESTHETIZED CATS. T.G. Waldrop* and D.E. Millhorn (SPON: D.C. German). Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801 and Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

Neurons located superficially in the ventrolateral medulla (VLM) are well known to be involved in the maintenance of resting arterial pressure. Superficial cooling, electrolytic lesions or chemical blockade of the VLM causes hypotension in anesthetized cats and rats. In addition, several cardiovascular responses including those elicited by baroreceptor stimulation, cerebral ischemia and stimulation of the fastigial nucleus are modulated by neuronal structures in the VLM. These findings have led to the suggestion that all sympathetic outflow from the medulla originates from the VLM. The purpose of this study was to test this hypothesis by determing the effects of blockade of the ventrolateral medulla upon cardiovascular responses to a known somatoautonomic reflex. Adult cats anesthetized with chloralose and urethane were studied. Arterial pressure and neural correlates for sympathetic outflow (cervical nerve activity) and respiratory output (phrenic nerve activity) were recorded in response to tibial nerve stimulation before and during neuronal blockade of the ventrolateral medulla. The tibial nerve was stimulated at an intensity that activates all categories of afferent fibers. Blockade of the VLM was performed by focal cooling in one group of cats and by flooding the VLM surface with lidocaine in a second group of animals. Both types of blockade produced immediate decreases in arterial pressure, phrenic nerve and cervical nerve activities. However, neither cold block nor anesthetic blockade prevented the increases in arterial pressure, phrenic nerve activity and cervical nerve activity elicited by stimulation of the tibial nerve. In contrast, the responses to tibial nerve stimulation were abolished by transection of the cervical spinal cord. The arterial pressure and cervical nerve responses to activation of baroreceptor afferents (stimulation of a carotid sinus nerve) were attenuated by focal cooling of the VLM. These findings show that not all somatoautonomic reflexes are dependent upon activity of neurons located superficially in the ventrolateral medulla. Therefore, sympathetic outflow from the brainstem is not limited solely to neural networks located superficially beneath the surface of the ventrolateral medulla. Thus, other brain sites must also contribute to sympathetic outflow to the spinal cord.

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78.23 EFFECTS OF CHEMICAL STIMULATION OF POSTERIOR HYPOTHALAMIC NEURONS UPON THE BARORECEPTOR REFLEX. R.M. Bauer* and T.G. Waldrop* (Spon: G.A. Iwamoto). Department of Physiology and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Several laboratories have shown that electrical stimulation in the posterior hypothalamus inhibits the baroreflex. However, these studies are difficult to interpret since it is not known if the attenuation of the baroreflex results from activation of fibers of passage or from stimulation of cell bodies located in the posterior hypothalamus. In addition, electrical stimulation of the hypothalamus often does not provide a stable baseline upon which the baroreflex can be tested. Therefore, the purpose of the present study was to determine the effects of chemical stimulation of posterior hypothalamic neurons upon the arterial pressure and heart rate response to baroreceptor stimulation. Experiments were performed in adult cats anesthetized with chloralose and urethane. Most of the cats were paralyzed with gallamine triethiodide and ventilated during the experiments. Arterial pressure and heart rate were recorded during activation of arterial baroreceptors before and after microinjection of the GABA antagonist picrotoxin (5 ng/nl) into the posterior hypothalamus. The baroreceptors were stimulated by increasing the pressure in a vascularly isolated carotid sinus and by intravenous injections of phenylephrine (20-50 micrograms). Picrotoxin was pressure injected through micropipettes (20-40 micron tips) positioned stereotaxically in the posterior hypothalamus (A9, L1.5, V-3). Injection sites were marked with fast green dye for subsequent histological verification of injection sites. The fall in heart rate produced by activation of the baroreceptors was attenuated by microinjections of picrotoxin into the posterior hypothalamus. The fall in arterial pressure evoked by increasing carotid sinus pressure (CSP) was slightly reduced by the picrotoxin injections. All of the effects of picrotoxin were reversed by microinjections of the GABA agonist muscimol (5 ng/nl) into the same site in the hypothalamus. Mean results ± SEM (n=10) are as follows:

	Control	Picrotoxin	Muscimol
Phenylephrine (Δbpm/ΔmmHg)	-0.642±0.2	-0.346±0.1	-0.637±0.2
Increased CSP (Δbpm)	-19.4±5.3	-8.1±2.0	-26.6±7.0

These results show that the depression of the baroreflex elicited by hypothalamic stimulation results from activation of cell bodies in the posterior hypothalamus. In addition, a GABAergic mechanism appears to be involved in the hypothalamic modulation of the baroreceptor reflex.

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CARDIOVASCULAR REGULATION: CNS PATHWAYS I

79.1 FUNCTIONAL RELATIONSHIP BETWEEN CARDIOVASCULAR AREAS IN THE MEDULLA.
R. Urbanski and H.N. Sapru, Dept. of Pharmacology and Section of Neurosurgery, Univ. of Med. & Dent. of NJ-New Jersey Med. School, Newark, NJ 07103.

In the medulla three areas with cardiovascular function have been identified. These are: the nucleus tractus solitarius (NTS), the ventrolateral medullary pressor (VLPA) and the ventrolateral medullary depressor area (VLDA). Anatomic interconnections between the NTS and the VLDA and VLPA have been demonstrated. Interconnections between the intermediolateral cell column of the thoraco-lumbar cord (IML) and the VLPA and the NTS have also been identified. However, the role of these projections has not been clearly established. In this investigation, a pharmacological approach was used to study the importance of these anatomic pathways in cardiovascular regulation. Urethane anesthetized male Wistar rats (n=30) were used. The NTS, VLPA and VLDA were identified bilaterally with microinjections of L-glutamate (L-glu; 1.77 nmole/site). Unilateral microinjections of L-glu into the NTS evoked a fall in BP (32.5 ±2.8 mmHg) and HR (42±6 bpm). Unilateral microinjections of muscimol (90 pmole) or lidocaine (8.5 nmole) into the VLPA or VLDA blocked the L-glu response from the ipsilateral (but not contralateral) NTS. A higher dose of L-glu (5 nmole) into the NTS evoked a rise (instead of a fall) in BP when the ipsilateral VLDA was inhibited with muscimol. Depressor responses to this dose of L-glu persisted in the contralateral NTS. These results suggest that the pathways: (i) from the NTS to the VLDA mediate hypotensive responses, (ii) from the NTS to the VLPA mediate hypertensive responses, (iii) from the NTS to the IML may not be involved in cardiovascular regulation.

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79.2 CARDIOVASCULAR ACTIONS OF CHOLINERGIC AGENTS IN THE DEPRESSOR AREA OF THE VENTROLATERAL MEDULLA: ROLE OF M_2 MUSCARINIC RECEPTORS. H.N. Sapru, A.J. Krieger* and K. Sundaram*. Neurosurgery & Pharmacology, UMDNJ - New Jersey Medical School, Newark, NJ 07103.

The depressor area in the ventrolateral medulla (VLDA) plays an important role in cardiovascular regulation. The role of cholinergic agents in this area has not been studied. This investigation was designed to study the cardiovascular actions of the following muscarinic agonists and antagonists in this area. Agonists used: cis-methyldioxolane (a specific agonist of M_2 receptors), McN A343 (a specific agonist of M_1 receptors), carbachol (non-specific agonist) and physostigmine (elevates the levels of endogenous acetylcholine). Antagonists used: AFDX 116 (a specific M_2 muscarinic receptor antagonist), pirenzipine (a specific antagonist of M_1 receptors), atropine (non-specific antagonist). The doses of agonists and antagonists varied between 0.004-4 nmole/site. Male Wistar rats (n=40) were anesthetized with pentobarbital or decerebrated at mid-collicular level. Blood pressure (BP), splanchnic nerve activity (SNA) and heart rate (HR) were recorded. The rats were artificially ventilated. Ventral medulla was exposed and VLDA identified bilaterally by microinjections of L-glutamate (1.77 nmole/site). Microinjections of cis-methyldioxolane into the VLDA evoked a decrease in BP, SNA and HR which lasted for 20-30 min. Intravenous injections of the same doses of this agent failed to evoke a response. Preliminary experiments indicate that microinjections of AFDX 116 into the VLDA do not evoke any response. However, these injections of AFDX 116 blocked as well as prevented the depressor responses evoked by cis-methyldioxolane. Microinjections of McN A343 or pirenzipine into the VLDA failed to evoke any response. These results indicate that the depressor and bradycardic responses evoked by cholinergic agonists in the VLDA are mediated via the M_2 receptors.

Support: N.I.H (HL 24347) and American Heart Assoc. (NJ).

79.3 M_2 MUSCARINIC RECEPTORS MEDIATE PRESSOR RESPONSES TO CHOLINERGIC AGONISTS IN THE VENTROLATERAL MEDULLARY PRESSOR AREA. K. Sundaram* and H.N. Sapru (SPON: R. Howland). Neurosurgery & Pharmacology, UMDNJ - New Jersey Medical School, Newark, NJ 07103.

Microinjections of cholinergic agonists into the ventrolateral medullary pressor area (VLPA) evoke increase in blood pressure (BP) and heart rate (HR). Recently two major subtypes of muscarinic receptors have been identified. This investigation was designed to study the role of these muscarinic receptor subtypes in pressor responses of cholinergic agonists in the VLPA. Male Wistar rats (n=30) were anesthetized with pentobarbital or decerebrated at mid-collicular level. BP, splanchnic nerve activity (SNA) and HR were recorded. The rats were artificially ventilated. Ventral medulla was exposed and VLPA identified bilaterally by microinjections of L-glutamate (1.77 nmole/site). Microinjections of cis-methyldioxolane (a specific agonist of M_2 receptors) in the doses of 0.004-4 nmole/site into the VLPA evoked increase in BP, SNA and HR which lasted for 20-30 min. Intravenous injections of the same doses of this agent failed to evoke a response. Microinjections of AFDX 116 (a specific M_2 muscarinic receptor antagonist) into the VLPA in the doses of 0.2-4 nmole/site evoked depressor responses. The depressor responses lasted for 15-20 min. These injections of AFDX 116 blocked as well as prevented the pressor responses of cis-methyldioxolane. Microinjections of AFDX 116 into the VLPA blocked the pressor responses to intravenously administered physostigmine. Microinjections of McN A343 (a specific agonist of M_1 receptors) or pirenzipine (a specific antagonist of M_1 receptors) in the doses Of 0.4-4 nmole/site into the VLPA failed to evoke any response. Microinjections of pirenzipine into the VLPA did not alter the pressor responses to intravenously administered physostigmine. These results indicate that the pressor and tachycardic responses evoked by cholinergic agonists in the VLPA are mediated via the M_2 receptors which may be under tonic excitatory cholinergic control because AFDX 116 caused a fall in BP and HR when microinjected at this site.

Support: N.I.H (HL 24347) and American Heart Assoc. (NJ).

79.4 ORTHOSTATIC AND HYPOXIC CHALLENGE DEFICITS FOLLOWING ROSTROVENTROLATERAL MEDULLARY LESIONS IN DOGS. K.J. Dormer, M.F. Wilson*, S.R. Ashlock* and D.J. Brackett*. Dept. Physiology and Biophysics and V.A. Medical Ctr., Okla. City, OK 73190.

In a continuing effort to evaluate the physiological role in conscious dogs of the subretrofacial region of epinephrine-containing cells in the rostroventrolateral medulla (C_1 area or nucleus reticularis rostroventrolateralis or RVL) we have presented various challenges to the conscious dog that elicit sympathoexcitatory responses (Fed. Proc. 46(3):676, 1987). If this putative vasomotor center, RVL, is universally important in cardiovascular control then any challenge should be affected following cytotoxic lesions. Thus, conscious dogs were instrumented to measure cardiac output (CO) and volumetric aortic blood flow (Transonic Systems, Inc.), aortic pressure (AP) and heart rate (HR, Konigsberg Inst.) and various peripheral blood flows using 20 MHz pulsed-Doppler. Orthostatic challenge was given using a supported tilt table from supine to 75° head-up tilt. Hypoxia (10% O_2 in N_2) was also administered through a mask assembly alternating with room air. Plasma catecholamines were also measured during quiet laboratory conditions. Experiments were conducted before and after small partial, unilateral 100 nl kainic acid lesions were made in the RVL. Histological verification of lesion sites showed that the analogous RVL area in the dog was injected. No changes in motor performance or behavior was noticed. The reduction in plasma catecholamines (n=4 dogs) as shown below in pg/ml of plasma

X±S.E.	Norepinephrine	Epinephrine
PRE	425±137	181±123
POST	281±107	64±51

was consistant with the lowered resting AP during rest (108 ± 12 vs 94 ± 9 pre/post) and in response to 75° tilt (increasing 16 ± 2 vs 14 ± 13 over resting values). Heart rate and CO both increased at rest post lesion but in response to 75° tilt the HR response was reduced (68 ± 23 vs 45 ± 3) while the CO increased (0.3 ± .6 vs 0.8 ± 0.9). This apparent decrease in peripheral resistance and afterload can attribute to the increased CO that is seen during tilting and exercise as shown in earlier studies. The CNS ischemic response to 10% O_2 elevated HR, AP and CO in 3/3 dogs but these responses were all reduced 10-50% following unilateral lesions. These data suggest that the RVL is involved in vasomotor control and that small, incomplete, cytotoxic lesions can produce small changes in responses to cardiovascular stressors in conscious dogs. Thus, we support anatomical and neurophysiological data of others which suggest RVL is a brainstem vasomotor center. Supported by Presbyterian Health Foundation and V.A. Research Service.

79.5 CHARACTERIZATION OF CARDIOVASCULAR AND RESPIRATORY ACTIVITY IN THE CAUDAL VENTROLATERAL MEDULLA A.C.Bonham, J.Jeske, and D.O.Nelson, Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611

The caudal ventrolateral medulla (CVLM) contains the ventral respiratory group (VRG); vagal preganglionic motor neurons; and neurons, including the A_1 cell group thought to be part of the central baroreflex circuitry and/or to provide tonic inhibitory input to the rostral VLM. Previous studies have shown that electrical or chemical stimulation in CVLM decreases arterial pressure (AP) and heart rate (HR); however, with the exception of one study in cats (McCrimmon et al., 1986), the region has not been systematically mapped for respiratory and cardiovascular effects. Accordingly, the first objective was to determine whether nanoliter injections of the excitatory amino acid agonist, DL-homocysteine (DLH, 20mM, pH7.4) produce selective effects on AP, HR or respiration. AP, HR, and diaphragm EMG were recorded in urethane-anesthetized rats. The mapped region extended 1mm rostral to 2mm caudal to calamus scriptorius, 1.5-2mm lateral to midline, and 1.5mm ventral to the dorsal surface of the brainstem to the base. Injections (3-20nl) were made via glass pipettes (10-20µm O.D) connected to a pressure injection system. Just medial to nucleus ambiguus, 1.8-2.4mm ventral to the dorsal surface and 1.5-1.8mm lateral to midline, DLH (20nl) decreased the amplitude and frequency of diaphragm EMG discharge with no effect on HR or AP. Lateral (0.2mm) to this site, from 1.5-2.2mm ventral to the dorsal surface, DLH produced a bradycardia (60±20bpm) that was blocked by iv atropine. When the pipette was displaced 0.2mm ventrally, DLH (3-15nl) decreased AP (10-40mmHg). In some cases, the depressor response was accompanied by a bradycardia (30-60bpm); however, the decrease in AP was not changed following prevention of the bradycardia with iv atropine.

To characterize neurons potentially involved in baroreflex function, the DLH-identifed depressor regions were explored using extracellular recording for units which were sensitive to changes in AP. AP was slowly raised or lowered, respectively, by graded infusions of phenylephrine or nitroprusside. In the ambiguual region in which DLH depressed diaphragm EMG, neurons were recorded which discharged with respiratory periodicity and were presumably part of the VRG. Approximately 0.5mm ventrolateral to the respiratory activity, in the region in which DLH decreased AP, neurons with various arterial pressure-activity profiles were identified: (1) spontaneously discharging neurons characterized by decreases in activity as AP increased and silence at AP 130-150mmHg. In some, but not all cases, unit activity was increased when AP was reduced to 50-60mmHg, (2) spontaneously firing units with discharges than increased when AP increased to 110-130mmHg and decreased when AP was lowered to 60mmHg, and (3) units which were silent at resting AP (85-110mmHg), but discharged when AP was lowered to 50-60mmHg. These results suggest that while excitation of cell bodies in CVLM decreases AP, indicating activation or facilitation of baroreflex inhibition of sympathetic outflow, the region contains neurons which are inhibited and excited by increases in AP. (HL 29033, NS 23423, and HS 07243).

79.6 NORMOTENSION IN CONSCIOUS RATS AFTER LESION OF ROSTRAL VENTROLATERAL MEDULLA (RVLM). K.L. Cochrane*, R.A. Buchholz and M.A. Nathan (SPON: L.P. Felpel). Dept. of Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284-7764

Bilateral electrolytic lesions of the RVLM causes hypotension in rats anesthetized with urethane but not in rats anesthetized with chloralose or pentobarbital (Fed. Proc. 45:293, 1986). This study extends those observations to conscious rats tested at 1 and 5 days after lesion of the RVLM. Captopril (5 mg/kg, iv) and chlorisondamine (1 mg/kg, iv) were used to assess the contribution of angiotension II and the sympathetic nervous system to the maintenance of mean arterial pressure (MAP) and heart rate (HR). Baroreflex sensitivity (BRS), defined as the slope of the inter-beat interval as a function of the change in systolic arterial pressure after phenylephrine (PE, 3 µg/kg, iv) or acetylcholine (ACh, 1 µg/kg, iv) was also assessed.

Cardiovascular Values - One Day Post Lesion

Group		Baseline MAP	Baseline HR	Captopril MAP	Captopril HR	Chlorisondamine MAP	Chlorisondamine HR
Lesion,	n=7	111±2	393±17	93±2*‡	400±26	54±2*	360±10*
Control,	n=18	117±3	405±11	107±4*	434±10*	56±2*	367±6*

Cardiovascular Values - Five Days Post Lesion

Group		Baseline MAP	Baseline HR	Captopril MAP	Captopril HR	Chlorisondamine MAP	Chlorisondamine HR
Lesion,	n=8	105±3	362±10	96±3*	378±14*	58±3*	290±7*
Control,	n=15	105±2	348±6	101±2*	373±9*	61±2*	304±5*

Baroreflex Sensitivity

Group		One Day Post ACh	One Day Post PE	Five Days Post ACh	Five Days Post PE
Lesion,	n=8	-.05±.15‡	1.34±.12	.28±.11‡#	.91±.08
Control,	n=13	.37±.04	1.26±.23	.51±.05#	1.07±.15

*p≤.05 from previous value; ‡p≤.05 versus control; #p≤.05 versus one day value; MAP=mmHg±SE; HR=bpm±SE; BRS=msec/mmHg

No differences were found between the groups during the baseline period or after chlorisondamine on postlesion days 1 and 5 and after captopril on postlesion day 5. However, MAP after captopril fell to a lower value in the lesion group as compared to the control group on postlesion day 1. The reduction in MAP after captopril 1 day postlesion was associated with an increased HR in the control group but not in the lesion group. The BRS to PE was similar in the lesion and control groups at 1 and 5 days postlesion. In contrast, BRS after ACh was abolished at 1 day and depressed at 5 days postlesion in the lesion group. These observations suggest that vasomotor and cardiomotor tone is unaffected by lesions of the RVLM and that, in the absence of the RVLM, other areas of the brain can maintain MAP and HR at normal values. (Supported by HL35635).

79.7 CATECHOLAMINERGIC METABOLISM RECORDED IN THE ROSTRAL VENTROLATERAL MEDULLA OBLONGATA DURING HEMODYNAMIC VARIATIONS IN ANESTHETIZED RATS. J.Y.Gillon*, L.Quintin, I.Colin*, B.Renaud and J.F.Pujol*. Lab.de Neuropharmacologie,UA CNRS 1196, Faculté de Pharmacie and Lab.de Neuropharmacologie Moléculaire, Faculté de Médecine Alexis Carrel, Université Claude Bernard,Lyon, France.

In vivo voltammetry using carbon fibre electrodes allows to record catecholaminergic (CA) metabolism in several dopaminergic and noradrenergic areas in the brain.By using this technique, we sought to determine if it was possible to detect an electrochemical signal in the C1 group of adrenergic cell bodies, a group wich lies in the rostral ventrolateral part of the medulla oblongata.In this region, a small electrochemical peak was recorded at +55 mV,a value which corresponds to the oxidation potential of 3,4 dihydroxyphenylacetic acid (DOPAC) in vitro.The site of electrochemical recording was always in the area where the adrenergic cell bodies are located, as evidenced after the end of experiments on sections of medulla incubated with antibodies against phenylethano-lamine-N-methyltransferase and revealed with peroxydase-antiperoxydase complex.Moreover, pharmacological characterizations showed that i)the tyrosine-hydroxylase inhibitor α-methylparatyrosine (αMPT,250 mg.kg^{-1} ip) suppressed totally this peak ii) the dopamine-ß-hydroxylase inhibitor FLA-63 (30 mg.kg^{-1} ip) produced a marked increase of the peak height and iii) the monoamine-oxydase inhibitor pargyline (75 mg.kg^{-1}) had an effect similar to αMPT.These results indicate that the most important contributor to the electrochemical signal should be DOPAC.Finally, the modifications of the oxidation peak were studied during hemodynamic variations induced in the circulatory system of anesthetized, ventilated and paralyzed rats. Major hemorrhage, maintained 30 min, induced a fall in arterial pressure below 50 mmHg and a marked increase in CA peak height. The same pattern of results was observed when the vasodilator sodium nitroprusside was infused. These results suggest that in vivo voltammetry combined with carbon fibre electrode could be a usefull technique to monitor the CA metabolic activity of the cardiovascular neurons of the C1 group.

79.8 **CATECHOLAMINERGIC NEURONS IN THE HUMAN ROSTRAL VENTROLATERAL MEDULLA.** *J.L. Callaway*, V. Arango, D.A. Ruggiero, M. Anwar*, J.J. Mann and D.J. Reis.* Div. of Neurobiology and Lab. of Psychopharmacology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

Adrenergic neurons in the C1 area of the rostral ventrolateral medulla (RVL),are of importance due to their presumed role in the tonic regulation of arterial pressure, baroreceptor reflex and the chemosensitivity associated with the ventral medullary surface. The connections of these neurons have been studied primarily in the rat, cat and non-human primate, whereas little is known about them in the human brain. As a first step in analyzing the functional biochemical anatomy of catecholamine (CA)-neurons in the human, we used antisera against tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) to localize medullary CA-containing neurons and processes, focusing on the C1 area of RVL. Samples from 3 neuropathologically normal human brains (< 9 hr postmortem) were collected at autopsy, fixed, sectioned frozen (-18°C) at 35 µm and incubated with antisera to TH and PNMT using a modification of the peroxidase-antiperoxidase method.

Cells staining for TH were located throughout the ventrolateral medulla (VLM). The majority of cells staining for TH and PNMT, and therefore adrenergic, occured in the RVL. Axons of TH-immunoreactive neurons in the RVL projected: 1) dorsally in a series of parallel trajectories towards the dorsomedial reticular formation and the nucleus of the solitary tract (NTS) and vagal complex, 2) longitudinally as fascicles running parallel to the neuraxis 3) ventrolaterally towards the ventral surface (VS) of the RVL where they terminated, and 4) medially into the raphe, where they arborized. Similar systems of fibers were labeled for PNMT; the longitudinal bundles were limited to the principal tegmental bundle and concentrated dorsally. Fibers containing PNMT were also identified in the medullary raphe and on the medullary VS. TH- and PNMT-labeled processes were observed making close contacts with blood vessels. In the dorsomedial medulla, neurons exhibited immunoreactivity to both TH and PNMT. In the NTS three principal subgroups of TH immunoreactive neurons were seen: a ventromedial, intermediate and dorsal group. Perikarya containing PNMT were restricted to the dorsolateral aspect of the NTS. TH- and PNMT-immunoreactive processes were identified in the medial and dorsolateral NTS; others appeared to project from NTS toward VLM. Immunoreactive fibers were observed within the solitary tract suggesting that axons of CA neurons in brain project peripherally, or that primary visceral afferents employ CAs as their neurotransmitters in the human.

We established several homologies between human and other species: a) we confirmed that the C1 area of RVL and a dorsolateral C2 segment of NTS harbor perikarya containing both TH and PNMT which are probably adrenergic. TH neurons in the caudal VLM (A1 area) and the other NTS subgroups of the A2 area do not contain PNMT and are probably noradrenergic. b) Processes containing both enzymes formed a pathway in the dorsal tegmentum equivalent to the C1-spinal tract seen in other species. c) The above pathway, and the presence of fibers of RVL interconnecting with NTS, raphe, intraparenchymal microvessels and the ventral surface suggest that the autonomic and chemoreceptor functions attributed to the C1 area also may apply to the human.

79.9 **ANTIBODIES TO A *p*-AMINOCLONIDINE CONJUGATE AS PROBES FOR IMIDAZOLE AND α_2-ADRENERGIC RECEPTOR BINDING SITES: IMPLICATIONS FOR THE STRUCTURE OF CLONIDINE-DISPLACING SUBSTANCE.** *M.P. Meeley, P. Ernsberger, P.M. McCauley*, A.C. Towle and D.J. Reis* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine interacts potently with both imidazole binding sites and α_2-adrenergic receptors in brain (Ernsberger et al., *Eur. J. Pharmacol. 134*: 1, 1987). An endogenous clonidine-displacing substance (CDS) also binds to both receptor types, with a 30-fold greater selectivity for imidazole sites (Ernsberger et al., *J. Hypertension 4 (Suppl.5)*: S109, 1986). Anti-drug antibodies have been used successfully as probes for specific recognition sites of receptor proteins. Recently, we have raised polyclonal antibodies to a conjugate of the clonidine analog *p*-aminoclonidine (PAC) (Meeley et al., *Soc. Neurosci. Abstr. 12*: 963, 1986). We sought to (a) determine the specificity of the binding of clonidine-related ligands to anti-PAC antibodies, relative to the specificities of imidazole and α_2-adrenergic receptors; and (b) examine the specific recognition by anti-PACs of endogenous CDS, the structure of which is unknown.

Polyclonal antibodies were raised in rats (anti-PAC_1) and in rabbits (anti-PAC_2) immunized with PAC coupled to hemocyanin as described previously (Meeley et al., *op. cit.*). The ligand specificity of binding to anti-PACs was examined using a competitive radioimmunoassay with ^{3}H-PAC (1nM) as the radioligand. Binding to both anti-PAC_1 and $_2$ was specific. Compounds shown to bind with high affinity to either (a) imidazole sites, such as the imidazoles cimetidine, histamine, and imidazole-4-acetic acid; (b) α_2-adrenergic receptors, such as the phenylethylamines norepinephrine and epinephrine; or (c) both receptor sites, such as idazoxan and phentolamine, tyramine, 1-benzylimidazole, and yohimbine, did not cross-react with anti-PAC antibodies. Only those agents which bind to both receptor sites, and which contain phenyl, as well as imidazol(in)e, ring moieties in their structures, competed for binding to specific antibody recognition sites, as indicated by their anti-PAC_1 IC_{50} values (nM): PAC (3.7 ± 0.5), clonidine (4.8 ± 1.0), oxymetazoline (3,400 ± 1,200), naphazoline (28,000 ± 14,000), tolazoline (110,000 ± 20,000) (n=4–24).

CDS, partially-purified from bovine brain (Meeley et al., *Life Sci. 38*: 1119, 1986), also inhibited specific ^{3}H-PAC binding to anti-PAC antibodies. The inhibition was dose-dependent (IC_{50}, 4.2 ± 0.2 Units, n=4); complete inhibition was obtained with 10 Units of CDS. Scatchard analysis of saturation binding data for ^{3}H-PAC in the presence of 3 Units of CDS showed that it had no effect on the apparent affinity of anti-PAC_2 for the radioligand (control: K_D, 0.45 ± .03 nM; with CDS: K_D, 0.44 ± .04 nM). However, a significant number of anti-PAC binding sites was blocked by CDS (control: B_{max}, 187 ± 10 nmol/mg IgG; with CDS: B_{max}, 130 ± 9 nmol/mg IgG). Immunoprecipitation experiments carried out by incubating increasing dilutions (1:100–1:100,000) of anti-PAC_2 with 2 Units of CDS showed that authentic CDS, i.e., that which displaces ^{3}H-PAC binding in a radioreceptor assay, is the same substance which is recognized by anti-PAC antibodies.

We conclude that (a) chemical determinants, critical for binding to anti-PAC antibodies, constitute a unique sub-set of structural requirements for interaction with both imidazole and α_2-adrenergic receptor sites; and (b) CDS may share structural elements with clonidine and related compounds since anti-PAC antibodies also recognize the endogenous substance.

79.10 ELECTRON MICROSCOPIC EVIDENCE THAT CELLS IN THE NUCLEUS TRACTUS SOLITARIUS PROJECT TO THE C1 EPINEPHRINE CELL GROUP. J. R. Carithers and D. P. Baker. Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011

Abundant evidence indicates that epinephrine producing cells in the rostral ventrolateral medulla (the C1 cell group) project to the intermediolateral cell column of the spinal cord and are involved in maintenance of cardiovascular sympathetic tone. The cardiovascular region of the nucleus tractus solitarius (NTS), the primary center for termination of baroreceptor afferent fibers, has been shown by light microscopic studies to project to the region of the C1 cells. It has been suggested that this projection from NTS to the rostral ventrolateral medulla is responsible for baroreflex inhibition of vasomotor tone.

This investigation was undertaken to determine if fibers from the NTS synapse directly on C1 cells. A craniotomy was performed on ten rats under ketamine anesthesia, and 20 nl of 2% horseradish peroxidase (HRP) was injected slowly into the cardiovascular portion of the left NTS. The animals were killed after 24 hours. HRP reaction product was developed using DAB as the chromogen, and the tissues were processed for electron microscopy. C1 cells were positively identified by the presence of phenylethanolamine-N-methyltransferase immunoreactivity at the electron microscopic level using colloidal gold.

Colloidal gold was localized in the cytoplasm of C1 cells in the rostral ventrolateral medulla. Terminals containing HRP reaction product synapsed upon neuronal somas and dendritic processes containing colloidal gold. These results demonstrate at the electron microscopic level that cells in the area of the NTS project directly to C1 cells. This projection may be a connection by which stimulation of the baroreceptors causes inhibition of the medullary vasomotor center.

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79.11 PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT (NTS) ONTO PNMT-IMMUNOREACTIVE CELLS IN THE VENTRAL MEDULLA OF THE RAT. M.B. Hancock* (SPON: J. Kitay). Department of Anatomy and Neurosciences, The University of Texas Medical Branch, Galveston, Texas 77550.

Phaseolus vulgaris-leucoagglutinin (PHA-L) was iontophoretically deposited (Gerfen, C.R. and P.E. Sawchenko, Brain Res., 290:219, 1984), into the nucleus of the solitary tract (NTS) of Sprague-Dawley rats anesthetized with Nembutal and urethane. Following survival times of 4-12 days, the rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde, the brains were removed, and sectioned (30 μm) on a Vibratome. The sections were stained first for PHA-L-immunoreactive (PHA-LI) elements with the PAP technique and nickel-intensified diaminobenzidine (Ni/DAB). Black-stained, PHA-LI cells and processes were present in NTS, and PHA-LI fibers could be traced into the ventrolateral medulla. The medullary sections were then immunostained for phenylethanolamine N-methyl transferase (PNMTI) with the PAP method and DAB alone. PNMT-immunoreactive (PNMTI) cells were stained amber. Black-stained PHA-LI terminal fields were present amongst amber-stained, PNMTI cells in the ventrolateral medulla. Varicose, PHA-LI fibers were observed coursing along the surface of PNMTI dendrites, and PHA-LI processes were seen on the somata of PNMTI cells.

These observations indicate that neurons in the solitary nucleus may have direct effects on adrenaline cells in the ventrolateral medulla. Adrenaline cells in the ventrolateral medulla have been linked to tonic vasomotor activity (Ross, C.A., et al., J. Neurosci. 4:474, 1984), and the close anatomical association between fibers originating in NTS and PNMTI cells in the ventral medulla suggests a direct functional relationship between these elements that may have a role in cardiovascular regulation.

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79.12 AN IN VITRO SLICE PREPARATION OF THE CANINE DORSOMEDIAL MEDULLA FOR ELECTROPHYSIOLOGICAL INVESTIGATION OF AUTONOMIC NUCLEI. W.D. Knowles, K.L. Barnes and C.M. Ferrario. Departments of Brain and Vascular Research and Neurology, The Research Institute of The Cleveland Clinic Foundation, Cleveland OH 44106.

We have developed an in vitro slice preparation of the canine dorsomedial medulla which is appropriate for extracellular and intracellular electrophysiological investigations of the autonomic nuclei in this region. The preparation allows stable intracellular penetrations of the larger neurons and stable extracellular recordings with excellent signal to noise ratio. The stability of the preparation allows applications of exogenous substances while maintaining the recordings. It is thus possible to make direct measurements of the effects of neuromodulators and receptor blockers on neural activity.

Dogs (8-12 kg mongrels) are anesthetized with halothane, loose ligatures are placed around the vertebral and carotid arteries, and the dorsal medulla is exposed. Anesthesia is tapered as tolerated during the later portion of the surgery. The medulla is cooled with ice-cold oxygenated physiological saline, and the neck arteries are tightly ligated. The brainstem is transected through the caudal pons and about 5 mm posterior to the obex and removed. After the pia and arachnoid are stripped, the dorsomedial medulla is blocked and then cut with a vibrating slicer into 400 μm thick horizontal sections while submerged in cold (3°-4°C) physiological saline. The sections are then placed in an interface type slice chamber at 36°C. The elapsed time from ligation of the arteries to placing the slices in the chamber is approximately 7 to 8 minutes. The slices are allowed to recover for one hour before recording.

Standard in vitro electrophysiological techniques are used to record neural activity for as long as 6 hours after slice preparation. Intracellular penetrations of good quality and long duration (30-60 minutes) are obtained from large neurons. Extracellular recordings with excellent signal to noise ratio (> 10:1) and long duration (> 45 minutes) are routinely obtained from the smaller neurons of the nucleus tractus solitarii and surrounding regions. Extracellular micropipettes are filled with 0.9% NaCl saturated with Fast Green dye, which is iontophoretically ejected to mark recording sites. We routinely apply exogenous substances in microdroplets on the surface of the slice while maintaining recordings. Our accompanying presentation (Barnes et al, this volume) shows the results of angiotensin peptides applied to nucleus tractus solitarii neurons. (Supported in part by NIHLBI grant HL-6385 and the Reinberger Foundation).

79.13 EFFECTS OF ANGIOTENSIN PEPTIDES ON SINGLE NEURONS RECORDED FROM AN IN VITRO PREPARATION OF THE CANINE DORSOMEDIAL MEDULLA. K.L. Barnes, W.D. Knowles, and C.M. Ferrario. Departments of Brain and Vascular Research and Neurology, The Research Institute of The Cleveland Clinic Foundation, Cleveland, OH, 44106.

We have previously obtained substantial physiological evidence that the area postrema (ap) and nucleus tractus solitarii (nTS) in the dorsomedial medulla (DMM) of the dog normally function as reciprocally counterbalanced influences on sympathetic vasomotor outflow. Chronic studies in conscious animals have also confirmed our hypothesis that removal or excessive activation of either structure may lead to dysregulation of arterial blood pressure. Recently, Averill et al. [Brain Res., 1987 (In press)] showed that microinjection of pmol doses of angiotensin II (Ang II) into the medial nTS of the dog causes pressor effects and tachycardia. In addition, we have localized within the canine ap, nTS, and dorsal motor nucleus of the vagus (dmnX) a discrete pattern of high affinity Ang II binding sites which is dependent on intact vagal innervation of the DMM (Diz et al., Brain Res Bull 17: 497-505, 1986). These findings have now led us to investigate directly the effects of Ang II on single neurons in an in vitro slice preparation of the canine medulla which we have developed.

Stable extracellular recordings were obtained from neurons in 400 μm thick horizontal slices of the DMM (for details, see W.D. Knowles et al., this volume). In 15 neurons located in or adjacent to the medial nTS (identified by Fast Green dye marks), the effects of microdrop application of either Ang II (8 pmol/μl) or artificial cerebrospinal fluid (aCSF) onto the surface of the slice surrounding the recording electrode were examined. The neurons were generally unresponsive to aCSF applied to the brain slice. Application of Ang II substantially increased the firing rate of 8 of the 15 neurons studied. The other 7 neurons were not responsive to Ang II. In most cases, the firing rate increased steadily to a peak at 1-2 min following Ang II application to the slice, then returned slowly to base line over the next 5-10 min. In 4 of the 15 neurons, the effects of both Ang II and its analogue [Sar^1,Thr^8] Ang II were tested. [Sar^1,Thr^8] Ang II produced a rapid onset, brief excitation in 3 of the 4 neurons. These 3 neurons were also excited by Ang II. Administration of [Sar^1,Thr^8] Ang II blocked the excitatory response to subsequent administration of Ang II in these 3 responsive cells. The remaining cell did not respond to application of either Ang peptide.

These experiments have begun to map the Ang II-responsive neural substrate for the cardiovascular actions of this peptide in the canine DMM. (Supported in part by NIHLBI grant HL-6835 and the Reinberger Foundation).

79.14 ORIGIN OF ATRIAL NATRIURETIC PEPTIDE-IMMUNOREACTIVE INNERVATION OF THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. K.M. Hurley, D.G. Standaert and C.B. Saper, Depts. of Pharm. & Physiol. and Neurol., University of Chicago, Chicago, IL 60637 and Dept. of Pharm., Washington Univ. Sch. Med., St. Louis, MO 63110.

Atriopeptin (AP), the atrial natriuretic peptide, is a 28 amino acid circulating hormone that is secreted by the atria of the heart, resulting in natriuresis, diuresis and vasodilatation. We recently described the distribution of AP-immunoreactive (APir) cell bodies and fibers in the rat brain, including the heavy APir innervation of the medial and commissural parts of the nucleus of the solitary tract (NTS) and the dorsal motor vagal nucleus (DMX). These areas have been associated with cardiovascular regulation, suggesting that AP may serve as a central neurotransmitter in this system. However, the origin of this APir innervation has not been established.

We therefore injected the dorsal vagal complex (DVC) in a series of rats with Fast Blue (2%) dye, 50-80 nl, and one week later treated them with 150 μg of colchicine, i.c.v. Thirty-six to forty hours later, the animals were pefused with 4% paraformaldehyde and sections through the brain and vagal ganglia were stained by an immunofluorescence method for AP. The injection sites were large, and included portions of the adjacent dorsal column nuclei, reticular formation and hypoglossal nucleus.

A relatively small number of double-labeled neurons was observed in the brain, mostly in the parvocellular part of the paraventricular nucleus of the hypothalamus and in the retrochiasmatic area. Other double-labeled cells were found in the laterodorsal and pedunculopontine tegmental nuclei; these cell groups project to the areas adjacent to, but not including, the DVC. No APir cell bodies were found in the vagal ganglia, and removing these ganglia did not effect the APir innervation of the DVC. Our technique did not allow us to determine whether intrinsic neurons within NTS contribute to the APir fiber innervation of the nucleus. However, the only extrinsic source of this innervation that we could identify consists of a small number of APir neurons in the hypothalamus.

Our results suggest that AP may serve as a neurotransmitter that subserves hypothalamic modulation of cardiovascular afferent transmission in the NTS.

79.15 CARDIOVASCULAR RESPONSES MEDIATED BY THE RELEASE OF GLYCINE INTO THE NUCLEUS TRACTUS SOLITARIUS OF RAT. S.C. Robertson* and W.T. Talman. (SPON: H. Damasio). Department of Neurology, VA Medical Center and University of Iowa, Iowa City, Iowa 52242.

The inhibitory amino acid glycine, found in high concentrations in the nucleus tractus solitarius (NTS) is released from the neurotransmitter pool into the NTS and is inactivated by high affinity uptake mechanisms. We sought to determine the role of glycine in the regulation of arterial pressure and heart rate by the medial subnucleus of the NTS. Thirty-eight adult male Sprague Dawley rats were anesthetized and instrumented for recording intra-arterial pressure, the administration of intravenous drugs, and the stereotaxic placement of microinjections into the brain stem. Glass micropipettes filled with artificial CSF as a control vehicle or with glycine, strychnine, or glutamate were positioned in the medial subnucleus of NTS and 25 nl injections were made into that nucleus. The site of all injections, marked by a vital stain, was confirmed postmortem. Glycine, like glutamate, an excitatory amino acid, elicited immediate dose-related hypotension and bradycardia. The threshold dose was 2 nmols and the maximally effective dose was 10 nmols. The maximal effect was a fall of mean arterial pressure of 35.5 ± 8.0 mmHg (mean ± SEM) from a baseline of 83.7 ± 12.5 mmHg and a fall of heart rate of 32.9 ± 16.0 bpm from a baseline of 324.7 ± 37.6 bpm. Strychnine, 67 pmols, completely blocked the cardiovascular responses of the maximally effective dose of glycine but when injected bilaterally into the NTS did not alter baroreflex mediated reflex bradycardia elicited by pressor doses of phenylephrine given intravenously. The hypotensive and bradycardic responses to glycine were highly localized to the dorsal medial subnucleus of the NTS and did not occur with injections outside the NTS. As previously reported, injections ventral to the NTS caused hypertension and tachycardia. When low doses of glycine (4 nmols) were injected prior to the injection of glutamate (250 pmols)into the same site, the excitatory response of glutamate was significantly attenuated or abolished. These data suggest that glycinergic mechanisms in the NTS may play an important role in central cardiovascular regulation. The responses to glycine are distinctly different from GABA, another inhibitory amino acid, and may be mediated through different reflex pathways. Supported by NIH-RO1 HL32205 and VA Merit Review Tab 18. WTT was supported in part by an Established Investigatorship with the American Heart Association.

79.16 INTRAPARENCHYMAL DISTRIBUTION OF OPIOIDS INJECTED INTO RAT DORSAL VAGAL COMPLEX J.W. Nemitz and A.H. Hassen, Depts. Anat. and Physiol., WVSOM, Lewisburg, W.Va. 24901.

The microinjection of a mu opioid receptor agonist into the rat hindbrain has been shown to elicit different cardiovascular responses depending upon the site of injection. In the present experiments we have used autoradiographic techniques to examine the distribution of a labelled (H^3) mu-agonist, D-Ala^2, $MePhe^4$, Gly-ol^5 enkephalin (DAGO, Amersham) after microinjection into the dorsal vagal complex (DVC) of anesthetized rats.

Experiments were conducted on male Sprague-Dawley rats anesthetized with urethane (1.6 g/kg, i.p., plus supplemental doses as needed), intubated, placed in a Kopf stereotaxic frame and ventilated with a mixture of air and O_2. A partial craniotomy was performed to allow a glass micropipette (OD = 28um), containing the H^3-DAGO (.3 nmol in 10 nl), to be inserted into the DVC. The H^3-DAGO was microinjected using a General Valve Corp. Picospritzer II. Continuous recording of systolic and diastolic blood pressure, mean arterial pressure, and heart rate was made throughout the experiment. The animals were sacrificed with an overdose of pentobarbital, the brain stems removed and frozen on dry ice. Serial frozen sections (20 um thick) were cut on a cryostat, mounted on subbed glass slides, dipped in photographic emulsion (Kodak NB-2), stored in lightproof boxes and developed after an exposure period of 2 - 6 weeks. Developed slides were analyzed for distribution of injected label.

Dense labelling in the DVC was observed after microinjection of H^3-DAGO into the nucleus of the tractus solitarius (nTS). Silver grains were observed to be arranged in circular patches over neuronal cell bodies of the nTS, ipsilateral to the injection site. Label distributed in a rostro-caudal orientation over an average distance of 1000 um. Labelling was also observed to occur in the dorsal motor nucleus of the vagus (DMnV) and the hypoglossal nucleus. Following microinjection into the DMnV, label again remained ipsilateral, was observed as circular patches over neuronal cell bodies and had a rostro-caudal orientation. Label was also observed in the nTS and the hypoglossal nucleus. No label was observed in the area postrema, regardless of the injection site. Although the labelled DAGO was never limited to the primary injection site, but was observed to distribute in an extensive rostro-caudal plane and to nuclei adjacent to the microinjection, the cardiovascular responses elicited from these sites are dissimilar. Thus the relative contributions of nTS and DMnV to specific cardiovascular responses elicited with the microinjection of DAGO remains to be determined.
Supported by WV Affiliate of the AHA.

79.17 **EFFECTS OF VAGAL RICIN INJECTION ON A2 CATECHOLAMINERGIC NEURONS OF THE RAT MEDULLA.** **T.N. Oeltmann* & R.G. Wiley**(SPON: W.Dettbarn) **Medicine & Neurology Dept, Vanderbilt U. and VAMC, Nashville, TN 37232.**

Concern exists as to whether or not suicide transport of ricin remains restricted to motor neurons originally transporting the toxin. Although double labelling studies have given conflicting results at levels rostral to the obex, A2 noradrenergic neurons caudal to the obex are not thought to project axons through the vagus nerve even though they reside among and adjacent to motor neurons of the the dorsal motor nucleus of the vagus. In the present study, we sought to determine if unilateral destruction of vagal motor neurons by suicide transport of the toxic lectin, ricin, injected into the cervical vagus nerve would also destroy A2 neurons. Either 50 or 270 ng of ricin was unilaterally pressure microinjected into the left cervical vagus nerve of 11 anesthetized, adult male Sprague-Dawley rats. After 3-14 days, rats were reanesthetized and transcardially perfused with aldehyde fixative (FAGLU for catecholamine histofluorescence, N=7, or formaldehyde for anti-tyrosine hydroxylase immunohistochemistry, N=4). Alternate transverse sections of the medulla were stained with cresyl violet to confirm complete destruction of the dorsal motor nucleus of the vagus. 4 control rats (2 for histofluorescence and 2 for anti-TH immunoperoxidase) were processed identically except that toxin injection was omitted. All fluorescent or immunoperoxidase labelled neurons in the dorsomedial medulla between the decussation of the medial lemniscus and the obex were counted without knowledge of which side was injected. There were no significant differences between right and left sides with respect to numbers of catecholaminergic neurons in the A2 of control and ricin-injected animals. Using FAGLU histofluorescence, A2 cell counts on the injected side were 101.3% +/- 3.7 (SEM) of the contralateral control side, and by anti-TH the injected side was 96.5% +/- 13.4 of control. We conclude: 1 - A2 neurons caudal to the obex do not project axons through the vagus, and 2 - using the doses in this study, the neuronal lesion subsequent to vagal ricin injection remains restricted to vagal motor neurons. (This work supported by the Veterans Administration.)

79.18 PROJECTIONS OF THE VAGAL MOTOR COMPLEX: A DOUBLE LABELING STUDY. D. P. Baker and J. R. Carithers. Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011

Labeled cells have been observed in the region of the medullary vagal complex after injection of horseradish peroxidase (HRP) into the spinal cord as far distally as the lumbar region. Our investigations have focused on identifying projections from the medulla that could be involved in the baroreflex. Therefore, we were interested in confirming these reports, and in determining whether cells projecting to the spinal cord were, indeed, parasympathetic preganglionic neurons that also project via the vagus nerve.

A dorsal laminectomy was performed on ten rats, and 200 nl of 4% HRP were injected into the second thoracic segment of the spinal cord. Although the needle was directed toward the left intermediolateral cell column, the injection solution diffused significantly, and in some rats most of the left half of the cord was labeled. Immediately thereafter, the cervical vagus was dissected free at the thoracic inlet and 1 ul of 2% Fluoro-Gold solution was injected into the perineurium. The rats were killed 24 to 48 hours later, and the tissues were processed for light microscopy, using TMB as the chromogen.

Cells double labeled with dark HRP reaction product and brilliantly fluorescent Fluoro-Gold were present in the nucleus ambiguus. Double labeled cells were also present in the dorsal motor nucleus of the vagus. Approximately 25% of the cells containing Fluoro-Gold also contained HRP reaction product in both nuclei. There were no cells in this nuclear group which were singly labeled with HRP.

The spinal projection from vagal preganglionic cells, which we have identified, could provide an anatomical substrate for parasympathetic modulation of the activity of preganglionic sympathetic neurons. Such modulation could contribute to inhibition of sympathetic vascular tone during activation of the baroreflex. Projections from the nucleus tractus solitarii, the primary projection target of baroreceptor afferent fibers, have been identified in association with cells in this group. Together, these connections may constitute one of the simplest pathways in the baroreflex complex.

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79.19 RESPIRATORY MODULATION OF SYMPATHETIC-RELATED RAPHE NEURONS. C.A. Connelly, D.C. Bolser*, and J.E. Remmers. Dept. of Medicine, University of Calgary, Health Sciences Centre, Calgary, Alberta CANADA, T2N 4N1

Raphe pallidus and obscurus neurons correlated with sympathetic activity were investigated in thirteen decerebrate, paralyzed, artificially ventilated cats. Phrenic and inferior cardiac sympathetic activities were recorded as indices of respiratory and sympathetic activities, respectively. Computer averaging techniques were used to assess sympathetic, cardiac, and respiratory-related activity patterns in the raphe neurons, recorded using intracellular methods. Raphe unit spike triggered averaging of sympathetic activity was used to detect the sympathetic-related activity patterns found in twenty-one of the thirty-six neurons recorded. The number of sympathetic-related cells primarily discharging at frequencies of 1-10 Hz, 11-30 Hz, and 31-70 Hz, respectively, was 7:9:5. Spike triggered averages from slowly discharging (2-3 Hz) neurons were indistinguishable from those of rapidly discharging (e.g. 20 Hz) neurons. We also recorded irregularly firing cells that discharged slowly with intermittent high frequency (greater than 20 Hz) bursts of activity correlated with decreased sympathetic activity. None of the sympathetic-related raphe neurons were antidromically activated by spinal cord stimulation. Respiratory cycle triggered averages of raphe neuron activities indicated that eleven of the sympathetic-related neurons exhibited respiratory characteristics; the usual pattern of respiratory modulation was relative hyperpolarization during late expiration and early inspiration. Respiratory cycle triggered histograms of the raphe unit spikes revealed that cell discharge was associated with the membrane depolarization during late inspiration/early expiration. ECG (R wave) triggered histograms of raphe unit spikes indicated cardiac-related activity patterns in nine neurons. Seven of the cardiac-related cells were also sympathetic-related. Five of the raphe neurons exhibited combined cardiac, respiratory and sympathetic-related activity patterns. Moment to moment variability of cardiorespiratory discharge patterns and the lack of bulbospinal projections suggest an integrative function for these neurons. (Supported by the Alberta Heritage Foundation for Medical Research).

CARDIOVASCULAR REGULATION: CNS PATHWAYS II

80.1 MICROINJECTIONS OF CALCITONIN-GENE-RELATED PEPTIDE IN THE CENTRAL NUCLEUS OF THE AMYDGALA BLOCKS NORADRENERGIC CARDIOVASCULAR EFFECTS. R. J. Leonzio*, J. P. Card, A. Zaspal*, W. Price*, P. Timmermans*, and J. S. Schwaber, (Spon: W. F. Herblin) E. I. du Pont de Nemours and Co., Medical Products Department, Wilmington, DE 19898.

The central nucleus of the amygdala (CeA) exerts a major influence on cardiovascular function through its interconnections with brainstem. Several chemically-distinct afferents terminate differentialy within subfields of the central nucleus. Understanding the interactions among these peptides and neurotransmitters is required to clarify the mechanisms by which the CeA regulates cardiovascular performance. The peptide calcitonin-gene-related peptide (CGRP) has been described by others to have both central and peripheral actions on the cardiovascular system. CGRP is also of particular interest due its very dense innervation of projection neurons in the CeA relevant to amydgaloid regulation of cardiovascular performance. CGRP-containing fibers that originate in the parabrachial nucleus form dense pericellular aborizations around neurons in the CeA that are particularly dense in the lateral central and lateral capsular subdivisions. The present study examined the interaction in the CeA of CGRP and NE in the control of blood pressure. Chronically implanted cannulae were stereotaxically positioned for bilateral injection of compounds into the CeA. The effects of CGRP (0, 0.16, 0.33, 0.66, or 1.32 pmole/0.5 µl) and/or NE (0 or 25 nmole/0.5 µl) on heart rate and blood pressure were measured with a chronic arterial cannula while the rats were awake and unrestrained. The administration of NE alone significantly increases mean arterial pressure by 30.6% with a peak effect 3 to 4 min after injection. CGRP blocked the NE-induced increase in blood pressure in a dose-related manner with an ED_{50} of 0.4 pmole/0.5 µl. CGRP alone had no effect in the range of doses used. Control placements of cannula outside the CeA also were without NE or CGRP effects. Our evidence suggests that CGRP-containing afferents from the parabrachial nucleus function to attenuate the hypertensive effects of endogenous NE in the CeA.

80.2 CALCITONIN-GENE-RELATED PEPTIDE CONTAINING NEURONS IN THE PARABRACHIAL NUCLEUS PROJECT TO THE CENTRAL NUCLEUS OF THE AMYGDALA. J.S. Schwaber, C. Sternini, N.C. Brecha, W. T. Rogers*, J.R. Dubin* and J.P. Card. E. I. du Pont de Nemours and Co. (JSS, JRD, JPC & WTR), Wilmington, DE 19898 and UCLA Sch. of Med. (NCB, CS), LA, CA 90034

The source, distribution and morphology of axons displaying calcitonin-gene-related peptide (CGRP) immunoreactivity in the central amygdaloid nucleus of the adult rat was investigated with immunohistochemical and combined immunohistochemical-axonal transport techniques. Immunoperoxidase methodology demonstrated an extremely dense plexus of CGRP immunoreactive axons that is differentially concentrated within the lateral capsular and lateral central subdivisions of the central nucleus. No immunoreactive neurons were observed in the central nucleus in any of the experimental animals. A characteristic feature of this fiber plexus is the prominent pericellular arborization of immunoreactive axons surrounding the somata of unlabeled neurons. The number of cells receiving this dense pericellular terminal arborization increases at caudal levels of the central nucleus. Retrograde transport of rhodamine-labeled microspheres or Fluorogold combined with immunohistochemical localization of CGRP immunoreactivity demonstrated that the major source of this fiber plexus arises bilaterally from multipolar CGRP-containing neurons in the ventrolateral aspect of the parabrachial nucleus. Combined retrograde dye transport and CGRP immunohistochemistry established that many of the neurons in the central nucleus which receive dense pericellular innervation from CGRP immunoreactive axons are projecting caudally to either the parabrachial nucleus or the nucleus tractus solitarius. These results strongly suggest the relevance of CGRP input to the central nucleus in cardiovascular and other autonomic function. Ascending CGRP-ergic influences may act directly or in concert with catecholaminergic or other peptidergic inputs to influence chemical-specific pathways mediating the effects of environmental stressors on cardiovascular function.

80.3 AMYGDALA MEDIATION OF CARDIOVASCULAR RESPONSES: EFFECTS OF ELECTRICAL STIMULATION AND INJECTIONS OF NEUROPEPTIDES. T.S. Gray and M.R. Brown. Dept. Anat., Loyola Univ. Sch. Med., Maywood, IL 60153 and Depts. Med. & Surg. UCSD Med Ctr., San Diego, CA

The central nucleus of the amygdala (Ce) mediates cardiovascular changes in response to defense- or fear-provoking stimuli through its connections with autonomic regions of the hypothalamus and brainstem. The Ce also contains at least sixteen different types of neuropeptide-containing terminals. The purpose of the present study is to: 1) To determine if the Ce is the only site within the amygdala that when stimulated will cause increases in heart rate (HR) and mean arterial pressure (MAP); 2) To determine which neuropeptides when injected into the Ce will affect HR and MAP.

The subjects of the study were male adult Sprague-Dawley rats. Prior to all surgical procedures animals were anesthetized with ether or sodium pentobarbital (40 mg/kg). Four days prior to experiments either a bipolar platinum electrode (Plastic Products) or a silastic-tipped polyethylene-50 cannula (Plastic Products) was implanted unilaterally within the amygdala of each animal. Femoral artery catheters were placed in all animals 3-4 h prior to experiments for measurement of HR and MAP. Electrical stimulation was conducted using a Grass S44 stimulator and a Grass SIU5 stimulus isolation unit. Stimulation was delivered over a 30-s period and consisted of monophasic pulses each 0.5 ms in duration at 50 hz and at currents of 15-75 uA. All experiments were performed in unanesthetized, unrestrained animals.

Electrical stimulation of the Ce consistently elicited both increases in HR and MAP. Increases in HR and MAP could be obtained using current levels as low as 15 uA with maximal responses typically obtained at current levels of 50 uA. Increases in HR and MAP were obtained from stimulation of other regions of the amygdala, but were not as consistent as those elicited from Ce stimulation. There were no sites where decreases in HR and MAP were consistently observed.

Results of neuropeptide injections into the Ce demonstrated that only 5 of the 16 neuropeptides caused changes in HR and/or MAP. Thyrotropin releasing factor (TRF) and calcitonin gene-related peptide (CGRP) induced increases in both MAP and HR. Angiotensin II and somatostatin-28 injections caused increases in MAP and decreases in HR. Bombesin injections in Ce induced increases in MAP, but did not alter HR. Thus, TRF and CGRP injections into the Ce produced increases in MAP and HR that most resemble those seen in learned responses to aversive stimuli. The results of these experiments lend further credence to the hypothesis that the Ce is responsible for amygdaloid-related changes in MAP and HR associated with defensive behaviors or emotional responses to environmental stressors. Supported by NS 20041, AM26741, HL32008, & HL37716.

80.4 BRADYCARDIC RESPONSE TO STIMULATION OF THE AMYGDALOID CENTRAL NUCLEUS IS PROPORTIONAL TO ARTERIAL BLOOD PRESSURE. J.P. Pascoe, D.J. Bradley* & K.M. Spyer*. Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill St., London NW3 2PF (UK)

Many findings suggest that the amygdaloid central nucleus (ACE) may contribute to cardiovascular regulation, particularly during emotional states (Kapp et al., 1984). Consistent with this notion are the existence of direct projections from the ACE to cardiovascular regulatory nuclei in the brainstem, including the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (NTS/DMN, Hopkins & Holstege, 1978), and the finding that stimulation of the ACE activates many neurons in these areas including a portion of those that receive an excitatory input from baroreceptor afferents (Cox et al., 1986). These and other observations (Pascoe & Kapp, 1985) have led us to hypothesize that increased activity in ACE projections to the NTS/DMN complex might serve to facilitate the baroreceptor-vagal reflex. Based upon this hypothesis we have tested the prediction that bradycardic responses to stimulation of the ACE should be proportional to arterial blood pressure (BP).

New Zealand white rabbits were anesthetized with α-chloralose, the trachea was intubated, a femoral vein was cannulated, and a Swan-Ganz catheter was inserted through a femoral artery until its tip was positioned in the descending aorta at the level of the diaphragm. A monopolar stimulating electrode was implanted in the ACE. Cardiovascular responses to stimulation of the ACE (150-500 μA, 0.5 ms, 100 Hz) then were assessed during periods in which BP was decreased using sodium nitroprusside (2-10 μg/kg) or increased in the rostral arterial compartment by inflating the tip of the Swan-Ganz catheter. Mean BPs ranging from 65-140 mmHg were obtained in this manner.

Resting heart rate was correlated inversely with BP (r's -.70 to -.88, $p<.01$) and ranged from 252-360 bpm. Stimulation of the ACE always evoked bradycardia and hypotension. In each experiment (N=7) bradycardic response magnitudes were correlated with the arterial BP at the onset of ACE stimulation, such that higher BPs were associated with larger bradycardic responses (r's from .67 to .86, $p<.01$, slopes from 0.5 to 1.1, intercepts from 43 to 91 mmHg). The durations of bradycardic responses to ACE stimulation when BP was increased were longer than those seen during periods of resting or low BP ($\bar{X}$'s= 12.0 vs. 7.2 and 7.0 s). Depressor responses to ACE stimulation were smaller when BP was increased or decreased, as compared to control responses ($\bar{X}$'s= 5.6 and 9.1 vs. 16.2 mmHg).

These data demonstrate that bradycardia to stimulation of the ACE is proportional to arterial BP and thus at least partially dependent upon baroreceptor afferent activity, and are consistent with the hypothesis that activity in the ACE projection to the NTS/DMN may facilitate the baroreceptor-vagal reflex in the rabbit. Supported by the British Heart Foundation. J.P.P. is a Fellow of the American Heart Association and the British Heart Foundation.

80.5 DIENCEPHALIC REGIONS CONTRIBUTING TO SYMPATHETIC NERVE DISCHARGE IN THE ANESTHETIZED CAT. K.J. Varner, Z-S. Huang*, S.M. Barman and G.L. Gebber. Depts. of Pharmacol. and Physiol., Michigan State Univ., East Lansing, MI 48824.

We (Huang et al., Am. J. Physiol., in press) have reported that midbrain transection in the chloralose anesthetized cat is accompanied by transient (lasting ≈30 min) decreases in blood pressure (33±4 mmHg) and inferior cardiac postganglionic sympathetic nerve discharge (SND; 38±7%). Two observations led us to conclude that the transient effects of midbrain transection reflected the loss of a forebrain-dependent component of SND rather than a nonspecific phenomenon such as generalized trauma. First, SND was correlated to frontal cortical activity prior to midbrain transection. Second, following recovery from the effect of midbrain transection, a second transection placed more caudally in the midbrain failed to affect blood pressure and SND.

The current study was initiated to determine whether the forebrain contribution to basal SND in baroreceptor-denervated cats anesthetized with chloralose is dependent upon the integrity of medial diencephalic structures. For this purpose, the effects of midbrain transection at stereotaxic plane A3 on blood pressure and SND (cumulatively integrated) in control cats were compared with those observed in cats in which radio frequency current was used to lesion bilaterally discrete regions of the hypothalamus or medial thalamus.

Lesions of the medial hypothalamus at a level including the paraventricular nucleus failed to alter the reduction of blood pressure and SND produced by midbrain transection. Extension of medial lesions caudally into the posterior hypothalamic region, however, significantly attenuated the effects of midbrain transection on blood pressure and SND. The effects of midbrain transection were similarly attenuated by lesions of the medial forebrain bundle and portions of the lateral hypothalamic region. Unexpectedly, lesions of the medial thalamus (0—3.5 mm lateral to midline) between stereotaxic planes A7 and A11 also significantly attenuated the effects of midbrain transection on blood pressure and SND. These lesions included portions of the following medial thalamic nuclei: centralis lateralis, centralis medialis and medialis dorsalis. These results have led us to conclude that the medial thalamus as well as the posterior and lateral hypothalamic regions are involved in mediating the forebrain-dependent component of basal SND in anesthetized cats. The relationships between medial thalamic and hypothalamic structures involved in sympathetic control remain to be elucidated. (Supported by HL33266 and HI13187.)

80.6 PREFRONTAL STIMULUS-PRODUCED HYPOTENSION. S.G.P. Hardy and D.E. Holmes*. Dept. of Anatomy, Univ. of Miss. Med. Ctr., Jackson, MS 39216.

It has recently been demonstrated, in anesthetized rats, that stimulus-produced hypotension (SPH) can be elicited from the medial prefrontal cortex (PFC). The present study was conducted in order to better understand the involvement of the PFC in cardiovascular control.

In urethane anesthetized (2g/Kg) rats, bipolar electrical stimulation (a 12 sec train of 1 ms square waves, delivered at 20 Hz with current intensity of 50-1500 uA) was administered to various sites within the frontal cortex, including medial PFC, lateral PFC, infralimbic cortex and frontal convexity cortex. The effects of stimulation upon blood pressure and heart rate were monitored. Stimulation of both the lateral PFC and ventral portion of the medial PFC (i.e. prelimbic cortex) consistently resulted in SPH. However, stimulation of the lateral PFC usually produced greater decreases in blood pressure (often decreases of 50% from baseline) and with lower stimulus thresholds (as small as 90uA). Within the lateral PFC, the most effective sites for supporting SPH were located caudally. Stimulus sites within the infralimbic cortex also consistently supported SPH (similar in magnitude as that elicited from the lateral PFC). Stimulation administered to the lateral PFC or infralimbic cortex also resulted in bradycardia, occurring in synchrony with SPH. Stimulation at sites in the frontal convexity cortex had no effect on blood pressure or heart rate.

SPH had a rapid onset (suggesting a neural substrate) with maximum hypotension observed at approximately 8 sec following the stimulus onset. Furthermore, SPH was a short-lived effect. Returns to baseline measurements usually were initiated before the electrical stimulus ceased. Hypertensive responses to frontal cortex stimulation were not observed.

To determine whether SPH is mediated via the vagus nerves, injections (1-3mg/Kg; i.v.) of either atropine methyl nitrate or gallamine triethiodide were administered to block vagal influences. Bilateral vagotomies were occasionally also performed. In none of these cases was SPH blocked; therefore, demonstrating that SPH is not mediated via the vagus nerves.

To determine whether SPH may be mediated via sympathetic inhibition, norepinephrine (NE) was administered (0.03 mg/Kg; i.p.). This produced a hypertensive state that remained steady over a 20 min. period. During this period, SPH was blocked. After the effects of NE had dissipated, SPH could again be elicited. This finding suggests that SPH may be mediated via sympathetic inhibition.

[Supported by grants from NIH (2-S07-RR05386) and the Miss. Affiliate of The American Heart Association].

80.7 ELECTROLYTIC LESIONS OF THE REGION OF THE PARAVENTRICULAR NUCLEUS TRANSIENTLY REDUCE CHRONIC RENAL HYPERTENSION. T.C. Herzig, R.A. Buchholz, J.R. Haywood. Department of Pharmacology, The University of Texas Health Science Center, San Antonio, Texas 78284-7764.

Hypertension has been attenuated by lesions of the paraventricular nucleus (PVN) region in both the Dahl salt-sensitive and SHR models of hypertension. Anatomical tracing studies have shown pathways between the anteroventral third ventricle (AV3V) region and the paraventricular nuclei, suggesting the importance of these brain regions in sodium-induced rises in arterial pressure. Since one-kidney, figure-8 renal hypertension is a sodium-dependent model of increased arterial pressure, the goal of the present study was to determine the contribution of the PVN to one-kidney, figure-8 renal wrap hypertension.

One-kidney, figure-8 renal wrap or sham operation (unilateral nephrectomy) was performed in male Sprague-Dawley rats. Rats were instrumented 26 days later with femoral artery and vein catheters for daily measurement of mean arterial pressure (MAP) and heart rate (HR) and administration of drugs. On day 28 post wrap or sham a bilateral electrolytic lesion of the PVN was performed. On day 7 post lesion, the contribution of the sympathetic nervous system, renin-angiotensin system, and arginine vasopressin to increased MAP was assessed sequentially by blockade of each system.

MAP was significantly elevated in the wrapped group prior to lesion; ablation of the PVN significantly reduced MAP from 150.0 ± 9.4 mmHg to 110.0 ± 2.9 mmHg. In the sham group, the lesion reduced MAP from 117.7 ± 1.8 mmHg to 98.9 ± 4.4 mmHg. However, in both groups MAP returned to pre-lesion values by day 7 post lesion. HR increased significantly in both groups on day 1 post lesion, but subsequently decreased to control levels. Histological examination indicated two types of lesions: complete ablation of the PVN or encroachment upon the rostral portion of the PVN. However, MAP and HR responses were similar with both types of lesions. Effects of ganglionic blockade on MAP were greater in wrapped animals (-43.6 ± 5.2 mmHg) than in sham animals (-26.2 ± 3.1 mmHg). Likewise, the response to combined blockade of the sympathetic nervous system, renin-angiotensin system and vasopressin was greater in wrapped animals (-77.8 ± 6.3 mmHg) than in sham animals (-43.0 ± 3.1 mmHg).

These studies suggest that the PVN contributes to renal hypertension. However, with ablation of the PVN region it appears that neurohumoral mechanisms compensate to return MAP to pre-lesion levels. (This work was supported by HL36080 and HL32977).

80.8 THE EFFECTS OF ISOFLURANE, HALOTHANE, MIDAZOLAM, AND ETOMIDATE ON PRESSOR RESPONSES INDUCED BY CNS STIMULATION IN CHRONICALLY IMPLANTED CATS. W. T. Schmeling, D.C. Warltier and J.P. Kampine. Departments of Anesthesiology and Pharmacology. Medical College of Wisconsin. Milwaukee, WI 53226

The systemic hemodynamic actions of the volatile anesthetics isoflurane and halothane and the intravenous agents etomidate and midazolam have been well documented. However, few studies have investigated the actions of these agents on central cardiovascular control sites. The purpose of the present investigation was to examine the actions of these agents on central nervous system (CNS) pressor (P) areas in chronically implanted cats. Chronic implantation allowed comparison of anesthetic effects to the conscious state. Cats (2.0-3.5 kg) were chronically implanted with bipolar stimulating electrodes in the regions of the ventrolateral hypothalamus (Hyp) (A=10.0, L=2.5, D= -4.0) and mesencephalic reticular formation (RF) (A=2.0, L=2.0, D= -1.0). The right carotid artery and external jugular vein were catheterized for measurement of arterial pressure and drug administration, respectively. In selected animals, a Statham electromagnetic flow probe was positioned around the abdominal aorta for measurement of aortic blood flow. All animals were allowed to recover for 7-10 days. Control experiments consisted of stimulation (S) sequences at 1,2, and 4 times "threshold" current levels to elicit pressor responses. Isoflurane (1.5, 2.5, and 3.0%), halothane (0.5, 1.0, and 2.0%), etomidate (3 mg/kg), or midazolam (7.5 mg/kg) were then administered in separate experimental groups. After establishing a steady hemodynamic state with each agent, S sequences were repeated. Despite minimal alterations in blood pressure, heart rate, or aortic flow, both etomidate and midazolam produced significant ($P < 0.05$) attenuation of P responses to S in RF. The Hyp P responses appeared more resistant, decreasing but not significantly. Both halothane and isoflurane markedly attenuated the evoked P responses, nearly abolishing responses at the highest concentrations. The action of isoflurane was more pronounced than halothane, particularly at RF sites. Significant changes in associated flow patterns were also observed with these agents, reflecting alterations in induced vasomotor tone to S. The results support the concept that disruption of CNS cardiovascular centers may contribute to the alterations in hemodynamic stability produced by these anesthetic agents. (Supported by grants from NIH HL 36144, the Veterans Administration, and the American Society of Anesthesiology.)

80.9 THE EFFECT OF PREOPTIC HYPOTHALAMIC LESIONS ON CEREBRAL BLOOD FLOW AND REDISTRIBUTION OF CARDIAC OUTPUT FOLLOWING HEMORRHAGE. S. L. Bealer and D. W. Busija*, Dept. Physiology and Biophysics, University of Tennessee, Memphis, TN 38163.

The periventricular tissue surrounding the anteroventral portion of the third cerebral ventricle (the AV3V region) is critical for cardiovascular regulation. Electrolytic ablation of this brain area reduces blood volume and decreases survival following severe hemorrhage. The present experiments were designed to determine if ablation of the AV3V region alters cardiac output and/or distribution of regional blood flow before and after hemorrhage in the conscious animal.

Rats underwent electrolytic ablation of the AV3V region or control surgical procedures and were allowed 2-3 wks to recover from the acute effects of these surgeries. Cardiac output and regional blood flow were then measured using 15 um radiolabelled microspheres before, and 2 and 15 min following hemorrhage. The hemorrhage procedure consisted of withdrawing blood from a femoral artery catheter until mean arterial blood pressure was approximately 65 mm Hg.

Under resting conditions, cerebral blood flow was significantly greater (216 ± 30 mm Hg/ml/min/100 g) and cerebral vascular resistance was lower (0.6 ± 0.09 mm Hg/ml/min/100 g) in rats with AV3V lesions than in control-operated animals (132 ± 16 mm Hg/ml/min/100 g; 0.92 ± 0.1 mm Hg/ml/min/100 g, respectively), while mean arterial pressure, cardiac output, and regional blood flow to other organs was similar between experimental groups. Hemorrhage that reduced blood pressure to 65 mm Hg in both groups decreased cerebrovascular resistance in control-operated rats (0.52 ± 0.1 mm Hg/ml/min/100 g), but not in rats with AV3V lesions (0.48 ± 0.1 mm Hg/ml/min/100 g). Cardiac output and redistribution of regional blood flow to other organs were similar between rats with AV3V lesions and control-operated animals following hemorrhage. These data demonstrate that electrolytic ablation of the AV3V region results in a selective increase in cerebral blood flow and decreased cerebral vascular resistance, but does not alter the reflex changes in regional blood flow evoked by hemorrhage. (Supported by USPHS grants HL-25877 and HL-30260 and a grant from the American Heart Association, Tennessee Affiliate.)

80.10 DEPRESSOR RESPONSES ELICITED FROM LATERAL HYPOTHALAMIC AREA AFTER L-GLUTAMATE MICROINJECTIONS. S.E. Spencer, W.B. Sawyer, and A.D. Loewy, Depts. of Neurology and Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

The role of the lateral hypothalamic area (LHA) in cardiovascular control was studied in Sprague-Dawley rats which were anesthetized with sodium pentobarbital (35 mg/kg). Microinjections of L-glutamate were made at different rostral-caudal levels of the LHA. In each experiment, 15 nl of a 500 mM solution containing 1.25 μCi of ^{3}H-glutamate was injected. The animals were maintained on a respirator and changes in arterial blood pressure and heart rate were monitored. At the end of the experiment, the brains were processed for autoradiography and the injection sites reconstructed.

The tuberal region of the LHA (LHA_t) which lies lateral to the ventromedial nucleus of the hypothalamus (VMH) was stimulated. This produced a decrease in arterial blood pressure of 20.4 ± 1.3 mmHg and a bradycardia of 34.8 ± 3.9 bpm ($\bar{x}$±SEM; n=49) Preliminary analysis of these experiments suggest that the ventrolateral portion of the LHA_t is concerned with blood pressure regulation, while injections in the vicinity of the perifornical region and dorsomedial part of the LHA_t appear to regulate heart rate. Stimulation of the posterior region of the LHA (LHA_p) which lies caudal to the region of the VMH and lateral to the mammillary nuclei produced smaller depressor changes: arterial blood pressure = -15.9 ± 2.8 mm/Hg and heart rate = -23.8 ± 5.8 bpm (n=17). Stimulation of the subthalamic nucleus (n=6) caused minimal changes: blood pressure = -7.5 ± 2.1 mmHg and heart rate = -8.3 ± 2.8 bpm.

In order to determine whether the bradycardic changes were mediated via the sympathetic and/or vagal fibers, pharmacological blockade experiments with either the β-blocker - timolol (0.5 mg/kg; n=5), or the muscarinic blocker - atropine methyl nitrate (2.0 mg/kg; n=7) were performed. Stimulation of the LHA_t region after either blocker caused approximately a 25% attenuation of the bradycardic response, suggesting the response is mediated by both limbs of the autonomic nervous system. When both drugs were administered, the heart rate response was attenuated by ~90% (n=4). The blood pressure response was attenuated by ~25% with atropine but not with timolol.

Our results demonstrate that when the LHA_t and LHA_p are activated by L-glutamate, it produces a depressor response -- not pressor responses as has been suggested by earlier electrical stimulaton studies. The latter findings are likely to be due to fibers-of-passage, not the cells of the LHA.
(Supported by USPHS grant 25449; ADL is an Established Investigator of the American Heart Association.)

80.11 CONNECTIONS BETWEEN CARDIOVASCULAR AREAS OF THE HYPOTHALAMUS AND THE PERIAQUEDUCTAL GREY REGION OF THE RABBIT D.R. Liskowsky, R.W. Winters, P.L. Vera, P.M. McCabe, & N. Schneiderman. Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124

The midbrain periaqueductal grey region (PAG) has been implicated in a constellation of behavioral and cardiovascular responses labelled the 'defense' response. The cardiovascular component of the defense response is in part characterized by an increase in blood pressure and heart rate. In the present study we examined the areas of PAG which elicited a cardiovascular response when electrically stimulated. The connections between cardiovascular related areas of the hypothalamus and PAG were also studied.

The PAG was examined using train electrical stimulation (100-300 uA, 100 pulses/sec, 10 sec.) in urethane anesthetized rabbits. Stimulation of the dorsal-lateral portion of the rostral PAG, at the level of the posterior commissure, resulted in a pressor response accompanied by either a sustained tachycardia or a tachycardia which converted to a bradycardia as blood pressure increased. Stimulation of the ventro-lateral PAG, at a level just posterior to the above area, resulted in a pressor response accompanied by a reflexive bradycardia. Injection of either glutamate (1 uM) or DL-homocysteic acid (1uM) elicited the same cardiovascular responses from this area. No other sites were identified in the PAG that produced cardiovascular changes when electrically stimulated.

We also examined the extent to which stimulation of cardiovascular related areas of the hypothalamus could activate single cells in the PAG. Single unit activity was recorded using glass micropipettes filled with 0.05 M sodium acetate and having an outside tip diameter of $\leq$ 2um. Electrical stimulation or application of glutamate to the perifornical nucleus and the region between the columns of the fornix and the paraventricular nucleus, resulted in a pressor response and tachycardia. Electrical stimulation (200-300 uA, 100 pulses/sec, 3 sec) of this region was found to alter the discharge rate of many single units in the PAG. The response of the units to hypothalamic stimulation was variable. Some units increased their firing rate only during stimulation while others maintained a higher than baseline rate of firing after stimulus offset. Other units were inhibited by hypothalamic stimulation. All of the units that responded to hypothalamic stimulation were localized to the rostral half of the PAG.

It is possible that cardiovascular responses elicited from PAG, which may be involved in the defense response, are modulated by these descending hypothalamic projections.

This work was supported by NIH grants HL36588 and HL07426-08

80.12 **CENTRAL NERVOUS SYSTEM ACTIONS AND INTERACTIONS OF CORTICOTROPIN-RELEASING FACTOR AND OPIOIDS ON CARDIOVASCULAR FUNCTION.** L. A. Fisher, Dept. of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

The neurochemical mechanisms by which the central nervous system (CNS) mediates stress-induced alterations of cardiovascular function are not fully understood. The rationale to investigate the role of corticotropin-releasing factor (CRF) and opioid peptides in generating CNS-initiated cardiovascular responses to stress is based on experimental evidence regarding: 1) the distribution of CRF, opioid peptides and their receptors in brain areas known to be critical loci for autonomic, endocrine and cardiovascular control; 2) the release of CRF and opioid peptides within the brain in response to physical and psychological stressors; 3) the potent CNS effects of CRF and opioid peptides on autonomic, endocrine and cardiovascular parameters; and 4) the co-localization of CRF and opioid peptides within individual neurons thus suggesting their potential simultaneous release upon neuronal firing. To examine the CNS interactions of CRF and opioid peptides on cardiovascular function, studies were performed in which CRF and receptor subtype selective opioid peptides were administered into the CNS, alone and in combination, while monitoring cardiovascular parameters. All experiments utilized conscious, unrestrained rats previously instrumented with lateral cerebroventricular (icv) cannulae for peptide administration and femoral arterial catheters for direct measurement of pulsatile arterial pressure (AP) and heart rate (HR). The following receptor subtype selective opioid compounds were tested: mu or universal/B-endorphin (B-Endo) and [D-Ala^2, NMePhe^4, Gly-ol^5]-enkephalin (DAGO); delta/leucine-enkephalin (Leu-Enk) and [D-Pen^2, D-Pen^5]-enkephalin (DPDPE); kappa/dynorphin-A(1-17) (DYN) and the nonpeptide agonist, U50,488H. CRF (0.015-1.5 nmol, icv) elicited enduring elevations of AP and HR. B-Endo (0.025-2.5 nmol, icv) produced negligible and inconsistent effects on AP and HR whereas DAGO (0.05-0.5 nmol, icv) increased both AP and HR. Leu-Enk (30-300 nmol, icv) and DPDPE (1.5-15 nmol, icv) evoked transient elevations of AP and HR. The nonpeptide kappa agonist, U50,488H (21.5-215 nmol, icv) had no effects on AP and HR except at the highest dose where it increased AP and decreased HR. DYN (0.1-3.0 nmol, icv) caused delayed and transient elevations of AP and HR. A dose of DYN (0.1 nmol, icv) that did not alter AP or HR when given alone, delayed and suppressed CRF-induced elevations of AP and HR. These results suggest: 1) agonists presumed selective for a particular opioid receptor subtype, e.g., mu or kappa, do not necessarily produce identical CNS cardiovascular actions; and 2) co-localized peptides, i.e., CRF and DYN, may modulate/modify the CNS actions of each other.

80.13 CARDIOVASCULAR RESPONSES IN CAT FROM ELECTRICAL STIMULATION IN THE DIAGONAL BAND OF BROCA. C.W. Dempesy and D.E. Richardson. Department of Neurosurgery, Tulane University School of Medicine, New Orleans, Louisiana 70112.

In attempting to learn how rostral limbic areas which subserve emotional behavior states might elicit concomitant cardiovascular expression, the rostral limbic forebrain of anesthetized cat was surveyed with stimulating electrodes. Modest cardiovascular changes could be generated in several regions, but stimulation within the diagonal band of Broca (DBB) invariably produced a spectrum of striking cardiovascular phenomena, including large blood pressure and heart rate changes and temporary suppression of the baroreflex. In cats in which the baroreflex was permanently suppressed, stimulation confined to the rostromedial horizontal limb of the DBB yielded a basic pattern of depressor (bradycardia) responses at low currents, shifting to pressor (tachycardia) responses (70 - 100 mmHg, 30 - 50 bpm) at high currents. Although electrical stimulation of forebrain areas immediately rostral and dorsal to the DBB generated cardiovascular changes, the responses were usually small in comparison. However, pressor-active sites in the DBB only yielded depressor responses when stimulated with glutamate, while some pressor-active sites in this rostrodorsal region responsed with pressors when chemically stimulated. Lesion of the DBB reduced the responses from these rostrodorsal pressor sites. Electrical stimulation of strong pressor (tachycardia) sites in the DBB generated large releases of circulating epinephrine (EPI). Pressor responses, tachycardia, and circulating EPI releases induced by DBB stimulation were significantly reduced by lesions either of the medial forebrain bundle (MFB) area in the ventrolateral hypothalamus, or of the C1-area in the medulla. Finally, DBB stimulation-induced pressor responses were significantly reduced by mechanical or chemical sympathectomies. These results suggest that the DBB pressor responses arise from stimulation of axons of passage, which originate from neurons located rostrodorsal to the DBB and which continue projecting caudally (directly or multisynaptically) via the MFB to the medullary C1-area, where the effects are expressed peripherally by ordinary sympathetic mechanisms. This model extends the cardiovascular control network in the basal brain proposed by S.M. Hilton (Brain Research, 87:213-219, 1975) to the rostral septal region, and suggests a possible mechanism by which the emotional substrate of the brain may recruit the cardiovascular system to meet emergency needs.

80.14 EFFECT OF STIMULATION OF FASTIGIAL NUCLEUS ON CEREBRAL BLOOD FLOW IN CATS. J.L. Williams, D.D. Heistad, J. Siems, and W.T. Talman. University of Iowa and V.A. Medical Center, Iowa City, IA 52242.

Electrical stimulation in the region of the rostral fastigial nucleus (FN) produces a pronounced pressor response. In rats, stimulation of the rostral or caudal FN has been reported to produce almost 2-fold increases in cerebral blood flow (CBF), even in sites which do not elicit a pressor response. In this study, we have used a new approach to directly measure blood flow through a cortical artery during stimulation of the FN in cats.

In 9 chloralose-anesthetized, paralyzed cats, a pulsed-Doppler crystal was placed under a pial artery (312±35 μm diameter, mean±SE). Thus, blood velocity was measured continuously in an artery to the parietal cortex. Vessel diameter was measured simultaneously. Changes in CBF were calculated as the product of velocity and cross-sectional area of the vessel.

In the rostral FN, electrical stimulation of 7 sites from which pressor responses could be elicited also increased CBF. Stimulation of 3 other rostral sites with a similar threshold for pressor responses produced no change in CBF. Threshold pressor responses (10 mmHg) were usually achieved at stimulus amplitudes $\geq$ 25 μA (50 Hz, 0.5 ms). With mean arterial blood pressure maintained at control levels by rapid withdrawal of arterial blood, stimulation at 100-250 μA increased CBF 19±5%. High amplitude stimulation (400-500 μA) of 2 sites increased CBF 41%. The changes in CBF were manifested by increased velocity, but vessel diameter did not change. CBF increased gradually after the onset of stimulation and reached a peak level in approximately 1 minute. This maximal level of flow was sustained until termination of the stimulus. Arterial blood gases and pH were maintained at baseline levels throughout the stimulation of the FN. Stimulation of areas outside the rostral FN, including caudal regions, elicited no changes in arterial blood pressure or CBF.

Autoregulation was examined periodically by measuring flow during modest hypertension produced by aortic occlusion. After an initial rise, CBF declined rapidly and approached control levels within 20-60 seconds. Intravenous administration of Evans blue indicated that the blood-brain barrier was intact at the end of the experiments. Sites of stimulation were confirmed histologically.

The results indicate that electrical stimulation of the pressor region of the rostral FN in the cat can produce a modest increase in CBF. Stimulation of caudal regions of the FN elicits neither a pressor response nor any change in CBF.

80.15 CHEMICAL ACTIVATION OF PARABRACHIAL NEURONS ELICITS A PRESSOR RESPONSE IN THE CAT. J.S. Hade*, T.S. Donta*, S.W. Mifflin* and R.B. Felder (SPON: A.K. Affifi). Cardiovascular Center, Dept. of Internal Medicine, University of Iowa, Iowa City, Iowa 52242.

Electrical stimulation of parabrachial nucleus (PBN) results in a pressor response characterized by an increase in arterial pressure and tachycardia (in cat) or bradycardia (in rabbit). Although these responses are presumed to be mediated by PBN neurons, the cardiovascular effects of chemical stimulation of neurons in this region have not previously been determined.

In pentobarbital anesthetized, paralyzed and mechanically ventilated cats, we recorded mean arterial pressure (MAP), heart rate (HR), and phrenic nerve activity (PNA) during low intensity (25-50 μA), electrical stimulation (ES) or microinjection (MI) of l-glutamate (l-glu, pH=7.4, 100 mM), kainic acid (KA, pH=7.4, 2 mM), or saline (isosmotic, pH=7.4) at the same site. Microinjections were made through two pipettes glued to a tungsten monopolar stimulating electrode (100 Kohm resistance), so that the tips of the stimulating electrode and the ejecting pipettes converged on approximately the same point. The responses to MI of either 40-100 (Group I: n=7) or 300-400 (Group II: n=11) nanoliters l-glu were compared with the responses to ES at PBN locations in 16 animals. In Group I, MAP (control 95.3±2.6, $\bar{x}$±SE) increased 12.7% and 20.7% with ES at 25 μA and 50 μA, respectively. In response to MI of l-glu, MAP increased 12.2% from 95.7±3.2 mmHg. In Group II, MAP (control = 96.2±2.9) increased 17.2% with 25 μA and 28.8% with 50 μA ES and 13.8% (from 100.7±3.2 mmHg) in response to l-glu MI. We observed no changes in HR and no consistent changes in PNA in response to ES or either volume of MI. Saline injected in comparable volumes (n=5) had no effect.

In four experiments, KA (300-400 nanoliters) transiently blocked the pressor response to ES of PBN. Prior to KA, MAP was 88.3±1.3 mmHg, and increased 22.9%, 39.3% and 62.7% on ES at 25 μA, 50 μA and 100 μA, respectively. MI of KA resulted in a transient increase (4.5%) in MAP. Following KA, MAP increased only 1.0%, 8.2% and 19.9% with ES at 25 μA, 50 μA and 100 μA respectively. Following a mean recovery time of 31.5±6.4 minutes, these responses were 12.4%, 28.7% and 41.8%, indicating a return toward control.

Microinjection in PBN of l-glu, used in these studies as a general neuronal excitant, elicited an increase in MAP comparable to low threshold electrical stimulation. KA blocked the pressor responses to low threshold electrical stimulation in PBN. These data provide evidence that the pressor response to electrical stimulation of PBN is mediated by neuronal elements within PBN and not by fibers of passage originating from neurons elsewhere.

80.16 PRESSOR AND DEPRESSOR FUNCTIONS OF THE A5 CELL GROUP. R. G. Drye*, D. L. Whittington*, R. H. Baisden and M. L. Woodruff. Department of Anatomy, Laboratory of Neurobehavioral Science, Quillen-Dishner College of Medicine, East Tennessee .State University, Johnson City, TN 37614.

The A5 region, located in the lateral brainstem tegmentum at the ponto-medullary junction, is a discrete area of norepinephrine-containing neurons having apparent influence on blood pressure (BP). Electrical stimulation studies (Loewy et al., Brain Res., 178, 196-200, 1979; Woodruff et al., Brain Res., 379, 10-23, 1986) suggest that A5 produces a BP pressor effect. Conversely, studies using excitatory amino acids to stimulate A5 (e.g. Neil and Loewy, Brain Res. 241, 271-278, 1982) report a depressor effect on BP. The present study examined the differential response of BP to electrical or L-glutamate stimulation of A5 in anesthetized New Zealand rabbits exposed to various experimental conditions. Aspiration lesions of the nucleus tractus solitarius (NTS) resulted in substantial reduction of the depressor response to microinjections of the neuroexcitant L-glutamate into A5. Administration of the catecholaminergic neurotoxin, 6-hydroxydopamine, resulted in a preservation of the L-glutamate-induced depressor response, but eliminated the pressor effect of electrical stimulation. Electrical stimulation, when presented after L-glutamate injections, produced a markedly decreased magnitude, or even a reversal, of the expected pressor response. Local injection of a L-glutamate antagonist resulted in a blocked depressive response to L-glutamate but had no effect on the increased BP of electrical stimulation. We conclude that the A5 region contains two neuronal systems. One is L-glutamate sensitive, interacts with the NTS and exerts a depressor effect on BP. The second is less sensitive to L-glutamate, highly sensitive to electrical stimulation, exerts its effect via the intermediolateral column of the spinal cord and produces a BP pressor response. (Supported by a Grant-in-Aid to MLW from the American Heart Association with funds contributed in part by the AHA Tennessee Affiliate.)

80.17 NEUROPEPTIDE Y, CENTRAL NERVOUS SYSTEM EFFECTS OF CARDIOVASCULAR FUNCTION. N. Scott*, V. Webb* and M.R. Brown (SPON: C. Cheung). Autonomic Physiology Lab., UCSD Medical Center, San Diego, CA 92103.

Neuropeptide Y (NPY) has been reported to exert variable effects on mean arterial pressure (MAP) and heart rate (HR), depending on the site of administration of this peptide into the central nervous system (CNS). This study has assessed the mechanism by which NPY given into the lateral ventricle (icv) decreases HR. Experiments have been carried out in awake, male Sprague-Dawley rats equipped with chronic icv, jugular venous and femoral artery catheters. NPY given intravenously (iv) produced a dose-dependent increase of MAP and decrease of HR. NPY (20-200 pmoles) and peptide YY, but not $[Phe^{27,36}]$-NPY^{21-36}, given icv produced a prolonged slowing of HR without changing MAP. Iv passive immunization against NPY prevented the changes of HR and MAP following NPY given iv, but did not prevent the decrease of HR when NPY was given icv. These observations support the conclusion that NPY-induced slowing of HR is secondary to a CNS rather than a peripheral action of the peptide secondary to its leaking out of the brain.

To evaluate the efferent mechanism by which NPY produced slowing of HR, experiments were performed using atropine methyl nitrate. Atropine given iv either before or after icv administration of NPY caused HR to return to those of control animals; results consistent with NPY producing sympathetic withdrawal rather than parasympathetic stimulation. Consistent with this conclusion, NPY given icv did not change plasma concentrations of epinephrine or norepinephrine despite dramatic reductions of HR. Thus, there was no compensatory increase of sympathoadrenomedullary activity following NPY-induced bradycardia.

Effects of NPY on baroreflex function were studied by constructing a MAP-pulse interval response curve using nitroprusside and phenylephrine infusions. NPY given icv resulted in a MAP-pulse interval curve that was upwardly displaced and parallel to the control curve. The changes in the MAP-pulse interval response curve induced by NPY are most consistent with a modulation of baroreflex set-point, without a change in baroreflex gain. Whether the effects of NPY on HR observed in these studies represent the summated actions of NPY exerted at different brain sites has not been determined. However, the relevance of summated versus single site actions of NPY or other peptides depends on the patterns of release and/or biodistribution of endogenous peptide under physiologic conditions. In conclusion, NPY acts within the CNS to slow HR. This effect is associated with decreased sympathetic influence on HR and a resetting of the baroreflex.

80.18 AN ADRENAL-MEDIATED, NALOXONE-REVERSIBLE ANTAGONISM OF THE INCREASE IN HEART RATE FOLLOWING INTRATHECAL ADMINISTRATION OF SUBSTANCE P AT THE LOWER THORACIC SPINAL LEVEL IN THE RAT. K. Yashpal* and J.L. Henry (SPON: R.B. Malmo). Departments of Physiology and Psychiatry, McGill University, Montreal, Quebec, H3G 1Y6

Intrathecal administration of substance P (SP) at the ninth thoracic spinal level increases the adrenal output of catecholamines (Neurosci. 15:529-536,1985) and increases heart rate and arterial pressure (J. Autonom. Nerv. Syst. 18:93-107,1987). As both humoral and neural mechanisms may mediate these cardiovascular responses a study was done to determine the effects on heart rate and arterial pressure in adrenalectomized (ADX) vs intact rats. Male Sprague Dawley rats (approx. 350 g) were anaesthetized with urethane (2.5 g/kg i.p.) and implanted acutely with an intrathecal PE-10 catheter passed via the atlanto-occipital junction to the T9 level. PE-60 catheters were implanted in the carotid artery to measure arterial presure and heart rate and in the femoral vein for injection of drugs. ADX was bilateral using a surgical method; control rats were sham operated. After 30 min, baseline readings were taken over 5 min and SP was delivered intrathecally in a dose of 6.5nmoles in 10ul of artificial CSF; the catheter was flushed with 10ul of CSF. In control experiments CSF replaced the SP solution. Readings were then taken over the next 30 min. SP increased heart rate and arterial pressure in both groups of rats: ADX (n=15), +394±16.6 (SEM) bpm and +21±4.0 mmHg; intact (n=10), +379±20.4 bpm and +20±4.8 mmHg, respectively. CSF administration failed to induce any changes in either parameter. While the magnitude of these responses was the same in the two groups, the increase in heart rate was significantly more abrupt in the ADX rats ($p<0.05$). This suggested that the adrenals are not necessary for the expression of the cardiovascular responses and that they might be secreting a factor which slows the neurally mediated cardioacceleration induced by administration of SP. To examine this possibility, as opioids are co-released with catecholamines from the adrenals, naloxone (10 mg/kg) was given i.v. 5 min before administration of SP (n=7); the SP-induced cardiovascular changes were similar in magnitude to those seen earlier. The time course of the cardioacceleration resembled that in the adrenalecotmized rats, suggesting that the factor secreted by the adrenals was opioid in nature. In addition, 8 rats were pretreated with nalorphine methochloride (10 mg/kg, s.c.), an opiate antagonist which does not cross the blood-brain barrier; the cardioacceleratory response to SP resembled that in ADX rats, sugesting that the site of action of the opioid factor is peripheral. These results suggest that intrathecal administration of SP induces the release from the adrenals of a factor which is opioid in nature and which slows a neurally-mediated cardioacceleration by an action in the periphery. (Supported by Quebec Heart Foundation)

80.19 ADRENAL AND NON-ADRENAL SYMPATHETIC PREGANGLIONIC NEURONES IN THE CAT: PHYSIOLOGICAL PROPERTIES AND RESPONSES TO SEROTONIN.
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Sympathetic preganglionic neurones (SPNs) projecting to the adrenal medullae receive a unique set of chemically identifiable synaptic terminals distinct from neighbouring, non-adrenal SPNs (Neuroscience 7: 1155, 1982). Adrenal SPNs vs non-adrenal SPNs lend themselves well to electrophysiological studies because adrenal SPNs, but not non-adrenal SPNs, can be activated antidromically from their target organ. Thus, in the present study, the two types of SPN were compared for their physiological properties and their response to serotonin. Cats were anaesthetized (chloralose), paralyzed (pancuronium bromide) and ventilated artificially (end-tidal CO_2 3-6%; phrenic nerve discharge regular and stable). Spinal segments T8-T10 were exposed. Extracellular single unit spike activity was recorded from the central barrel (containing 2.7M NaCl) of multi-barrelled micropipettes; barrels for iontophoresis contained serotonin creatinine sulphate (50mM in 0.16M NaCl, pH 3.3 or 4.5), methysergide maleate (10mM in 0.16M NaCl, pH 4.0) and control solution (0.16M NaCl, pH 3.3 or 4.5). Positive sites of recording were identified by Pontamine Blue deposits; all were in the intermediolateral nucleus. A unit was classified as SPN if it responded antidromically to electrical stimulation of the greater splanchnic nerve or adrenal medulla, with non-adrenal SPNs responding only to stimulation of the greater splanchnic nerve and adrenal SPNs to adrenal stimulation. Of the 19 adrenal SPNs, 17 discharged spontaneously (mean rate of discharge ±S.D., 1.3±0.5 spikes/s); the pattern of activity was irregular and none discharged with a phasic pattern. Of the 83 non-adrenal SPNs 70 discharged spontaneously (mean rate 2.0±1.5); 10 of these units demonstrated a phasic pattern of activity clearly related to phrenic nerve discharge, lung inflation or oscillations in arterial pressure. Mean latencies of antidromic responses were 19.5±6.2ms (adrenal SPNs) and 15.9±4.8 (non-adrenal SPNs). Serotonin (10-100nA) increased the firing rate of 7/9 adrenal SPNs and 32/35 non-adrenal SPNs; depression was never observed. Excitation was similar for both types of SPN, being typically slow in onset (mean latency 58.6±46.6s) and prolonged in after-discharge (mean duration 131±75s). Responses were similar whether using serotonin at a pH of 3.3 or 4.5, suggesting that the absence of a depressant effect cannot be accounted for by pH, as observed with cortical neurones (Brain Res.40:552,1972). The excitatory response to serotonin was antagonized by methysergide (60-100nA) with 2/3 non-adrenal SPNs and with the one adrenal SPN tested. These data suggest that the two types of SPN share some physiological properties and show similar responses to serotonin. (Support: Canadian and Quebec Heart Foundations. Stairs Foundation of McGill Univ. & NATO)

80.20 TRANSNEURONAL AND AFFERENT TERMINAL LABELING OF SYMPATHETIC PREGANGLIONIC NEURONS WITH THE C-FRAGMENT OF TETANUS TOXIN.
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Sympathetic preganglionic neurons(SPN's) exhibit several somatic shapes and dendritic alignments. It is unknown if morphological diversity presages peripheral functional specificity. This issue is approachable anatomically, but requires the identification of a "marker" molecule that is transported retrogradely, transsynaptically and transneuronally. Recent evidence suggests that the C-fragment of tetanus toxin (CTTx) is such a "marker" and that it can be immunohistochemically recognized using a monoclonal antibody (Evinger C. and Erichsen, J., Brain Res.,**380**:383, 1986). Our preliminary light and electron microscopic data confirm that CTTx is transported retrogradely, transsynaptically and transneuronally following injections in the peripheral autonomic nervous system.

Experiments were performed in anesthetized pigeons. Injections of 1-2ul of 15% CTTx were made in paravertebral ganglion 14. Pigeons were sacrificed (pentobarbital, 1mg/kg) at 5-15 hrs; 1,3, 5 days; and 1-5 wks. CTTx was localized immunohistochemically (monoclonal antibody provided by Drs. Erichsen and Evinger). Light microscopically, the following observations have been made: (1) SPN's are transsynaptically, transneuronally labeled in T4 and T5 and retrogradely labeled in caudal C14, T1-3; (2) The aggregate transsynaptic, transneuronal transport rate is > 4mm/hr; (3) Retrograde CTTx labeling of SPN's is most apparent at 5hrs and gradually appears to diminish by 3 days; (4) Terminal labeling within the SPN neuropil (C14-T5) is evident at 5 hrs and persists for at least 4 wks before becoming immunohistochemically undetectable at 5wks postinjection.

Ultrastructural localization of CTTx (survival intervals of 5,10 and 15hrs) revealed that retrogradely labeled SPN's exhibited somatic, dendritic and axonal accumulations of CTTx. Reaction product was localized: (1) as granular appearing, diffusely distributed cytoplasmic inclusions; (2) within individual membrane bound vesicles; and (3) in multivesicular bodies. Afferent terminal labeling of synaptic input onto CTTx-labeled SPN somas as well as on large and small dendrites was present at 5hrs; the density of such terminal labeling increases with survival time (10 and 15hrs) and persists for at least 21 days. At all time points examined (5,10,15 hrs and 21 days) it has been repeatedly observed that unlabeled synaptic boutons are interposed between CTTx containing terminals. These data suggest transsynaptic specificity of CTTx transport may exist. (JBC is an Established Investigator of AHA; supported in part by HL24103).

TRANSPLANTATION II

81.1 ADHESIVE PROPERTIES OF NEURAL CELL AGGREGATES FORMED 'IN VITRO'.
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Aging of neural cell populations constitute a limiting factor in the probability of such cells to grow outside their natural environment. In addition to severe mechanical trauma inflicted by procedures during cell dissociation of adult brains, changes in cell adaptability and viability as they may relate to time have also been suggested. Somewhat similar conditions may ensue when dissociating monolayer cultures for transplantation purposes. The possibility of using floating cell aggregates cultured 'in vitro' previous to transplantation provides a relatively safe procedure to avoid additional trauma (Colombo and Molina, 1986). The work reported here deals with further testing such procedures. Transfer of floating aggregates from rotating to stationary conditions was utilized to assess their ability to attach and grow, after various times in rotating culture.

Mechanically dissociated septal, and mes-metencephalic fetal tissue (E15-E17) was plated in 35mm uncoated dishes at $1X10^6$ cells per dish, in several culture media. Dishes were placed in rotating conditions (approximately 80 rpm), in a 5% CO_2/air atmosphere at 37°C. Three-day tests were run to evaluate attachment of aggregates after three or seven days in rotating cultures. Dishes on stationary cultures were coated with polylysine or Matrigel. Percent of aggregates attached to the substrate was significantly reduced when media was not supplemented with 10% fetal calf and horse defined sera, or when cytosine arabinoside was added to the culture media during rotating conditions.

Culturing cells for seven days also resulted in a decrease of the number of attaching aggregates, although DMEM with sera was used. Septal cells, under present conditions, appeared to be more affected by time than mes-metencephalic cells in rotating culture.

While eight day old, first generation aggregates failed to attach, second generation aggregates formed after trypsination of the former resulted in aggregates that regained their attachment capacity, followed by neuritic growth. The association of preculturing and cytosine arabinoside treatment with reduced glial population, supports the contention that glial cells subserve a critical role for the attachment of these cell aggregates, under present culture conditions. The observed 'inhibition' with time followed by 'disinhibition' after trypsin treatment, suggests a change in molecules bound to cell membranes. In conclusion, present results confirm the potential viability of early cell cultures in aggregate form, suggest that under standard culture conditions changes take place with time that reduce their ability to adhere 'in vitro' to certain substrates, and show that trypsin can restore such capacity.
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81.2 **SYNAPTIC CONNECTIVITY OF DOPAMINE AFFERENTS IN THE DORSAL STRIATUM OF WEAVER MUTANT MICE**
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Striatal dopamine (DA) deficiency in weaver mutant mice is associated with a substantial loss of mesencephalic DA neurons. The concentration of striatal DA in weaver mutants reaches its maximal value on postnatal day 20 and eventually declines to 25% of the control value. Accordingly, the number of DA neurons in the substantia nigra represents 60% of the normal number on day 20 and becomes 30% of the normal value by day 90. Aim of the present study was to examine the form of synaptic connectivity established by DA afferents in the weaver striatum at the time when striatal DA concentration is at its peak level. To this end, 20-day-old weaver homozygotes, along with age-matched weaver heterozygotes and wild-type mice were studied by electron microscopy after immunocytochemical labeling for tyrosine hydroxylase. Quantitative analyses of the relations of tyrosine hydroxylase immunoreactive nerve terminals were carried out in the dorsolateral striatum, which receives DA innervation from the substantia nigra proper. In wild-type mice, 73% of the contacts were non-junctional, 1% was junctional asymmetrical, and 26% were junctional symmetrical. The majority of contacts (92%) were with dendrites and spines. In weaver heterozygotes, 71% of the contacts were non-junctional, 4% were junctional asymmetrical, and 25% were junctional symmetrical. Again, 91% of the contacts were with dendrites and spines. In homozygous weaver mutants, 83% of the contacts were non-junctional, 1% was junctional asymmetrical, and only 16% were junctional symmetrical. The majority of contacts (88%) were with dendrites and spines. The proportion of axosomatic contacts in the striatum of weaver homozygotes was double of that found in normal animals.

The reduced incidence of junctional synapses in weaver homozygotes is in all likelihood suggestive of inadequate synaptogenesis. Further, the increased incidence of axosomatic contacts is indicative of synaptic immaturity, as such contacts are commonly seen in early developmental stages. Our results support the developmental nature of the nigrostriatal deficit in weaver mutants, since the synaptic investment of striatal neuronal elements by DA afferents appears to be immature at the time when nigrostriatal synaptogenesis is normally complete.

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81.3 **SYNAPTOLOGY OF DOPAMINE AFFERENTS IN THE DORSAL STRIATUM OF WEAVER MUTANT MICE FOLLOWING GRAFTING OF VENTRAL MESENCEPHALIC TRANSPLANTS**
L.C. Triarhou, W.C. Low and B. Ghetti. Dept. of Pathology, Div. of Neuropathology, and Dept. of Physiology and Biophysics, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

We have previously reported that ventral mesencephalic anlagen survive following grafting to the striatum of weaver mutant mice and reinnervate the dopamine (DA) depleted basal ganglia of the recipients (Proc. Natl. Acad. Sci. USA 83: 8789-8793, 1986). Aim of the present study was to examine the form of connectivity established by graft deriving DA afferents in the host striatum. Grafts were obtained from normal embryos at E15 and implanted into a surgical cavity overlying the dorsal striatum of adult weaver recipients. Tissues were processed for electron microscopic immunocytochemistry using a primary antiserum against tyrosine hydroxylase. At the time of examination, recipient weaver mutants were 8.5 months old and the grafts had survived for 4.5 months. Grafts were found to contain an estimated 100-1,000 tyrosine hydroxylase immunoreactive neurons. Tyrosine hydroxylase immunoreactive fibers, displaying the characteristic varicosities, innervated the dorsal striatum to a depth of 1,000 μm. In the non-grafted striatum, 91% of the contacts of tyrosine hydroxylase immunoreactive nerve terminals were non-junctional, 1% was junctional asymmetrical, and only 8% were junctional symmetrical. These proportions contrasted with the corresponding percentages found in normal animals, which are respectively 73%, 1%, and 26%. In the grafted striatum, 70% of the contacts were non-junctional, 2% were junctional asymmetrical, and 28% were junctional symmetrical. These percentages approximated the corresponding values in normal animals. The majority of contacts in the reinnervated striatum were with dendrites and spines. However, the proportion of axosomatic synapses in the reinnervated striatum was three times higher than that found in the striatum of normal animals. We conclude that: (1) In spite of a naturally occurring degenerative process, the basal ganglia of weaver mutant mice are receptive to synaptic investment by DA afferents originating in normal donor tissue. (2) In repopulating the previously denervated weaver striatum, graft deriving DA afferents display a connectional selectivity, i.e., they establish synaptic relations preferentially with those cellular domains that are normally innervated by DA nerve terminals. In this context, it is possible that DA fibers originating in the grafts invest postsynaptic sites that had either been vacated from their presynaptic DA input or had never received one. (3) A certain degree of anatomical immaturity may be suggested by the increased incidence of axosomatic contacts, which is a feature of the developmental morphology of the neostriatal anlage.

(Supported by USPHS grant RO1-NS14426).

81.4 **FUNCTIONAL INNERVATION OF THE NEOSTRIATUM IN WEAVER MUTANT MICE BY GRAFTED MESENCEPHALIC DOPAMINE NEURONS**
W.C. Low, L.C. Triarhou, Y. Kaseda, J. Norton, and B. Ghetti, Depts. of Physiology and Biophysics, Pathology (Neuropathology), Psychiatry, and Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46223.

Transplants of dopamine neurons have been extensively studied using various experimental models of parkinsonism. These animal models have used neurotoxins such as 6-hydroxydopamine (6-OHDA) and N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to destroy dopamine (DA) nerve fibers and neurons in order to replicate the degeneration of the nigrostriatal DA pathway seen in patients with Parkinson's disease.

The inherited loss of dopamine neurons in the substantia nigra pars compacta has been documented as part of the phenotypic expression in weaver mutant mice. A previous study has reported that DA-containing ventral mesencephalic grafts were capable of surviving and innervating the striatum of host weaver mutants (Proc. Natl. Acad. Sci., 83:8789-8793, 1986). In the present study, we provide evidence for the functional reinnervation of the striatum of weaver mutants by grafts of fetal ventral mesencephalic tissue.

Adult weaver mutants received ventral mesencephalic tissue from normal fetuses, placed on the surface of the dorsal striatum on the right side of the brain. Animals were tested for methamphetamine-induced circling behavior 113 - 141 days after transplantation. They were given methamphetamine (2.5 mg/kg body weight, i.p.) and placed in a stainless steel cylinder (17 cm in diameter and 18 cm high). Turning behavior was monitored by three observers during 1 minute intervals at 4-5, 9-10, 14-15, 19-20, and 24-25 minutes after the injection of methamphetamine. For each animal, the total number of rotations was determined for both left and right turns in increments of half body turns. Mean values for rotations to the left and right were calculated for each animal.

As a group, unoperated weaver mutants circled to the left 10.0 ± 1.6 (mean ± SEM) times, and to the right 8.7 ± 1.6 times during the 5 minutes of observation. In comparison, mutants with lesions and without grafts circled slightly more frequently to the left and slightly less frequently to the right (13.4 ± 3.3 and 5.0 ± 1.2 respectively). Weavers mutants with non-surviving nigral grafts circled 12.6 ± 2.6 times to the left and 8.71 ± 2.58 times to the right. No significant differences in rotational behavior were determined among these three groups. Weaver mutants with surviving tyrosine hydroxylase-immunoreactive neurons, on the other hand, displayed a marked increase in the number of rotations to the left (35.8 ± 8.5) in comparison to the three other groups along with a slight decrease in the number of rotations to the right (3.6 ± 1.9). The rotations to the left in animals with surviving implants represented a 358% increase above that of unoperated weaver mutants, a 266% increase above that of weavers mutants with lesions, and a 283% increase above that of weavers mutants with non-surviving grafts.

These results demonstrate that grafted dopamine-containing neurons establish a functional innervation of the weaver striatum and suggest that grafting of neural tissue is a viable approach in restoring motor function in genetic degenerative disorders of the nigrostriatal system.

81.5 FETAL CORTEX TRANSPLANTS DO NOT ENHANCE RECOVERY OF LOCOMOTION FOLLOWING SENSORIMOTOR CORTEX LESIONS IN RATS.
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Two studies were carried out to evaluate whether fetal brain tissue transplants could enhance recovery of locomotion following sensorimotor (S-M) cortex damage in rats. In the first study, 23 mature male Charles River Rats were pre- and postoperatively trained and tested on a narrow elevated runway. Seven rats were sham-operated (SHAM), and 16 sustained bilateral sensorimotor cortex lesions. Seven days later, one group of 8 animals received fetal transplants from sensorimotor cortex of 19 day old fetuses (S-M 19) while the other 8 lesioned (LES) plus the SHAM rats sustained a sham operation. Postoperatively, both the LES and S-M 19 rats were impaired on two levels when compared to SHAMS, but LES and S-M 19 did not differ from each other. The two levels of analysis were: behavioral (Mean Run Time, No. of Errors, Days to Pre- operative Criterion) and movement (Quantitative Measures of Movement Patterns of the hindlimb). Histiology revealed that most of the transplants did not survive.

Since sensorimotor cortex differentiates early, we hypothesized that S-M transplants taken earlier, or frontal (F) cortex transplants, would survive better. Therefore, we trained and lesioned three more groups of rats, and seven days later performed transplants from S-M cortex (fetal age 15 days, 7 rats; fetal age 17 days, 8 rats) or frontal cortex (fetal age 19 days, 6 rats). All three groups were clearly impaired postoperatively, but did not differ from each other on either the behavioral or the movement level of analysis.

When comparing across the two experiments, there is a suggestion that fetal tissue transplants may in fact have been detrimental. First, 70-80% of rats in all transplanted groups have longer Mean Run Times than lesioned animals. In addition, a more aberrant movement pattern consisting of a very low initial flexion phase of the swing cycle of the hindlimb was observed in at least one transplant group after the rats had achieved preoperative performance levels. Preliminary evidence suggests that some of the transplants in the three groups of the second experiment did not survive. Further histological analysis is being carried out. Apparent differences may be more evident after adjusting for presence/absence of transplants.

Thus, fetal brain tissue transplants from different sites (S-M or F cortex) or of different fetal ages (15,17, or 19 days) failed to produce a demonstrable enhancement in recovery. In fact, the transplants may have been disruptive to the normal recovery process.

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81.6 ABNORMAL POPULATIONS OF MUSCARINIC CHOLINERGIC RECEPTORS IN FETAL STRIATAL TRANSPLANTS CORRELATE WITH TRANSPLANT-INDUCED LOCOMOTOR RECOVERY.
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Past work in various laboratories has demonstrated that fetal striatal transplants, when placed into the kainic acid or ibotenic acid lesioned striatum, partially remediate the locomotor and "cognitive" deficits caused by the lesion, but do not remediate locomotor deficits under conditions of DA or muscarinic cholinergic drug stimulation. The current experiment examined to what extent muscarinic cholinergic receptor systems develop in the transplant, and the relationship between these transplants and transplant-induced locomotor recovery.

Thirty six adult female rats were divided into four groups, including control (sham lesion/sham tran), lesion only (kainic acid lesion/sham tran), lesion and transplant (ka lesion and E18 fetal striatum (str)), and tran only (sham lesion/str tran). Kainiate lesions (0.8 ug delivered in 0.4 ul PBS, ph=7.4) were made bilaterally in the anterior-medial str (coordinates A1.5, L2.2., H 4.5). One week later, 2.0 ul of E18 fetal str was mechanically aspirated into a capillary tube and implanted back into the lesioned adult str.

Six months following surgery, animals were run in an activity monitor that assessed a wide range of locomotor activities. Each animal received, on an every other week basis, one of 6 drugs, including saline (1ml/kg), amphetamine (1 mg/kg), apomorphine (0.2 mg/kg), haldol (1 mg/kg), scopolamine (0.8 mg/kg) and pilocarpine (1 mg/kg). Following completion of the locomotor assessments, muscarinic cholinergic receptor binding was determined. Twenty five um slices were incubated either directly in 1nM tritiated N-methyl scopolamine to give a measure of total binding, or scopolamine plus 100 uM carbechol or 1uM atropine, respectively. Binding values for M1 and M2 receptors were calculated via densitometry.

The kainic acid lesions caused a pronounced hyperactivity under the saline, amphetamine, apomorphine, and scopolamine drug conditions. The fetal strital transplants reversed the hyperactivity in the saline condition, but had little effect on locomotor behavior under the various drug conditions. M1 receptor density was found to be significantly higher in the transplanted tissue compared to control str, while M2 receptors were significantly reduced in density in the transplants. M1 receptor density was not correlated with locomotor behavior, however M2 receptors significantly correlated with locomotor activity in each of the drug groups except for the saline condition.

81.7 DEVELOPMENT AND FUNCTION OF FETAL HUMAN BRAIN TISSUE IN IMMUNOCOMPROMISED RODENT CARRIERS: THE USE OF ATHYMIC NUDE RATS. A. Seiger, L. Olson[1]*, I. Stromberg[1]*, M. Bygdeman[2]*, P. Bickford-Wimer[3], A.-C. Granholm[3]*, J. Stevens[3]*, B. Hoffer[3]. Dept. of Neurological Surgery, Univ. of Miami Sch. of Med., Miami, FL; [1]Dept. of Histology, Karolinska Institute, Stockholm, Sweden; [2]Dept. of OB-GYN, Karolinska Hospital, Stockholm, Sweden; [3]Dept. of Pharmacology, Univ. Colorado Health Sciences Ctr., Denver, CO.

Human fetal tissue fragments were collected following elective abortions in the seventh to eleventh week of gestation using procedures which were approved by the Ethical Committee of the Karolinska Hospital and conformed to guidelines of the USPHS. Under a stereomicroscope, it was possible to identify most areas of the developing human brain and prepare tissues suitable for xenografts to the anterior chamber of the eye or the brain of immunocompromised rodent hosts. We have previously shown structural and functional maturation of cortical and subcortical areas in the anterior chamber of the eye and of dopamine neurons grafted to the dopamine-denervated striatum in rats receiving daily cyclosporin injections. Recently, similar transplantations have been carried out in athymic nude rats. Intraocularly, human brain tissue survives and develops significantly better in nude rats than in Sprague-Dawley rats treated with cyclosporin (10 mg/kg/day). Cortex cerebri, cortex cerebelli, the hippocampal formation and the spinal cord develop characteristic structural and functional properties in oculo as evidenced by immunohistochemistry using antibodies against GFA, neurofilament, laminin, tyrosine hydroxylase, NPY, other neuropeptides, and by extracellular recording techniques. Data from cyclosporin-treated as well as nude rats suggest that development occurs according to a human rather than a rodent timetable. Electrophysiological evidence suggests development of an intrinsic circuitry in cerebellar grafts and of sensitivity to noradrenaline. In cortex cerebri grafts, penicillin superfusion increased discharge rates of pyramidal neurons. We conclude that xenografting from tissue fragments of human fetuses to nude athymic rats provides a unqiue model in which to perform invasive studies of defined areas of the developing human nervous system.

81.8 CROSS-SPECIES SEPTO-HIPPOCAMPAL TRANSPLANTS OF THY-1.2 IMMUNOREACTIVE CELLS. R.J. McKeon, J. Wells, B.P. Vietje,* and D.G. Wells.* Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

A variety of experiments have suggested that cross-species transplants of embryonic neurons survive, differentiate, and may innervate an adult host. One limitation of these studies has been the difficulty in differentiating between host and donor cells in long term transplants. In the present experiments, embryonic cells (E 15-17) from the septal-basal forebrain region of Thy-1.2 positive C57Bl/6 mice were transplanted to the dentate gyrus of Thy-1.2 negative adult Sprague-Dawley rat hosts. In situ, the Thy-1.2 antigen is not fully expressed until 20 days postnatally. Consequently, host animals were sacrificed between 3 and 8 weeks following transplantation in order to allow the Thy-1.2 antigen to reach adult levels. Hippocampal sections were processed immunocytochemically for the presence of Thy-1.2 reactive cells. Staining was seen as early as 4 weeks, corresponding to the delayed expression of Thy-1.2 in situ. The greatest intensity of staining was noted at 8 weeks. The transplant site consisted of a large mass of reactive tissue in the dorsal leaf of the dentate gyrus. Reactive fibers were seen extending from the transplant into the host neuropil. In some animals, these fibers appeared to begin organizing in a laminar fashion. However, this organization differs from the pattern of AChE histochemical staining, leaving the relative contributions of host and donor cells in the cholinergic relamination of the hippocampus in question. Frequently, Thy-1.2 reactive cells were found away from the body of the transplant, primarily in the hilus and in the molecular layer of the dentate gyrus. These cells were large, in a variety of shapes, with long processes which ramified extensively within the molecular layer. Experiments are in progress to determine the feasibility of using Thy-1.2 immunoreacitvity to identify donor cells at later time points, measure the survivability of transplanted cells, and study the potential for xenogenic transplants to form functional synapses with the host.
Supported by NS 23266.

TRANSPLANTATION II

81.9 FLUORESCENT MICROSPHERES IMPLANTED IN THE HIPPOCAMPUS SHOW "MIGRATION." B.P. Vietje,* J. Wells, D.G. Wells,* and M.E. Dunn* (SPON: M. Ariano). Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

In our previous studies of xenogenic transplants, injected cells appeared to have migrated along the cleavage planes of the hippocampus. In order to differentiate between active migration and passive displacement, we injected fluorescent polystyrene microspheres (6.49 ± 0.4 um) into the hippocampal formation. The fluorescent microspheres were found at substantial distances from the injection site in a pattern which might be interpreted as migration. However, because the beads can reasonably be assumed to have no capacity for active migration, the observed distribution could be due to passive displacement. The fluorescent beads outside of the injection site were distributed characteristically in three ways. 1) The beads were found along the cleavage planes of the hippocampal formation. The cleavage planes are found above the stratum oriens, along the obliterated hippocampal fissure and on the hilar side of the dentate granule cell layer. The beads spread along these planes singly or in small groups with no apparent connection to the main injection site. This appeared as "migration." From the cleavage planes, microspheres frequently gained access to the ventricles. 2) Beads also were seen at considerable distances from the main injection site; e.g., the diencephalon and cortex. Most of these beads were associated with blood vessels, and we assume the apparent "migration" occurred by paravascular circulation. 3) A few beads were found neither in the cleavage planes nor associated with blood vessels. The beads that were found in the hilus were frequently in this category. To control for artifactual displacement of beads, pairs of brains were sectioned in opposite directions and we did not consider in our analysis any beads that appeared on either surface of the sections. We conclude that transplanted neurons, which appear outside of the injection site, may not necessarily have arrived there by active migration but by other means as demonstrated by fluorescent microspheres.
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81.10 REPAIR OF THE BLOOD-BRAIN BARRIER DURING THE FIRST WEEK AFTER SEPTO-HIPPOCAMPAL TRANSPLANTS. J. Wells, B.P. Vietje,* D.G. Wells* and M. Fiala.* Department of Anatomy & Neurobiology, University of Vermont, Burlington, VT 05405.

The act of transplanting neurons into a host brain breaks the blood-brain barrier (BBB) allowing the host to begin its immunological response to the injected antigens. Since it takes a few days to build up a pool of activated killer lymphocytes, the rapid repair of the BBB would seem to be critical for the survival of the transplanted cells--particularly for xenogenic transplants. In the present experiment, we determine the characteristics of the repair of the BBB during the first week after transplantation. Embryonic mouse or rat cells from the septal-basal forebrain region were transplanted into the dentate gyrus of rat hosts. Using the procedure of Rosenstein and Brightman (Science 221:879, 1983), HRP was injected into the femoral vein before the animal was perfused. Brain sections were then processed to demonstrate TMB reaction product. Within a few hours of the transplantation, the HRP was found in the host to be uniformly distributed around the perimeter of the injection site and extended out into the surrounding tissue in a gradually decreasing concentration. Similar reactions were seen at the surface of the cortex where the injection needle first penetrated and along the needle tract. One day after transplantation, there was a partial repair of the BBB at the injection site and along the needle tract, but not at the cortical surface. The distribution of the HRP became discontinuous around the perimeter of the injection site in the hippocampal formation. The thinner, more peripheral, parts of the injection site and the needle tract appeared to be repaired first. In the bulbous portion of the injection site, HRP continued to infiltrate the host hippocampus but was usually less dense and less extensive than at the earlier time points. There was little change in the distribution of HRP during the remainder of the first week after transplantation. The changes in the BBB were the same in both xenogenic and homogenic transplants. In summary, the BBB was partially repaired within the first day. The repair of the BBB was never complete around the transplant site during the first week, but the areas that were repaired--the thinner perimeters of the injection sites-- were the areas where the survival of labelled xenogenic neurons were also observed.
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81.11 INVOLVEMENT OF MHC ANTIGENS IN NEURAL ALLOGRAFT REJECTION. K. Rao,* H. W. Kunz,*° T. J. Gill,*° and R. D. Lund (SPON: J. Hermanson), Dept. of Neurobiology, Anatomy and Cell Science, and the °Dept. of Pathology, Uni. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

We have shown previously that xenografts of embryonic (E13-14) CD-1 mouse retinae that had integrated and survived for prolonged periods in a host rat brain, are rejected when a CD-1 mouse skin graft was placed on the flank of the recipient animal.

We have extended these studies using highly inbred congeneic strains of rats. Embryonic (E14-15) dark agouti (DA) rat retinae were transplanted into the brains of neonatal brown Norway (BN) rats. The host immune system was subsequently challenged with skin grafts of animals which differed from the recipients at (i) the MHC loci only, (ii) the non-MHC loci only, and (iii) both the MHC and the non-MHC loci. Serum samples drawn before and after skin grafting were analysed for antibodies directed against cells expressing MHC or non-MHC antigens alone, or in combination. This was done in order to determine the significance of these antigens in the host immune response to the neural graft, as well as the subsequent skin graft. Appropriate controls were incorporated into the experimental design.

Animals were sacrificed within two weeks following skin grafting, and frozen sections were examined after using the following stains: cresyl violet for cell bodies, a neurofibrillar stain for fiber projections from the transplanted retina, and the Fink Heimer stain to show degenerating fibers.

Serum antibody data indicated that in 7 of 17 cases studied, the host immune system had been sensitized by the neural graft. Rejection of the neural grafts was served after skin grafting when the skin differed in either MHC or non-MHC antigens with respect to the host.

These results emphasize an instability of neural allografts even when placed in the brains of neonatal rats prior to the development of full immunocompetence.

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81.12 COURSE OF TRANSPLANT REJECTION WITHIN THE CNS. M. B. Houston, H. W. Kunz,*° T. J. Gill,*° and R. D. Lund. Dept. of Neurobiology, Anatomy and Cell Science, and °Dept. of Pathology, Uni. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Recently it has been shown both in vitro and in the CNS, in vivo, that neurons and astrocytes are capable of expressing the major histocompatibility complex (MHC) antigens which, in peripheral tissues, are required for recognition by the immune system (L. Lampson, TINS, 10:211, 1987). Our experiment was designed to elucidate the sequence of events leading to immuno-rejection of a neural graft placed into the brain.

Retinae or cerebral cortices were removed from CD-1 mice at embryonic day 13 and transplanted into either the midbrain or cerebral cortex of neonatal Sprague-Dawley rats. At 30 days of age, one group of rats received a graft of CD-1 skin approximately 1 cm^2. Animals were perfused 1 to 8 days following skin grafting, and adjacent 25μm frozen sections reacted immunocytochemically for murine MHC, rat-specific Common Lymphocyte Antigen (T and B cells), GFAP (astrocytes), and a mouse-specific neuronal cell-surface antigen designated M6 (viable transplanted neurons). The same protocol was followed for control animals, which received a neural transplant but no skin graft.

MHC labeling in control animals was restricted to areas along the vasculature within the transplant but no lymphocytes were observed. Numerous astrocytes were present within the transplant. Two days after skin grafting, MHC labeling was evident in parenchyma of the graft and surrounding host brain, but lymphocytes were still not observed. GFAP positive astrocytes became prominent in the graft, areas containing transplant innervation, and surrounding host tissue. At 8 days post-skin grafting, there was heavy lymphocytic infiltration into the transplant, as well as into surrounding host tissues. Vascular cuffing was prominent, and the transplant shows signs of degeneration.

This study provides insite into how immunological privilege becomes compromised in the brain and indicates that a complex interaction involving MHC antigens, the immune system (lymphocytes) and CNS glia (fibrillary astrocytes) occurs during transplant rejection. Supported by NIH grants EY05283, CA18659, and a grant from the Winters Foundation.

81.13 EVIDENCE FOR THE SURVIVAL OF RETINAL GANGLION CELLS IN NEONATAL RETINAL GRAFTS BY THY-1.1 IMMUNOCYTOCHEMISTRY. J.R.Blair and J.E.Turner. Dept. of Anatomy, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27103

Thy-1 is a 17,500 Kd cell surface glycoprotein found on the surface of a number of different cell types in mammalian tissues. In the retina only the retinal ganglion cells express this particular cell surface protein. The rat exhibits the Thy-1.1 form of the protein in contrast to the Thy-1.2 form produced by certain strains of mice. Utilizing the OX-7 monoclonal antibody to Thy-1.1, we have examined neonatal retinal grafts made to the lesioned retinas of adult hosts using a previously described paradigm (Turner, J.E. and J.R. Blair, Dev. Brain Res., 1986), in order to establish the presence of surviving ganglion cells in the grafts. The OX-7 antibody was employed in conjunction with the avidin-biotin technique for antigen identification using HRP/DAB as the enzymatic marker for localization in tissue sections. Initial work with adult retinal control tissues showed the OX-7 antibody to be very specific in localizing the Thy-1.1 antigen to the optic fiber layer (OFL), ganglion cell layer (GCL) and inner plexiform layer (IPL) of the retina. No labeling was seen when either the 1° or 2° antibody was omitted. Nor was there any labeling when a monoclonal antibody for Thy-1.2 was substituted for OX-7. Neonatal grafts were processed for Thy-1.1 immunocytochemistry 1 and 12 weeks after grafting. In both instances specific positive labeling was observed in the graft tissues. Utilizing the host retina as a positive internal control, the labeling was shown to be localized to the same regions of the graft tissue as in the host. While not all cells in the ganglion cell layer were positively labeled, many of the larger cells were, corresponding to those with the characteristics of GCs seen in counterstained sections. The staining in 1 week grafts was more diffuse than that of the older grafts suggesting that the ganglion cells may still have been undergoing maturation or remodeling connections with other cells in the graft. The importance of these findings lies in the survival of the retinal ganglion cell as the only output cell of the retinal graft. Thus the presence of ganglion cells is critical if retinal grafts are to play any role in the repair of damage due to disease or trauma with concomitant with regain of function.

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81.14 DONOR AGE INFLUENCES THE SUCCESS OF RETINAL TRANSPLANTS TO ADULT RAT RETINA. R. Aramant, M. Seiler and J. E. Turner. Dept. of Anatomy, Bowman Gray School of Med., Wake Forest Univ., Winston-Salem, NC 27103.

We have recently developed a technique for grafting embryonic rat retina into an adult rat retinal lesion site (Turner, J.E. and J.R. Blair, Dev. Brain Res., 26: 91-104, 1986). A penetrating lesion through retina on the dorsal surface of the eye creates an area devoid of retina into which the graft is placed. Retinal donor tissue is inserted by a 26 gauge needle attached to a 10 μl syringe through the lateral edge of the sutured lesion into the vitreous side. We report in this study that the rat retina can be successfully grafted within a long time period which extends into the first two weeks of postnatal life. At 4-7 weeks after transplantation E15 and PN1-2 (postnatal) grafts were organized in layered folded sheets and rosettes. Donor tissue from PN1-2 rats demonstrate no significant differences in their ability to form successful transplants. However, grafting success begins to diminish gradually starting between PN2-4 and reaches a low point in organization and survival by PN14. PN21 grafts illicit an immunological rejection-like response. Although early postnatal retinal tissue can be successfully grafted, E15 embryonic retinas make better transplants for their ability to form consistent laminae and to integrate with host tissue in a fresh lesion site when observed 7 weeks after transplantation. E15 grafts exhibited an increase in the number of laminae (i.e., 6-7 of the 9 retinal layers, excluding the retinal pigment epithelium) compared to only 3-5 for PN1 grafts. Light microscopic observations indicated in E15 grafts the presence of ganglion cell (GCL), inner plexiform (IPL), inner nuclear (INL), outer plexiform (OPL), outer nuclear (ONL), and outer limiting membrane (OLM) layers. Occasionally evidence of developing inner photoreceptor cell segments was found in E15 graft rosettes. In contrast, PN1 grafts possessed only a GCL, IPL, INL and ONL. Characteristically absent from both E15 and PN1 grafts was a continuous optic fiber layer and inner limiting membrane. In this study it was also confirmed by the use of a Fast Blue labeling technique that retinal tissue which was found in the lesion site indeed belonged to the graft and not to the host.

This research was supported by a grant, EY04377, from the National Eye Institute awarded to JET.

81.15 DEVELOPMENT OF GLIAL MARKERS IN HOST AND GRAFT RAT RETINA AFTER TRANSPLANTATION. M. Seiler and J. E. Turner, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

E15 retinae were transplanted into fresh retinal lesion sites of adult hosts (see Turner and Blair, Dev. Brain Res., 26:91-104, 1986). Animals were sacrificed 3-5 hrs, 1-15 days, 5 and 7 wks after surgery. After vascular perfusion with saline and fixation with 4% Paraformaldehyde, the eyes were processed for immunocytochemistry on frozen sections with a monoclonal mouse GFAP antibody (Debus et al., EMBO J., 1: 41-45, 1982) and with a polyclonal rabbit antiserum against S-100 protein (ICN, former Miles Inc.). In normal retina, only Astrocytes (AS) in the optic fiber layer and few Mueller cell (MC) fibers in the inner plexiform layer stained for GFAP. 3-5 hrs after transplantation, GFAP-filled reactive MC appeared in the peripheral part of the lesioned dorsal retina where ganglion cells (GC) axons had been axotomized. After 1 day, GFAP immunoreactivity of MC was also found in the central part of the ventral retina. After 2 days, MC stained for GFAP throughout the entire (dorso-ventral) extent of the host retina; but up to seven weeks after transplantation, there remained a dorso-ventral gradient of MC GFAP immunoreactivity in response to the lesion (denser and more intense staining of MC in dorsal retina). Thus, MC injury response seemed to be initiated by axotomized GC and to spread in a declining "wave" through the entire retina. S-100 staining in host retina did not change after injury; the antiserum stained AS and MC cell bodies, fibers and the internal and external limiting membrane (ILM + ELM). At the host-graft interface, occasionally a dissolution of the ILM could be seen. Within the graft, S-100 stained cells were found first 4 days and GFAP+ fibers 8 days after transplantation. 15 days after transplantation, the ELM within graft rosettes stained faintly for GFAP and S100, indicating MC maturation and reactivity. By 5 and 7 wks after transplantation, the graft was filled with intensely staining GFAP+ and S-100+ multipolar AS and bipolar MC, thus exhibiting a reactive appearance. Within graft rosettes MC were distributed radially, indicating their importance for the formation of retinal layers. AS were found everywhere in the graft (not restricted to the vitreal surface), concentrated along blood vessels, but less frequent in graft rosettes. This abnormal AS distribution might explain the lack of a continuous ILM within the graft. In the graft, the two retinal glial cell types develop from undifferentiated precursors into injury-reactive glia. This work was supported by grant No. EY04377 (National Eye Institute) awarded to JET.

81.16 NEOCORTICAL TRANSPLANTS GRAFTED INTO THE NEWBORN RAT BRAIN DEMONSTRATE A BLOOD-BRAIN BARRIER TO MACROMOLECULES. R.S. Swenson, J.C. Sørensen*, J. Zimmer* and A.J. Castro (Spon: G. Celesia), Univ. of Illinois Sch. of Med., Chicago, IL 60612; Instit. of Anat. B, Aarhus Univ., Denmark and Dept. of Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

Systemically injected HRP and endogenous serum albumin was recently found to penetrate the blood brain barrier (BBB) and permeate fetal neocortical tissue grafted into adult host rats (Science 235:772-774, 1987). In the present study we examined BBB permeability in adult rats that received fetal neocortical grafts at birth. Accordingly, fetal (E14-17) cortical tissue dissected from fetuses removed from sodium pentobarbital anesthetized (50 mg/kg) dams was transplanted into hypothermic-anesthetized newborn (0-2 day old) rats. Using a glass pipette, transplants were placed just posterior to a cortical lesion made by aspiration rostral to bregma immediately before grafting. Transplant BBB permeability to systemically injected HRP and endogenous immunoglobulin (Ig) was examined 3-5 months later. HRP was administered via a jugular venous canula in a dosage of 1mg/5g body weight and animals were sacrificed 30 minutes post-injection by overdose of pentobarbital followed by cardiac perfusion with 1% paraformaldehyde, 1.25% glutaraldehyde. HRP histochemistry on frozen sections through the host brain and transplant was done according to routine methods using TMB as the chromogen. Another group of animals was perfused with 4% paraformaldehyde, and sections through the host brain and transplant were examined immunocytochemically using HRP conjugated goat anti-rat immunoglobulin (Sigma).

In HRP injected animals, TMB reaction product was observed within the ventromedial hypothalamus, subfornical organ and area postrema. These areas which normally lack a BBB to macromolecules were also found to be immunoreactive to Ig. However, neither HRP nor endogenous Ig were found within the transplants.

These findings demonstrate the establishment of an effective barrier to the penetration of macromolecules into transplants of fetal neocortical tissues placed within the cerebral hemisphere of newborn recipients. These findings are in contrast with the results of previous studies involving the grafting of neocortical tissue into adult recipients and imply that host age affects the vascular development within transplanted neuronal tissue. (Supported by NIH Grant NS 13230 and by funds from the Danish MRC.)

81.17 VASCULARIZATION OF THE NEONUCLEUS CONSTRUCTED BY EMBRYONIC NEURONS GRAFTED IN THE ADULT CNS AS A CELL SUSPENSION. I. Dusart, F. Nothias, F. Roudier and M. Peschanski INSERM U 161, 2 rue d'Alésia 75014 Paris, France

This study is part of a general project considering the nervous tissue reconstructed by fetal cells grafted homotopically as a cell suspension into an area of the central nervous system previously neuron-depleted. Results presented here concern the morphology of capillaries and the function of the blood-brain-barrier (BBB).

Rats have been studied after neuron-depletion due to the injection of kainic acid into the dorsal thalamus (n=7) or after neuron-depletion followed by grafting of E 15-16 cells (labeled with ^{3}H-thymidine) taken from the thalamic primordium (n=10). Two additional rats (1 lesion and 1 lesion-grafted) have been used for regular electron microscopy analysis. Two types of techniques have been used : perfusion with Indian ink followed by freeze-cutting on a cryostat (n=9) and circulation of horseradish peroxidase followed by aldehyde fixation and regular peroxidase histochemistry using 3 3' 5 5' tetramethylbenzidine (n=8)

In the intact thalamus, there is a regular vascular network of relatively narrow capillaries (average diameter 5 µm). HRP does not cross the BBB. Ultrastructurally, the capillaries are bordered by a thin epithelial cell, a basal membrane and small astrocytic expansions successively. In the lesioned zone, the vascularization is quite different : (i) numerous capillaries have enlarged diameter (up to 30 µm) and irregular caliber ; (ii) HRP is present in extravascular spaces and, in particular, in pericytes ; (iii) ultrastructurally, capillaries are surrounded by a thick mantle formed by loosely arranged astrocytic processes. The neonucleus formed by grafted neurons exhibits a normal vascular network both in terms of morphology of the capillaries and in the function of the BBB.

Whatever the origin of the epithelial cells which form the capillaries of the neonucleus (fetal thalamic primordium or adult neuron-depleted thalamus), they have probably their genetic program in common. This permits to suggest that the neuronal environment these cells encounter in the neonucleus is the major incitation for them to reorganize a vascularization comparable to that of the intact tissue. Development of capillaries in the neo-nucleus is presently under study.

81.18 INTACT BLOOD-BRAIN BARRIER IN LARGE NEOCORTICAL TRANSPLANTS IN THE RAT BRAIN. M.H. Lee, A.S. Lossinsky, A. Rabe and H.M. Wisniewski*. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

The mammalian brain is protected by a blood-brain barrier (BBB) which isolates it from plasma and provides the brain with a tightly controlled homeostatic environment. Introduction of a transplant with a dysfunctioning BBB could not only affect the development and neurophysiological function of the transplant, but also allow serum proteins and neurotoxins to gain a direct access to the host brain. A recent study (Rosenstein, Science, 235:721, 1987) found that small transplants of fetal neocortical tissue in juvenile rats lacked a BBB to macromolecules. It is important to know whether the BBB is absent under other transplantation conditions. To determine whether a large fetal neocortical transplant introduced into a host brain during the early period of postnatal development would also fail to retain the BBB, we examined the brains of rats with prenatally induced micrencephaly bearing large fetal (E17-18) neocortical transplants given at 7-12 days of age. A slow infusion (0.5cc/min) of 50 mg of horseradish peroxidase (HRP) (Sigma VI) in 0.5ml of Ringer's solution was administered into the femoral vein of 1 year old male rats weighing 480g on average, and allowed to circulate for 1 hour before sacrifice by transcardiac perfusion with aldehydes. The brains were sectioned either 50-80µ on a Vibratome or frozen at 25µ, and incubated for HRP detection with diaminobenzidine. All animals in the transplant group had a large (over 200mm^3) tranplant occupying the intracranial space formed as a result of cerebral hypoplasia. In each animal, the transplant had established parenchymal interface at numerous sites with the host brain. No obvious sign of protein exudation was seen in any part of the transplant or in the host brain. Many disorganized blood vessels were identified in each transplant, but all were free of HRP; isolated areas of focal leakage of HRP from small segments of individual vessels were observed both in the transplants as well as in the host brains of a few animals. In one animal whose brain contained a huge abscess, involving over half of the basal ganglia and cerebral cortex of one side of the hemisphere with which the transplant interfaced, HRP filled the entire surface of the parenchyma surrounding the abscess to a depth of 1mm, while the transplant, 2-3mm away from the exudate, remained unaffected. It was concluded from these observations that the BBB in large fetal neocortical transplants retains its normal function, at least with regard to macromolecules.

81.19 RETINAL TRANSPLANTS MEDIATE A PUPILLARY REFLEX IN HOST RATS. H. Klassen,* R. D. Lund. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

To examine the functional efficacy of retinal transplants, retinae from E14 rat embryos were dissected free of investing membranes and placed over the midbrain of neonatal rats from which the right eye had been removed prior to transplantation. Five months later the left optic nerve was cut intracranially to eliminate afferent visual input while sparing the efferent pupilloconstrictor pathway running in the oculomotor nerve. Three days later the skull was opened and the transplant was exposed lying either rostral to the superior colliculus or displaced to a position over the cerebellum. When the transplant was illuminated, pupilloconstriction of the host eye resulted, with the degree of constriction dependent upon the level of illumination. Dilation followed cessation of illumination. These pupillary responses were abolished by damaging the transplant and by lesions localized to the pretectal region. No pupillary responses could be elicited in control animals that had undergone similar surgery to the experimental group, but had not received transplants.

The results indicate that retinal transplants are not only able to respond to changes in light intensity but also to relay this information to the pretectal region to effect an appropriate pupillary response in the host eye. Therefore, besides modulating complex behavior patterns as has been shown for nigral and basal forebrain grafts, neural transplants can also drive simple reflex pathways in response to natural stimuli. Supported by NIH grant EY05283 and Mellon Fellowship (HK).

REGENERATION I

82.1 3-DIMENSIONAL CULTURE OF NEONATAL AND ADULT ASTROGLIAL CELLS IN NITROCELLULOSE IMPLANTS : A NEW MODEL FOR EXAMINING GLIAL-NEURONAL INTERACTIONS.
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The mammalian central nervous system loses the capacity for axonal outgrowth as it ages such that regenerating axons in damaged CNS will only grow short distances into adult brain tissue. We are studying the mechanisms which allow axons to regenerate in young but not in adult CNS. To examine this question we have used the astrocyte coated nitrocellulose prosthesis model developed by Silver (Smith et al. 1986 JCN 251:23). A 0.45μ nitrocellulose filter implanted into the region of the corpus callosum in acallosal neonates is invaded by glial cells and other non-neuronal elements. Prior to 8 days of age these cells present a conducive surface to regenerating axons (Critical Period) but after 8 days (Post-critical Period), implantation results in the formation of a glial-fibroblastic scar around the nitrocellulose. We are interested in the changing properties and interactions of the young and old cell populations on the implant with respect to their capacity to promote axon outgrowth. To study these cell changes in a defined system we have increased the pore size of the nitrocellulose to 8μ, removed the implants from critical period and post-critical period brain and placed them into serum-free culture. The cells migrate on and into the nitrocellulose which protects them upon removal from the brain as well as dispensing with the need for an enzymatic dissociation step. The result is a stable population of cells derived from neonatal and adult rat brain which are viable for several weeks in culture and present in the same state as they were in situ. Immunocytochemical characterization of cells associated with the implants shows that at the critical period, cells are predominantly astrocytes with a minority of fibroblasts, oligodendrocytes and macrophages. The astrocyte cell bodies are found primarily at the surface of the implant inserting processes into the pores of the filter but also in arrays along the surface. In contrast, the post-critical period implants exhibit a greater percentage of fibroblasts, oligodendrocytes and macrophages. The fibroblasts are found at the surface of the filter while the macrophages migrate deep into the pores. The astrocytes which are still in the majority, have increased in size and have thick entangled processes. We intend to use this model to examine the ability of cells in and on the critical and post-critical period implants to promote neurite outgrowth in coculture with purified populations of neurons.
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82.2 CAN IMMATURE GLIA IN THE RAT OPTIC NERVE PROMOTE AXONAL REGENERATION? N. Giftochristos* and S. David. Neurosciences Unit, The Montreal General Hospital Research Institute and McGill University, 1650 Cedar Avenue, Montreal, Canada, H3G 1A4.

There is increasing evidence that the CNS glia might play a crucial role in the failure of axonal regeneration in the adult mammalian CNS. Recent studies suggest that in contrast to glia in the adult rodent corpus callosum (CC), glia from the developing CC may be capable of promoting axonal regeneration.

We have carried out experiments to determine whether CNS glia in the developing rat optic nerve are also capable of promoting the growth of injured axons. The rat optic nerve was also chosen because much is known about the development of the various glial cell types in the nerve. Segments of optic nerve 3-4 mm in length, from E_{20}, P1, 5, 10 and adult Lewis rats were grafted into the peroneal nerve of adult female Lewis rats. After 8-12 months, longitudinal cryostat sections through the graft and adjacent peripheral nerve (PN) were double labeled with a rabbit anti-GFAP and a monoclonal antineurofilament (RT97) antibodies and visualized with appropriate fluorescein and rhodamine conjugated secondary antibodies, respectively. The majority of the PN axons bypassed the CNS grafts to re-enter the distal PN segment. This was true of grafts of all ages. However, a very small number of axons penetrated the optic grafts (up to 20/graft) and of these, 40% extended through the entire length of the grafts. There were no differences in the number of axons growing through the embryonic, neonatal and adult optic nerve segments.

There are at least 4 possible explanations for these results: (1) Injury-induced changes in immature and mature optic nerve glia may not favour axonal growth. (2) Glia from optic nerves of E_{20} and older rats may be incapable of supporting axonal growth but glia from younger rats may do so. (3) An increased number of axons may have grown through the embryonic and neonatal optic grafts, but may have degenerated at long survival times. (4) Greater affinity of the growing axons for Schwann cells rather than immature CNS glia. We are currently doing experiments to exclude the last 3 possibilities.

82.3 PC12 NEURITE PROMOTING FACTOR FROM ADULT TRANSECTED NERVE IS DISTINCT FROM NERVE GROWTH FACTOR.

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Endoneurial explants from permanently transected, adult rat sciatic nerve secrete a factor which induces dorsal root ganglion neurons to extend neurites in the absence of exogenous nerve growth factor (NGF). This factor (SN) is found in low amounts in explants removed 0-2 weeks after transection but reaches high levels in explants cultured after 3 and up to 5 weeks post-injury (Windebank and Poduslo, 1986, Brain Res. 385:197-200). We have compared SN with NGF in its ability to induce neurite outgrowth from a pheochromocytoma cell line (PC12). The explant-derived factor appears to be distinct from NGF in several important characteristics. Neurite outgrowth obtained from saturating levels of SN (81±51μ) was significantly less than with saturating concentrations of NGF (50 ng/ml (176±256μ), compared to untreated PC12 cells (59±25μ) (Means ± SD). Unlike NGF, this SN induced outgrowth could not be blocked with anti-NGF antibody (86±78μ). When saturating amounts of NGF and SN were used in combination, they demonstrated an additive effect of longer neurite outgrowth (209±206μ), which suggests two different growth factors using separate receptors. This was confirmed by the finding that SN does not compete with ^{125}I-NGF in a receptor-binding assay on PC12 cells or dorsal root ganglion neurons. PC12 cells in the presence of 50 ng/ml NGF showed only 57±0.08% of the ^{125}I-NGF binding found in the absence of unlabeled NGF; however, with saturating levels of SN, binding was 99.0±0.1% of control. With dissociated dorsal root ganglion neurons, the respective values were 64±0.1% (50 ng/ml NGF) and 107±0.2% (SN). We conclude that SN is a neurite-promoting factor distinct from NGF whose timing of expression after transection may indicate a role in regeneration. Additionally, PC12 cells provide a convenient system in which to study the biochemical action of SN. (Supported by NINCDS Grants NS-14304 and-NS 20551).

82.4 AXOTOMISED TROCHLEAR MOTONEURONS:THEIR MORPHOLOGY AND SYNAPTIC ORGANIZATION.

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In earlier studies of axotomized cat trochlear motoneurons (TMn), we reported that the physiological profiles of these neurons were not distinguishable from normal 2-3 weeks after axotomy (Murphy et al, Neurosc.Abst.1985). However, after a longer survival (2-4 months), at least 50% of the axotomised TMns die. Of those neurons that survive, many regenerate to their original target and their mean soma size of is significantly larger than normal (Murphy et al Neurosci. Abst. 1986). The consequences of such changes might also be evident in the dendritic arborizations and the physiological profiles of these long term surviving TMns. Our aims in the present study, therefore, were to characterise the dendritic arborizations and establish their physiological profiles.Intracellular recordings were made in axotomised TMns in each nucleus 2 - 4 months following unilateral trochlear nerve section. Stimulating electrodes were placed on the ipsi and contra IVth and vestibular nerves. Following penetration and physiological recording of individual neurons, one or two TMns were filled intracellularly with 10% HRP to permit reconstruction and measurement of the dendritic arborizations. In axotomised, HRP filled neurons, the number of proximal dendrites was not changed, but the mean size of the proximal dendrites was decreased compared with normal, even in TMns in which the soma size was much larger than normal. In 2 neurons in which the entire dendritic arborization was reconstructed, its extent and the number of secondary and tertiary dendrites was not noticeably different from normal.Of all HRP filled neurons, only one was not antidromically activated. This TMn was also the only neuron in which an axon collateral was observed, and it exhibited the smallest mean dendrite diameter of all neurons studied. Antidromic field potentials in axotomised nuclei were localised and of small amplitude.By contrast, intracellular records from axotomised TMns showed in all cases normal antidromic SD invasion. However, the antidromic potential amplitude and the resting membrane potential were noticeably less than in the contralateral control TMns. Vestibular inhibitory and excitatory field potentials were nearly normal in amplitude and duration. The intracellular amplitude and duration of EPSPs and IPSPs in all axotomised TMns were not distinguishable from normal. Since the soma constitutes a small part of the total cell surface area, the results indicate that, despite an increased mean soma size, the total cell membrane area of axotomised TMns is decreased because the dendrites are narrower (see Gustaffson & Pinter, J. Physiol.'84). Thus, although the ratio of soma size to dendrite is altered, the presence of normal synaptic excitation and inhibition suggests there may be little alteration in distal dendritic membrane properties. NIH EY02007 and NS24707.

82.5 MORPHOLOGICAL AND METABOLIC INDICES OF THE RESPONSE TO AXOTOMY OF ABDUCENS MOTONEURONS.

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The distribution of cytochrome oxidase (CO), a mitochondrial enzyme involved in the electron transfer chain, has previously been shown to correspond well with areas of high metabolic activity, as revealed by 2-deoxyglucose uptake. We hypothesised that the response to axotomy might be associated with an increase in metabolic activity. In the present study, therefore, we have studied the relationship between changes in density and distribution of CO staining and changes in morphological characteristics observable in Nissl stained material in abducens motoneurons at various time periods after axotomy.

In anesthetised Sprague Dawley rats, the abducens nerve was transected unilaterally below the sinus cavernosus. Animals were allowed to recover for survival periods from 1 to 5 weeks and then sacrificed and their brainstem processed for CO (Wong-Riley,Brain Res. 79) and Nissl. In the Nissl material, the number of neurons in the abducens nucleus was counted and their cross sectional area measured on the axotomised and control side. In CO material the density of CO staining in the control and axotomised side was compared, using quantitative densitometry.

Chromatolytic changes in the axotomised motoneurons were not observed. However, other changes detectable in Nissl material, as listed below, were first observed 2 weeks after axotomy. Axotomised cells tended to fall into 2 extreme groups in the distribution of Nissl substance. Some were very faintly stained and appeared to be undergoing cell death. Others were more densely stained than normal. Cells in the control nucleus appeared normal in the range of Nissl staining observed. By 4 weeks after the axotomy, the axotomised nucleus evidenced significant cell death. On average the number of neurons in the axotomised nucleus was reduced by approximately 50% compared with normal. At 5 weeks postoperative the soma size of surviving axotomised cells was significantly larger than normal. Changes in CO staining were evident earlier than changes in Nissl material but were not in the predicted direction. Within one week of axotomy, a significant decrease in CO staining was observed in the neuropil of the axotomised nucleus, and this difference increased progressively during the the first 4 postoperative weeks. At 4 and 5 weeks post-axotomy, the axotomised nucleus appeared greatly decreased in the intensity and area of CO staining.

The changes in CO suggest that there are indeed changes in energy metabolism following axotomy. The observation that, within the nucleus, there are at least 2 subpopulations, only one of which survives axotomy, suggests that these metabolic changes may not be uniform among the entire population.

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82.6 A CONDITIONING LESION DOES NOT FURTHER STIMULATE TUBULIN AND ACTIN SYNTHESIS BUT FURTHER DECREASES NEUROFILAMENT SYNTHESIS IN THE FACIAL NUCLEUS OF THE RAT.

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We have demonstrated previously that in the axotomized rat facial nucleus the synthesis of actin and tubulin is increased while synthesis of the neurofilament proteins (NfP) is decreased. Here, we analyzed whether a conditioning lesion (CL) of the facial nerve, which speeds up the velocity of axonal elongation by 40%, further stimulates actin or tubulin synthesis or decreases NfP synthesis in the facial motoneurons.

These cytoskeletal proteins were separated by 2-dimensional gel electrophoresis of the isolated facial nuclei 2 h after administration of [35S]-Methionine into the brainstem. Synthesis was measured by quantitative densitometry of the fluorographs taken from these gels. In a complementary approach, we used cDNA probes β-α1-tubulin (α-tubulin) and neurofilament-68 (NF68) to evaluate mRNA expression of these components using in situ hybridization. For the CL the left facial nerve was crushed; 7 days later, the right and left nerves were crushed (test lesion). This allowed a comparison of the single lesioned (SL) and CL facial nucleus in the same animal.

There was no difference in actin or tubulin [35S]-methionine incorporation when comparing the SL to the CL facial nucleus, 7 days after test lesion. In situ hybridization with the tubulin cDNA followed by film autoradiography did not show an overall difference of the SL facial nucleus versus CL. In contrast, neurofilament [35S]-methionine incorporation (NF68, NF150) was further decreased on the CL side. This was also evident by in situ hybridization. Immunocytochemistry with a monoclonal antibody to NF68 and NF150 (Boehringer) revealed significantly less immunostaining in the facial axons at the inner genu of the CL side 7 days after axotomy.

Thus, the CL resulted in an additional decrease of neurofilament synthesis, which was already decreased by a SL; in contrast, elevated tubulin synthesis did not further increase with a preceding CL. Neurofilaments are believed to interact with microtubules during axonal transport. Our findings support the hypothesis that less neurofilament-tubulin interactions might occur in conditioned axons, which could allow a greater proportion of the tubulin to travel in the faster SCb component rather than with the neurofilaments in the SCa component of axonal transport.

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82.7 **SLOW AXONAL TRANSPORT AND SYNAPTIC PLASTICITY**

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We have been studying the modification of the axonal transport of the axonal cytoskeleton, labelled after intraspinal or intraganglionic injection of ^{35}S-methionine, during post-crush regeneration and in streptozocin (STZ) diabetic rats. The cytoskeletal proteins, first separated into a polymer-enriched and a soluble-enriched fraction, were analyzed by autoradiography of polyacrylamide gel electrophoresis. Our results indicate that the cytoskeletal components carried with SCa (whose polymeric fraction were composed mostly of neurofilaments and microtubules) undergoes very litle modification when they cross the crushed region of the nerve inside the new neurites stemming from the severed axons. As if, for SCa, entering a newly made axonal sprout was just the same as advancing into the intact axon. On the other hand, our results on early STZ-diabetic rats indicate that the speed of the axonal transport of neurofilaments in sensory axons is reduced by 70%. Which could be correlated with the sensitivity loss often observed in diabetic neuropathy.
These results could indicate that slow axonal transport is more implicated in maintaining synaptic integrity than is currently thought. Synaptic integrity and function is generally associated to rapid axonal transport, which carries membrane proteins and enzymes necessary to synaptic maintenance and transmission. Yet, if synaptic integrity were to depend on synaptic plasticity, then slow axonal transport would become a crucial mechanism and its impairment would result in synaptic "rigidity" and eventually degeneration. Quantitatively, slow axonal transport could afford to renew a nerve ending from several times a day to once every few days for large endings.

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82.8 MORPHOMETRIC ANALYSIS OF EARLY CELL BODY CHANGES EXHIBITED BY AXOTOMIZED MOTOR NEURONS. L.L.Hall, K.R.Latham* and R.C.Borke. Depts. of Physiology, Medicine and Anatomy, USUHS, Bethesda, MD 20814-4799.

The retrograde response of different neuronal populations following axotomy is extremely variable. Regenerative and degenerative phenomena contribute to the cell body response, but the significance of specific somatic changes is unclear. Quantification of the nuclear and perikaryal post-injury events has been limited. A morphometric approach may highlight aspects of the retrograde reaction important for successful regeneration. In the present study, we have applied morphometric techniques to the analysis of hypoglossal motor neurons 7 days post-axotomy (dpa).

The left hypoglossal nerve was transected in adult, male, Sprague-Dawley rats. Sham-operated and normal control rats were also used. At 7 dpa the animals were transcardially perfused with dilute and concentrated aldehydes. Sections were cut through the extent of the hypoglossal nucleus, post-fixed in osmium tetroxide, incubated in tannic acid and processed routinely for plastic embedding. Ultrathin sections were collected on copper grids and stained with lead citrate. Eight neurons were analyzed per hypoglossal nucleus. A micrograph of each cell (2500X) was used to estimate the nuclear fractional volume (Vv) and surface density (Sv) per cell and nucleolar Vv per nucleus. Four micrographs of the perikaryon (11,700X) per cell were used to estimate mitochondrial and lysosomal Vv and rough endoplasmic reticulum (RER), Golgi apparatus (GA) and smooth membrane Sv of the cytoplasm.

Neurons from control and sham-operated rats were typical multipolar neurons with spherical, centrally located nuclei and parallel cisterns of RER in the cytoplasm. Injured neurons displayed classical features of the retrograde reaction. Chromatolysis was ubiquitous while nuclear eccentricity and nuclear membrane invaginations were seen in some cells. The nuclear Vv and Sv were not altered by axotomy. The average nuclear Vv was 27% and 24% in control and injured neurons. The average nuclear Sv ranged from 1313.0/cm to 1136.3/cm in the control and injured groups. The nucleolar Vv was increased significantly from 4.3% among controls to 7.4% in the injured cells. A significant reduction of the average RER Sv from 42,453.3/cm to 29,119.3/cm occurred. Smooth membrane Sv significantly increased from 2,114.3/cm in normal to 4,966.8/cm in axotomized cells. The GA Sv and mitochondrial and lysosomal Vv did not change 7 dpa.

The morphometric techniques used in this study have provided specific information about the ultrastructural changes induced by axotomy. Notably, chromatolysis, or the dissolution of the long parallel cisterns of RER, corresponded to a 25% loss of RER surface area. Also, an increased nucleolar Vv and smooth membrane Sv were measured. Other organelles were not significantly altered 7 dpa.

82.9 ROLE OF MONOAMINERGIC INNERVATION IN CEREBELLAR ECTOPIA AFTER POSTNATAL X-IRRADIATION OF POSTNATAL RATS. W.J. Anderson, P.E. Kunkler*, and D. Cooper*. Indiana University School of Medicine and Indiana State University, Terre Haute, IN 47809.

The finding of heterotopic cell nests in the cerebellum of the human and animals has received a vast amount of attention in the literature. Heterotopic cell nests in normal cerebella are quite common, and often transient. They have all the appearances of an arrested migration of granule cells from the external granular layer (EGL) to their ultimate destination in the internal granular layer. Studies by Ebels et al. and Altman have found there is a consistent heterotopia (ectopia) of migrating granule cells in the molecular layer of x-rayed rat pup cerebella, and concluded that the normal timing of migration of these cells from the EGL was disrupted so that normal mossy fiber projections were not inhibited, resulting in their projection into the molecular layer where they contacted the delayed migrating granule cells to form normal synaptic relationships with them. This study was performed to see whether monoaminergic innervation of the cerebellum was altered in x-ray produced regeneration of the EGL, where granule cell ectopia is consistently found in adult animals. Long-Evans hooded rats were bred and at birth were assigned to eleven conditions: x-rayed at birth with 1 to 5 200r daily doses; x-rayed at day 4 with 1 ti 5 200r daily doses; and controls. All animals were sacrificed at 30 days of age and prepared for routine histology or histofluorescence histochemistry using the glyoxylic condensation method of de la Torre. The newborn group receiving 2 to 5 daily doses of x-ray displayed the maximal ectopia. These cerebella displayed most granule cells arrested in the molecular layer. Noradrenergic innervation was intense in all ectopia of all experimental conditions. Noradrenergic fibers increased as a function of increased ectopia and did not appear to be related to vasculature. Increased monoaminergic innervation of Pukinje cell somata, indicated by pericellular terminal endings were common in most regenerated cerebella. Whether the pericellular terminations was due to a direct effect upon growth cones, or is a restoration of neurotransmitter homeostasis is presently unknown. This study suggests that although mossy fibers are present in ectopia, noradrenergic innervation may play a major role in the altered migration of granule cells.

82.10 NORADRENERGIC INNERVATION OF THE REGENERATING EXTERNAL GRANULAR LAYER OF THE RAT AFTER POSTNATAL X-IRRADIATION. P.E. Kunkler*, W.J. Anderson and D. Cooper*. (Spon. N.A. O'Connell) Indiana University School of Medicine and Indiana State University, Terre Haute, IN 47809

In previous studies, our laboratory indicated that fractionated x-irradiation in rat pups begun at: birth, 4 days, 8 days, and 12 days and continued until the external granular layer of the cerebellum was destroyed and not reconstituted resulted in an alteration in the number of noradrenergic fibers in the cerebellum of the 4-day and 8-day treatment group when observed at 30 days of age. This indicated that x-irradiation may induce axon sprouting of noradrenergic fibers from the locus coeruleus at ages older than the first appearance of climbing fibers and might be a compensatory mechanism for the altered neurochemistry of the cerebellum due to the loss of various postnatal neurons due to the x-irradiation. This compensation in recircuitry was considered in conjunction with previous findings by Crepel et al. for Purkinje cell axon collaterals, and Anderson and Stromberg for basket cell axons. This study was performed to see whether monoaminergic innervation of the cerebellum was altered after varying x-irradiation doses that allowed regeneration of the EGL to occur. Long-Evans hooded rats were bred, and at birth were assigned to eleven conditions; x-irradiated at birth with 1 to 5 200r daily doses; x-irradiated at 4 days of age with 1 to 5 200r daily doses; and control animals for each age group. Rat pups were sacrificed at 24, 48, 72 hours after their last dose and at 10 days of age. All animals were prepared for routine histology or the histofluorescence histochemistry using the glyoxylic acid condensation method of de la Torre. Control animals never revealed any noradrenergic fibers reaching the EGL. During regeneration at both birth and 4-day conditions, increased noradrenergic innervation of the EGL could be seen 24 to 48 hours after the last treatment. These results indicate that in the regenerating EGL, cell migration is inhibited and cell multiplication is increased due to the radiation effect and the noradrenergic fibers may play a major role in this development phenomenon.

82.11 **A Chronic Chamber for Direct Observation Of Nervous System Function in vivo** W. J. Levy, R. Rumpf, M. Humphries, Neurosurgery, Univ. of Mo. School of Med. Columbia, MO 65212

Nervous system function can be studied by direct observation in tissue culture, or by in vivo studies which usually do not allow the study of individual neural elements. Tissue culture does not allow the study of cells in their normal enviornment or with the effect of a blood supply. We are reporting the use of a chamber which allows individual cell and axons to be observed in vivo. It consists of two glass coverslips 5mm square spaced 20-70 microns apart. sealed on their edges with silicone elastomer, except for two ports 2mm long at opposite ends of the chamber. The peroneal nerve in the hindlimb of a 250 gm Sprague-Dawley rat is sectioned and run into the two ports on each side. The chamber is then left implanted in the hindlimb of the rat, and repeatedly inspected by reanesthetizing the animal and opening the wound under sterile conditions. The system can be studied on a Nikon Optiphot Nomarski microscope with image enhancement.

Chambers in 13 rats had an average growth rate of 0.6 mm/day. A fan of material could be observed growing from the proximal to the distal port, and neovascularization occurred from both ports. The system under microscopic observation allowed visualization of individual axons and cells in an environment which resembled tissue culture in appearance, with the addition of blood flow. The nerve regenerated across the chamber and innervated the distal muscles, with an observable twitch, nerve signal and EMG signal distal to the chamber from stimulation proximal to the chamber. Section or selective xylocaine block of the chamber at the proximal port resulted in a loss of these responses. EM of the chamber contents revealed axons and Schwann cells. This system may allow useful studies of neuronal function, as well as effects of blood flow.

82.12 EFFECTS OF CUTANEOUS SENSORY NERVE CRUSH AND REGENERATION ON NEURONS IN CORONAL CORTEX (AREA 3b) OF CATS. G.A. Brandenberg and M.D. Mann. Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, NE 68105.

Previous investigations of nerve crush and repair have emphasized the transient nature of changes in cortical activity and the completeness of restoration to preinjury conditions. These changes include encroachment of activity from skin areas neighboring the denervated field into the cortex which normally contains the representation of that field and enlargement of receptive fields of individual neurons. In no case, has the enlargement of receptive fields included ipsilateral skin fields, nor has an ipsilateral representation been described for SI cortex. We present evidence for unexpected changes in receptive fields of SI cortical neurons which persist after functional recovery is completed.

Extracellular recordings were made of the activity, evoked by electrical stimulation of each paw, in neurons in the forepaw focus of somatosensory cerebral cortex of control cats and cats in which the sensory nerves to the contralateral forepaw had been crushed 31 to 63 days previously. Neurons that responded only to stimulation of the contralateral forepaw were classified as sa neurons; those that responded to stimulation of both forepaws were classified as sb neurons; those that responded to stimulation of both contralateral paws were classified as sc neurons; and those that responded to stimulation of at least three paws were classified as m neurons. The ratio of sa : sb : sc : m neurons in control cats was 46 : 3 : 0 : 0, and in cats that had undergone nerve crush it was 104 : 15 : 3 : 26. The distribution of sa neurons across the thickness of the cortex was similar in both control and experimental animals, with a mode at 0.5 mm. Sa neurons from experimental cats responded with more spikes per discharge, longer latency, and higher threshold than sa neurons in control cats. Frequency-following ability was similar for both groups of neurons. M neurons from experimental cats were distributed deeper in the cortex than sa neurons. When compared with experimental sa neurons, the m neurons responded with longer latencies and poorer frequency-following ability, however, the number of spikes per discharge and threshold were not significantly different. The appearance of wide-field (i.e., sc or m) neurons after temporary loss of sensory input was not expected because 1) there normally is no detectable excitatory input to this tissue from the hind paws, 2) no previous investigation of damage and regeneration had found any such neurons in this tissue, and 3) previous investigators had claimed that the cortex returns to control conditions after crush and regeneration. Of several possible explanations for the appearance of wide-field neurons in the tissue, the most likely appears to be that deafferentation strengthens previously subthreshold inputs to neurons somewhere along the somatosensory system.

82.13 CENTRAL ARBORS OF NEONATALLY AXOTOMIZED TRIGEMINAL PRIMARY AFFERENTS THAT FAIL TO REFORM NORMAL PERIPHERAL CONNECTIONS. N.L. Chiaia and R.W. Rhoades. Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

We have employed intra-axonal recording and horseradish peroxidase (HRP) injection techniques to ask whether a failure to reform normal peripheral receptor associations after axotomy results in abnormal central arbors for sensory ganglion cells. We have transected the infraorbital (IO) nerve in hamsters on the day of birth, allowed the animals to survive to adulthood, and then recorded and filled with HRP the central axons of fibers that were determined, by electrical stimulation, to have regenerated axons into the IO nerve. All injections were made in the trigeminal spinal tract near the border between subnucleus interpolaris and subnucleus caudalis.

We recovered 13 IO primary afferents that had no identifiable peripheral fields and obtained physiological data from an additional 19 such fibers. These axons had an average peripheral conduction velocity (determined by two-point stimulation) of 29.8 ± 10.0 m/s. This was slower than that for normal IO axons (40.3 ± 8.5 m/s), but not significantly different from that of regenerate IO fibers that did have peripheral fields (27.4 ± 10.3 m/s).

The central morphology of "no-field" primary afferents was quite variable, but there were a number of differences between them and IO axons in interpolaris and caudalis of normal hamsters (Chiaia, N.L. et al., J. Comp. Neurol., in press). The average number of collaterals for primary afferents with no peripheral fields was 4.0 ± 1.7 in interpolaris and 3.3 ± 2.3 in caudalis. The value for caudalis was lower than that for any functional class (vibrissa-related, skin, or guard hair) of primary afferent we have analyzed in normal, adult animals, but that in interpolaris was nearly the same as that for normal, slowly adapting vibrissa afferents and guard hair related fibers. The average cross-sectional areas for the largest interpolaris and caudalis arbors of each no field axon were 0.022 ± 0.008 mm^2, and 0.037 ± .023 mm^2, respectively. The value for the interpolaris arbors was about one-half of that for normal V primary afferents while that for the caudalis arbors was nearly normal.

These data thus demonstrate that failure to reform normal receptor associations after neonatal axotomy does result in abnormalities in central arbor morphology for trigeminal primary afferents.

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82.14 EFFECTS OF TEMPERATURE ON THE LONG TERM SURVIVAL OF ENUCLEATED AXONS. R.A. Sheller, J.W. Moehlenbruck*, J.A. Blundon*, S.A. Halls, G.I. Garcia*, G.D. Bittner. Department of Zoology and Institute for Neurological Sciences, The University of Texas at Austin, Austin, TX 78712

When mammalian axons are severed from their cell bodies, the severed distal stump degenerates within a couple of days, a process termed Wallerian degeneration. When many lower vertebrate or invertebrate axons are severed, the distal stump often survives for months or years, a phenomenon known as long term survival (LTS) (see Bittner, Amer. Zool., 1987, in press). Cancalon (Progress in Neurobiology, 25: 27-92, 1985) has reported that axonal degeneration during LTS is a temperature dependent process which occurs in a proximal to distal direction at a rate equivalent to slow axonal transport in garfish olfactory axons. We are examining various characteristics of LTS as a function of temperature in crayfish medial giant axons (MGA's), crayfish segmental lateral giant axons (SLGA's), and goldfish Mauthner axons.

Crayfish (4-9cm in body length) are acclimated at temperatures which they experience in their environmental range (5°C, 10°C, 15°C, 20°C, and 25°C) for at least two weeks before the entire ventral nerve cord is severed between the first and second abdominal ganglion. The animals are sacrificed weeks to months later and the ventral nerve cord is removed in order to sample resting potentials, action potentials, and conduction velocities at several different points in MGA's and SLGA's. The ventral nerve cord is then fixed and processed for light and electron microscopy observations of axonal morphology. Preliminary results from these studies indicate that there are no physiological differences in the LTS of MGA's or SLGA's at the different temperatures. We find no evidence of a proximal to distal temperature-dependent degeneration.

Concurrently, we are examining the temperature dependence of LTS in severed Mauthner axons of goldfish kept at 10°C, 20°C, and 30°C. Goldfish are equilibrated for 1-2 weeks at each temperature before the spinal cord is severed, effectively enucleating the Mauthner axons. Mauthner axons are sampled at post-operative intervals of 10 days for 70-80 days and LTS determined using electrophysiological and morphological criteria. Mauthner axons survive for at least 35 days at 20°C. Degeneration of severed Mauthner axons does not continuously proceed in a proximal to distal direction at any temperature.

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82.15 A CONDITIONING LESION ACCELERATES RECOVERY OF ONLY SOME OF THE ELECTRICAL PROPERTIES OF BULLFROG B-CELLS WHICH ARE ALTERED BY AXOTOMY. M.E.M. Kelly, M.A. Bisby and K. Lukowiak. Dept. Med. Physiology, Univ of Calgary, Calgary, Alta. T2N 4N1, Canada.

Axons which have been subjected to a previous conditioning lesion (CL) regenerate more rapidly following a subsequent test lesion (TL) than axons which have only received a single TL. The CL effect may be due to both metabolic changes in the cell body and environmental influences derived from non-neuronal elements at the site of injury (1). We have investigated the effect of a prior CL on axotomy-induced alterations in the membrane properties of bullfrog B-type sympathetic neurones. In response to a TL alone, these include a decrease in the duration of the afterhyperpolarization (AHP) following an action potential (AP), a decreased AHP amplitude and a slowing of AP repolarization (2).

To determine whether conditioning influences (i) the magnitude of the axotomy-induced changes observed in somal membrane properties and (ii) the time course of their recovery, we compared the effects of a single TL with those of a CL followed by a TL. At intervals following either a TL alone, or a CL followed 7 days later by a TL made at the same site, the IXth and Xth ganglia were removed for electrophysiological investigation. Growth of axons was confirmed by retrograde axonal transport of rhodamine-tagged beads injected into the nerve distal to the TL.

AHP duration recovered much earlier in conditioned cells (14 days after TL) than in unconditioned cells (42 days). A decrease in AHP amplitude did not occur until 21 days after the TL in conditioned cells, 28 days in unconditioned cells, so the time interval from the first lesion was the same. Increases in AP duration began somewhat earlier in conditioned cells (14 days) than in unconditioned cells (28 days). Neither AHP amplitude nor AP duration recovered to normal by 63 days. In no instance was the response to a single lesion further increased by the second lesion.

The distinction between the behavior of AHP duration and that of AHP amplitude and AP duration following conditioning suggests that the underlying membrane conductances are distinct, and differentially regulated during regeneration. The accelerated recovery of AHP duration in conditioned axons which regenerate faster suggests that the underlying conductance is regulated by retrograde trophic factors derived from cells of the distal nerve. Acknowledgements:Supported by the MRC of Canada. M.E.M.K. is an AHFMR Fellow.

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82.16 REGROWTH OF NEUROSECRETORY AXONS TO FENESTRATED VESSELS OF TRANSPLANTED TISSUES Y. Kadota and M. Brightman (Spon. J. Bressler) Laboratory of Neurobiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

A major target of neurosecretory axons (NSA) is perivascular basal laminae around fenestrated capillaries (FC) in the posterior lobe of the pituitary gland. Do mature, regenerating NSA terminate selectively on the FC of the posterior lobe compared with the FC of other tissues that, normally, are not innervated by NSA? Fragments about $1mm^3$, of pineal gland, adrenal medulla and posterior lobe, taken from 2 - 5 week old, inbred, Fisher rats were grafted bilaterally to the hypothalamic, retro- chiasmatic area of adult Fisher rats. This area includes axons from both neurosecretory nuclei but no FC. Two to four weeks later, the grafts were processed for immunohistochemical localization of NSA and for electronmicroscopy. Vascular approximation with NSA was defined as direct apposition to the FC or 4 μm from them, associations characteristic of neurosecretory terminals and FC in normal posterior lobes. At 2 weeks, a few regenerating NSA entered all three types of grafts. Most of the NSA were encapsulated by non-neuronal cells, some of them being Schwann cells. Partially encapsulated or free NSA were closely associated with the FC of the 3 grafts : 72 ± 27/mm^2 in pineal, 20 ± 7/mm^2 in medulla, and 261 ± 200/um^2 in posterior lobe. According to the Kruskal - Wallis test for statistical significance, there was a significantly greater number of NSA - FC associations in the pineal and posterior lobe grafts than in the adrenal medulla transplants ($p < 0.05$). However, 5 out of 8 adrenal grafts had NSA - FC approximations ranging from 10 to 25/mm^2 in frequency. Thus, even in adrenal tissue, which normally is not innervated by NSA, close neurovascular associations can be formed. At 4 weeks, the number of FC, NSA and vascular associations in pineal and adrenal tissues did not change significantly. However, in posterior lobe grafts, there was a greater number of NSA - FC associations (2095 ± 891/mm^2) than in 2 week old grafts (261 ± 200/mm^2; $p < 0.05$). We conclude that regenerating NSA can form close associations with FC from different sources to the same degree, but that posterior lobe tissue is more conducive to the ingrowth of many NSA so that there are more of these axons available to establish perivascular associations.

82.17 EXPRESSION OF APOLIPOPROTEIN B,E(LDL) RECEPTORS AND UPTAKE OF APOLIPOPROTEIN E-CONTAINING LIPOPROTEINS BY THE REGENERATING RAT SCIATIC NERVE. J.K. Boyles*, D.Y. Hui*, K.H. Weisgraber*, R.E. Pitas*, and R.W. Mahley*. (SPON: T.W. Kraft). Gladstone Foundation Laboratories, Univ. of California, San Francisco, CA 94140-0608.

Regeneration of peripheral nerves, including axon sprouting, elongation, and remyelinization, requires the biosynthesis of large quantities of cholesterol-rich membranes. Therefore, a key component of regeneration may be an efficient lipid transport mechanism. Apolipoprotein (apo-) E, a lipid transport protein synthesized by macrophages entering the injured nerve, has previously been shown to accumulate in high concentration in the distal segment. Apolipoprotein E has the capacity to bind to apo-B,E(LDL) receptors, resulting in the delivery of cholesterol to the cells for use in membrane biosynthesis. Immunocytochemistry was used in the present study to elucidate the role of the apo-B,E(LDL) receptor and apo-E in regeneration of the crushed sciatic nerve of adult rats. Anti-bovine apo-B,E(LDL) receptor and anti-rat apo-E-specific antisera were used to evaluate the distribution of these antigens in crushed nerves taken 1 day to 15 weeks after injury.

In the normal sciatic nerve, low levels of reactivity specific for apo-B,E(LDL) receptors are found along the cell membranes of Schwann cells, whereas the cells of the neurolemma and nerve axons show little reactivity. Just 1 day after a crush injury, a high level of reactivity for the apo-B,E(LDL) receptor is detected immediately proximal to the injury in the axonal stumps. As axonal sprouts grow, they show intense reactivity for apo-B,E(LDL) receptors. Reactivity within the regenerating nerve axons dissapears from the wound site by about 1 week after injury, but it can still be identified in the growing tips farther distal in the nerve. As myelinization of regenerated axons begins, Schwann cells express apo-B,E(LDL) receptors along their membranes.

Apolipoprotein E production within the wound is seen within 24 hours of injury. Tissue macrophages that migrate into the wound secrete apo-E, as shown by the intense reactivity for apo-E within their Golgi apparatus. By day 3 monocyte-derived macrophages begin apo-E production as well. Nerve sprouts take up apo-E, as shown by the presence of apo-E within their cytoplasm. This apo-E is contained in a granular compartment, perhaps a lysozome or prelysozome. Fluorescently labeled apo-E-containing lipoproteins infused at the site of a cut nerve are also taken up by regenerating axons. At the time of active myelinization of regenerated axons, apo-E accumulates within the nerve.

These results suggest that the environment of the peripheral nerve supports nerve regeneration by supplying cholesterol and phospholipids through a specific receptor-mediated process.

82.18 **GALVANOTROPIC REGENERATION FOLLOWING DELAYED TREATMENT OF THE DAMAGED MAMMALIAN PERIPHERAL NERVE.** **P.A. Fornano, M.F. Zanakis, and B.J. Albala (SPON: M.A. Palmatier) American Biointerface Corp., New York, NY**

The use of *galvanotropic neural guides*, specifically galvanotropic nerve cuffs to facilitate peripheral nerve regeneration is efficacious when applied immediately after injury. Clinically, however, most nerve injuries are not treated immediately, and significant amounts of delay (from days to months) may occur before surgical intervention. The present study outlines experiments designed to assess the efficacy of the TRAXON™ model galvanotropic nerve cuff compared to a standard (passive) nerve cuff following delayed treatment. The sciatic nerves of rats were crushed at mid-thigh using smooth tipped needle holders. This method results in a significant lesion in continuity of the nerve. A piece of 9-0 suture was placed through the epineurium at the injury site for marking purposes. The wound was closed and the animals returned to their cages. After 10 days, the wounded area was re-opened to expose the damaged nerve. At this time, a 13mm long active galvanotropic nerve cuff (TRAXON™) was placed over the nerve, with the anode oriented just proximal to the lesion site, while the cathode was oriented 12mm distal to the lesion. The cuff is designed to deliver 1μ ampere to the tissue. Control animals received an inactive device (ie., a standard silicone nerve cuff). All animals were allowed to survive for 12 days post-implantation (22 days post lesion). The portion of the sciatic nerve at the cathodal electrode (12mm from the lesion) was removed, cut in cross sections and treated with fluorescent antibodies to neurofilament protein. Counts of neurofilament-positive profiles were made and compared between groups in a blinded fashion. The results indicate clear evidence of axons in both groups of animals. However, in nerves treated with an active device, the mean number of axons was 767 (±203), while the mean number of axons in controls was 250 (±218). The difference was statistically significant with $p<0.05$. These results indicate that galvanotropic nerve cuff treatment is efficacious in facilitating the regeneration of damaged axons of the mammalian peripheral nervous system even after a delay of many days between injury and treatment. Thus, the clinical use of such a device to aid in the repair of damaged human peripheral nerves is supported by this study.

82.19 **GALVANOTROPIC REGENERATION OF THE MAMMALIAN PERIPHERAL NERVE.** **B. J. Albala, M. J. Politis and M. F. Zenakis (SPON: R. Meibach) American Biointerface Corp., New York, NY and Dept. of Anatomy, Univ. of Saskatchewan, Canada**

The application of static electric fields (EF's) to damaged peripheral nerves will result in axonal regeneration at an accelerated rate toward the cathodal electrode. However, detailed characterization of this response has been lacking. The following study utilizes the lesioned rat sciatic nerve model in order to demonstrate histologically that the mammalian PNS is capable of a more rapid rate of regeneration following the application of EF's. The sciatic nerves of rats were transected at mid-thigh, and 1cm of the distal stump was lifted and frozen on a piece of dry ice. The cut ends were then sutured together, and a *galvanotropic neural guide* device (TRAXON™) was placed over the lesion with the anode just proximal to the coaptation site, and the cathode 13mm distal to it (delivering 1μA to the nerve). At the appropriate time points, the nerves were removed and analyzed for the presence of axons using fluorescent antibodies to neurofilament protein. Neurofilament-positive (NFP) profiles 1mm distal to the cathode were determined at 6, 12 and 18 days after lesion/implantation. Control animals received either an inactive electrode implant or a reverse current (anode distal to the lesion) device. Histological analysis indicated that the NFP counts of the "cathode distal" treated groups were consistently greater than both controls at each time point. The NFP counts between the "cathode distal" group and "inactive electrode" controls were statistically significantly different at 6 days (X±SEM: 45±6 vs 10±9; $p < .05$) and at 12 days (311±29 vs 77±29; $p < .01$), respectively. The "cathode distal" treated rats also had more NFP counts in the distal nerve at 18 days (439±65) than did the matched "inactive electrode" controls (196±68), but this difference was not statistically signficant. The "anode distal" control animals were not significantly different than the "inactive electrode" controls at any time point studied. Thus, the efficacy of *galvanotropic neural guide* devices in facilitating regeneration of damaged peripheral nerves following immediate application is supported by these studies.

PEPTIDES: ANATOMICAL LOCALIZATION I

83.1 LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF IMMUNOREACTIVE DYNORPHIN IN THE SPINAL DORSAL HORN OF THE RAT H.J. Cho* and A.I. Basbaum. Departments of Anatomy and Physiology, UCSF, San Francisco 94143.

Light microscopic studies have established that immunoreactive dynorphin cells and terminals are concentrated in laminae I, outer II and V of the spinal dorsal horn. This pattern is more restricted than that seen for enkephalin and suggests that dynorphin cells may have a more selective association with the transmission and/or control of nociceptive messages than the enkephalin neurons. In this study we addressed the relationship of primary afferent fibers to the dynorphin synaptic organization in the superficial dorsal horn of the rat.

To identify degenerating primary afferent terminals, several rats underwent a multiple dorsal rhizotomy (L5-S1). Some of those rats were also treated with an intrathecal injection of colchicine (20ug/ul; 10ul). Twenty four hours later all rats were perfused with a mixed paraformaldehyde/glutaraldehyde fixative. Coronal and transverse sections of the lumbar cord were immunoreacted with an antiserum directed against dynorphin B (courtesy of Dr. Ekhard Weber).

Dynorphin immunoreactive cell bodies were common in lamina I and outer IIo. Surprisingly, both the number and density of labelled cells were significantly greater on the deafferented side of the cord. At the EM level, labelled cell bodies were found postsynaptic to both normal and degenerating axon terminals. Symmetrical synaptic contacts predominated on labelled cells. Immunoreactive dendrites also received synaptic inputs from normal and degenerating primary afferents. In this case, however, asymmetric synaptic contacts predominated. Dynorphin immunoreactive axons were associated with a variety of unlabelled profiles. Seventy-six percent were found presynaptic to unlabelled dendrites. Asymmetric axodendritic contacts slightly outnumbered those with symmetrical contacts. Ten percent of the labelled axon terminals were presynaptic to unlabelled spines; eight percent were presynaptic to unlabelled cell bodies. All of the latter contacts were symmetrical. Finally, a small number of associations between immunoreactive dynorphin terminals and unlabelled terminals, including degenerating primary afferent terminals, were seen. Some labelled dynorphin terminals were unequivocally postsynaptic to degenerating primary afferent fibers. A few examples in which dynorphin terminals appeared presynaptic to primary afferents were found, but synaptic specializations were not clear.

The large increase in dynorphin cell staining in deafferented, colchicine treated rats, indicates that dynorphin synthesis is increased under these conditions. This observation parallels studies which showed increases in dynorphin message in arthritic rats. Although a quantitative estimate is not available, it appears that dynorphin neurons are more closely associated with primary afferent fibers than are enkephalin neurons. It is unlikely, however, that spinal dynorphin neurons provide a significant input to primary afferent fibers.

Supported by PHS NS 14627 and 21445.

83.2 DISTRIBUTION OF SEVEN DIFFERENT PEPTIDES IN HUMAN SPINAL CORD. K. Chung, R.P. Briner*, S.M. Carlton and K.N. Westlund-High. (SPON: R.L. Weiner). Marine Biomedical Institute and Departments of Anatomy & Neurosciences and Division of Neurosurgery, University of Texas Medical Branch, Galveston, TX 77550.

Immunohistochemical methods permit the precise anatomical study of neurotransmitter specific pathways in the peripheral and central nervous systems of many vertebrates. The present study describes the comparative distribution of seven different peptides in four levels (cervical, thoracic, lumbar and sacral) of human spinal cord using the peroxidase-antiperoxidase technique (PAP) at the light microscopic level. The peptides examined were substance P (SP), vasoactive intestinal polypeptide (VIP), methionine-enkephalin (M-ENK), bombesin (Bomb), somatostatin (SOM), cholecystokinin (CCK) and thyrotropin releasing hormone (TRH). Each of the above peptides has been previously identified in the spinal cord of other vertebrate species including rat, cat and monkey. Tissue was obtained from six human cadavers with a short post-mortem interval (4-10 hr.) and no spinal cord pathology. The spinal cord was removed, sliced transversely into 0.5 cm in lengths and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2-5 days. The fixative was then replaced with a 20% sucrose solution in 0.1 M PB. Frozen sections (50 μm) were cut in the transverse plane and collected in PB. The sections were treated with 1% sodium borohydride for 30 minutes and immunostained according to the protocol of Sternberger ('79). An absence of immunoreactivity was observed when each antibody was preabsorbed with its antigen. The distribution of Bomb-, SP-, CCK-, and M-ENK-like immunoreactivity was localized in three regions: the superficial dorsal horn (laminae I, II and outer III), the intermediate gray matter (especially lateral laminae V to VII) and the gray matter adjacent to the central canal in all four levels of the spinal cord. The distribution of immunoreactivity for SOM in the spinal cord was very similar to that of SP and CCK, with the exception that only the inner portions of lamina II and the outer parts of lamina III were stained. The VIP-like immunoreactivity was visualized in Lissauer's tract, marginal layer of dorsal horn, in the intermediate gray and the central gray but only in the sacral spinal cord. The TRH-immunoreactivity is observed in teminals and neurons in the ventral horn of spinal cord. This study extends our knowledge of chemical anatomy of spinal cord to human system.

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83.3 ULTRASTRUCTURAL CHARACTERIZATION OF CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVE FIBERS INNERVATING THE RAT PANCREAS. C. Sternini, N. Brecha and J. P. Card. Depts. of Medicine and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024 and Medical Products Department E. I. Dupont de Nemours and Company, Willmington, DE 19898.

In a previous investigation (Sternini and Brecha, Gastroenterology, 90:1155, 1986) we have demonstrated that the rat pancreas is innervated by capsaicin-sensitive sensory afferents containing calcitonin gene-related peptide immunoreactivity (CGRP-I). In the present study we have utilized electron microscopic immunocytochemical techniques to characterize the fine structure of this afferent innervation. Rat pancreata were fixed with aldehyde solutions, cut in small pieces, processed for immunohistochemistry using a CGRP antiserum raised in rabbit against [Tyr] rat CGRP23-37 and the avidin-biotin method, embedded in plastic and cut on an ultramicrotome. Immunoperoxidase reaction product was confined to axons and terminals located within the stromal compartment and the perivascular space of arterioles of the exocrine pancreas. Immunoreactive fibers in the stromal compartment were a prominent component of nerve bundles that coursed among the pancreatic acini and were surrounded by collagen and fibroblast processes. Within these bundles, CGRP-I was confined to thin, unmyelinated axons which coursed among other unlabeled axons and dendrites. The dendrites, which apparently arise from neurons in the intrapancreatic ganglia, were often in close proximity to immunoreactive axons and terminals. However, we were not able to identify any synaptic specialization between these profiles. Abundant immunoreactive axons and terminals were also evident within bundles of nerve fibers in the perivascular space surrounding small arterioles. These fiber bundles consisted of numerous thin, unmyelinated axons which were separated from the smooth muscle of the arterioles by a distinct basal lamina. Once again, no synaptic specialization was apparent between the nerve fibers comprising individual bundles. In both the stromal compartment and the perivascular space immunoreactive afferents characteristically contained lucent, spherical vesicles which were approximately 40 nm in diameter. CGRP is a biologically active peptide which has been shown to affect exocrine pancreatic secretion and to be a potent vasodilator. The present findings, demonstrating the presence of CGRP terminals and axons in close vicinity to the exocrine pancreatic structures and vasculature provides a morphological basis for a CGRP-mediated control of pancreatic functions, perhaps acting directly on the acini or indirectly via control of blood flow.

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83.4 CALCITONIN-GENE RELATED PEPTIDE-LIKE IMMUNOREACTIVITY (CGRP-LI) IS FOUND IN PRIMARY AFFERENT NEURONES WITH A- AS WELL AS THOSE WITH C-FIBRES, IN THE RAT. P.W.McCarthy* and S.N.Lawson*. (SPON: M.R. Duchen). Dept. of Physiology, Medical School, Bristol BS8 1TD

Intracellular recordings were made in vitro at 37 ± 1°C, from neurones of L4, L5 and L6 DRGs of 6-8 week female wistar rats. Conduction velocities (CVs) were calculated from the conduction time from stimulating electrodes on the peripheral nerve or dorsal root to the cell body. After electrophysiological recordings were complete, an intracellular injection of fluorescent dye (either ethidium bromide or Lucifer yellow) was made. The ganglia were fixed with Zamboni's fixative and 7 µm frozen serial sections were cut. The indirect immunofluorescent technique was used to examine the CGRP-LI of the dye labelled neuronal somata, with a polyclonal anti-CGRP antibody, (see Gibson et al. J. Neurosci. 4, 3101-3111, 1984).

Sixty labelled neurones were tested for CGRP-LI. Of those with C fibre CVs (<1.3 m/s), 4/11 showed CGRP-LI and these had a mean CV of 0.91 m/s, range 0.61-1.3 m/s. These fibres conducted rather faster than those of CGRP-LI negative C fibre neurones, which had a mean CV of 0.64 m/s, range 0.43-0.87 m/s. Of the neurones tested which had Aδ fibres (CV 2-12 m/s), 7/19 showed CGRP-LI and had a mean CV of 7.1 m/s, range 2.4-12 m/s. The neurones negative for CGRP-LI in this CV range had a mean CV of 9.32 m/s, with a range of 2-12 m/s. Of the neurones with Aα/β CVs (>12 m/s), 5/29 showed CGRP-LI. These had a mean CV of 18.35 m/s, range 13-26 m/s, very similar to the mean and range of those with Aα/β fibres which did not show CGRP-LI.

In conclusion, we have direct evidence that CGRP-LI can be shown not only in primary afferent neurones with C fibres, but also in a substantial proportion of primary afferents with A fibres, both in the Aδ and Aα/β CV ranges.

Acknowledgements

The anti-CGRP antibody was a gift from J.Polak. Technical assistance was provided by S. Nickels and D.Martin.

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83.5 A LIGHT AND ELECTRON MICROSCOPIC LEVEL ANALYSIS OF CGRP IN THE SPINAL CORD OF THE PRIMATE. S.M. Carlton, D.L. McNeill*, K. Chung and R.E. Coggeshall. Dept. of Anatomy & Neurosciences and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

Calcitonin gene-related peptide (CGRP), a substance predicted from a nucleotide sequence derived from a gene for calcitonin, is located immunocytochemically in dorsal root ganglion (DRG) cells and fibers in laminae I and II of the dorsal horn (DH) of the spinal cord. The above findings suggest a possible role of CGRP in primary afferent systems. The present study describes the light and electron microscopic localization of this peptide in the DH of the monkey.

Three adult monkeys (M. fascicularis) were perfused with 3% paraformaldehyde, 3% glutaraldehyde and 0.1% picric acid in 0.1M cacodylate buffer. Spinal cord segments from the lumbar enlargement were removed and post-fixed overnight. Vibratomed sections (25 µm) were rinsed in 1% sodium borohydride. Tissue for EM analysis was exposed to low concentrations of alcohol to increase antibody penetration prior to immunostaining. Tissue for LM analysis was immunostained with solutions containing Triton-X. Antiserum was raised in rabbits against synthetic human CGRP and used at a dilution of 1:2000 with the PAP method.

LM analysis demonstrated immunostained fibers and varicosities throughout lamina I, II outer, lateral V and X. No cell bodies stained positive for CGRP in the DH. Analysis at the EM level demonstrated that CGRP positive terminals contained an abundance of dense core vesicles (DCV) as well as round clear vesicles. Two major terminal types were observed: a round to ovoid shaped terminal participating in only one synaptic interaction and a glomerular type terminal arrangement with one CGRP terminal indented by several postsynaptic profiles. Post-synaptic profiles consisted of small to medium sized dendrites. However, two CGRP-positive profiles were observed synapsing directly onto somae in lamina I. Two labeled terminals were often seen abutting each other however, a functional interaction could not be determined. These findings are steps towards unravelling the synaptic architecture of the CGRP system in the primate DH. (Supported by NS 11255, NS 17039 and the Florence and Marie Hall Endowment.)

83.6 DISTRIBUTION AND COLOCALIZATION OF NEUROPEPTIDE Y AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE GUINEA PIG LIVER. L. Goehler, C. Sternini and N. Brecha, Depts. of Psychology, Anatomy, and Medicine, UCLA and Center for Ulcer Research and Education, VA-Wadsworth, Los Angeles, California 90024.

Neuropeptide Y (NPY), the neuronal component of the pancreatic polypeptide family, has been localized in the central nervous system and in the periphery, including the cardiovascular and digestive systems. Colocalization studies have demonstrated that NPY immunoreactivity is expressed in catecholamine fibers innervating the heart and the splanchnic vasculature. Catecholamine nerve fibers have been found to innervate the liver of several species. In this study we have investigated the distribution of NPY immunoreactivity in the liver of the guinea pig and its possible colocalization with tyrosine hydroxylase (TH), a marker for catecholamine neurons. Guinea pig livers were fixed in a paraformaldehye-picric acid solution and sectioned at 15-30 µm on a cryostat. The cryostat sections were processed for immunohistochemistry by standard immunofluorescence techniques using rabbit antisera to NPY (kindly provided by J. K. McDonald) and to TH (kindly provided by A. W. Tank and N. Weiner). For colocalization studies, tissue was stained for NPY immunoreactivity, photographed, and the antigen-antibody complex eluted in a sulphuric acid-potassium permanganate solution. Tissue was then restained using TH antisera. NPY immunoreactivity was observed in single, varicose fibers and bundles innervating the hepatic vasculature. Single varicose fibers were also observed in the liver parenchyma, running through the spaces of Disse and the central acini. Thin, single fibers were often observed to surround the hepatocytes. The distribution of TH immunoreactivity was very similar to that of NPY immunoreactivity. Colocalization studies demonstrated that most of NPY immunoreactive fibers also contained TH immunoreactivity, which, taken together with the observation of NPY immunoreactivity in sympathetic ganglion cells, suggest that the hepatic NPY immunoreactive fibers are sympathetic. Sympathetic fibers innervating the liver have been implicated in the control of hepatic blood flow and glucose metabolism. The results of this study, showing the presence of NPY immunoreactivity in nerve fibers in the liver parenchyma and vasculature and its colocalization with TH, suggest that NPY may have a role in these hepatic functions, perhaps acting in concert with noradrenaline.

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83.7 CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVITY APPEARS TO BE PRESENT IN PREGANGLIONIC SYMPATHETIC B-TYPE, BUT NOT C-TYPE, NEURONS IN THE BULLFROG. J.P. Horn and W.D. Stofer. Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

When paravertebral sympathetic ganglia in the bullfrog were stained using an antiserum to rat calcitonin gene-related peptide (CGRP), many axons, but not cell bodies, were found to contain CGRP-like immunoreactivity. Although many of the stained axons had the appearance of sensory through-fibers, closer examination revealed immunoreactive synaptic boutons making contacts with most of the larger profiles of ganglion cell bodies. To examine the possible origins of the immunoreactive axons and boutons, sections of dorsal root ganglia (DRGs) and the spinal cord were stained. As expected from earlier work by others (eg. Gibson et al., J.Neurosci. 12,3101(1984)), the DRGs were found to contain a subpopulation of neurons with CGRP-like immunoreactivity and, in the spinal cord, CGRP-like immunoreactivity was present in ventral motoneurons and in fibers in the dorsal horn. In addition, however, we found immunoreactive cell bodies in the intermediolateral nucleus. Moreover, stained cells in the intermediolateral cell column were present only in sections cut rostral to the level of spinal nerve 6. All staining in ganglia and the cord was blocked by pre-absorbing the antiserum with 10^{-7}M rat CGRP and was unaltered by pre-absorption with 10^{-7}M to 10^{-5}M substance P, LHRH, bombesin or neurotensin.

To interpret further these findings, additional experiments were focused upon paravertebral sympathetic ganglia 9 and 10. In previous work, it has been shown that sympathetic neurons in these ganglia can be classified as B- or C-type based on the conduction velocities of their axons. In addition, B cell bodies are larger, as a group, than C cell bodies, and ganglionic B cells are selectively innervated by preganglionic neurons in the intermediolateral nucleus between spinal nerves 4 and 6 while ganglionic C cells are selectively innervated by preganglionic neurons in the intermediolateral nucleus between spinal nerves 6 and 8 (Stofer and Horn, Soc.Neurosci.Abstr. 12,542(1986)). To determine the origin of CGRP-like immunoreactivity in ganglia 9 and 10, spinal nerves were cut between DRGs and rami communicantes and allowed to degenerate for 2 weeks. After nerves 3 and 4 were cut, the staining of boutons on large cell bodies was eliminated, but not that of through-fibers. By contrast, cutting nerves 7 and 8 had no effect on staining in ganglia 9 and 10. When the sympathetic ganglia at the level of the nerve cuts were examined, the numbers of immunoreactive through-fibers were reduced. Together, these results support the hypothesis that CGRP-like immunoreactivity in ganglia 9 and 10 is present in sensory axons and in preganglionic sympathetic B-type, but not C-type, fibers.

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83.8 IMMUNOHISTOCHEMICAL CORRELATION OF CALCITONIN-GENE-RELATED PEPTIDE AND OTHER REGULATORY PEPTIDES IN SYMPATHETIC GANGLIA. Ch. Heym*, W. Kummer*. Dept. of Anatomy, Univ. of Heidelberg, D-6900 Heidelberg (SPON: R.W. Rieck)

The occurrence of calcitonin-gene-related peptide (CGRP) immunoreactive (IR) nerve fibres and ganglion cells in paravertebral sympathetic ganglia of various species (rat, guinea pig, cat, dog) was investigated applying the biotin-streptavidin-technique. In 7µm section series the distribution of CGRP-IR structures was correlated with those being IR to antibodies against vasoactive intestinal peptide (VIP), enkephalins (ENK), neuropeptide y (NPY), somatostatin (SOM) and dopamin-ß-hydroxilase (DBH).

In rat and guinea pig, CGRP-IR fibres were present in all investigated ganglia. CGRP-IR cell bodies were visible only in the guinea pig superior cervical ganglion (SCG) after preganglionic denervation. CGRP-IR in these neurons exhibited co-occurrence with VIP-IR but did not show co-existence with IR to NPY or the catecholamine-synthetizing enzyme DBH.

In the untreated cat, CGRP-IR perikarya were scattered throughout the SCG and stellate ganglion. These neurons were also VIP-IR and a subpopulation of them was supplied by DBH-IR and ENK-IR fibre baskets. Other CGRP-IR/VIP-IR perikarya received either SOM-IR- or both, DBH-IR and NT-IR fibre supply. In addition to singly scattered CGRP-IR/VIP-IR neurons, clustered VIP-IR perikarya,being nonreactive to CGRP were observed in the stellate ganglia. These cell bodies received a dense SOM-IR innervation. The upper thoracic ganglia contained clustered VIP-IR nerve cell bodies, similar to those seen in the stellate ganglion, whereas CGRP-IR/VIP-IR neurons were very infrequent. CGRP-IR structures in the dog SCG resembled those in cat.

The findings indicate the existence of a population of CGRP-IR postganglionic sympathetic neurons which, in part, represent a subpopulation of VIP-IR neurons.

83.9 DISTRIBUTION OF SUBSTANCE P (SP), CALCITONIN GENE-RELATED PEPTIDE (CGRP), GALANIN (GAL), SOMATOSTATIN (SOM), ENKEPHALIN (ENK), C-FLANKING NEUROPEPTIDE Y (CPON), VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AND THYROTROPIN-RELEASING HORMONE (TRH) IN HUMAN SPINAL CORD. PRESERVATION OF IMMUNOREACTIVITY IN DORSAL HORN, AUTONOMIC NUCLEI AND ONUF'S NUCLEUS IN MOTOR NEURONE DISEASE (MND). S.J. Gibson*, J.M. Polak, H-C. Su*, T. Katagiri*, [1]J. Ball*, [1]S.R. Bloom*, [2]S. Holland*, [2]J.T. Hughes*, [3]D.B. Brownell*, and [4]R.O. Weller*. Depts. of Histochemistry and [1]Medicine, RPMS., London W12 OHS, U.K, [2]Dept. of Neuropathology, Radcliffe Infirmary, Oxford, U.K., [3]Dept. of Neuropathology, Frenchay Hosp., Bristol, U.K., [4]Dept. of Neuropathology, Southampton Gen.Hosp., Southampton, U.K.

A major feature of motor neurone disease (MND) is loss of motoneurones from the ventral horn, although certain groups, eg. Onuf's nucleus which innervates external anal and urethral spincters, are spared. In the spinal cord the majority of peptides are localised to sensory and autonomic areas but are present in significant concentrations in the ventral horn where some (SP, TRH) modulate motoneurone excitability. To determine which peptides are possibly implicated in the aetiology of MND, sections from 36 MND and 26 control spinal cords were immunostained with antisera to SP, CGRP, GAL, SOM, CPON, ENK, VIP and TRH using the PAP method.

Peptide distribution was apparently unaltered in the dorsal horn and autonomic nuclei. In the dorsal horn, immunoreactive (IR) fibres were concentrated in laminae I-III with relative densities CGRP> SP>GAL>SOM>ENK>CPON>VIP. Cell bodies IR for SOM and ENK were observed in laminae I-III. All peptides studied were localised to fibres in autonomic nuclei with TRH, SOM, ENK and CPON being the most abundant. In the ventral horn of controls some CGRP-IR motoneurones were present. SP-, GAL-, SOM-, ENK-, TRH- and CPON-IR fibres were scattered throughout the grey matter but were most apparent apposing motoneurones. Onuf's nucleus was densely innervated with SOM-, ENK-, CPON- and VIP-IR fibres. In the ventral cord of MND cases, no CGRP-IR soma were seen and there was a reduction in number of IR fibres compared to controls (due to loss of motoneurones). In contrast, Onuf's nucleus was unaffected. Since vesicorectal function is maintained in MND we may speculate that not only is Onuf's nucleus but also the peptides found associated with it involved in regulation of bladder and rectal function. Further studies are required to establish the possible trophic relationships between motoneurones and peptides.

This work was supported by the Motor Neurone Disease Association.

83.10 CRANIAL MOTOR NEURONS CONTAIN GALANIN-LIKE IMMUNOREACTIVITY IN RAT BRAIN. Robert Y. Moore. Depts. of Neurology and Neurobiology, SUNY-Stony Brook, Stony Brook, NY 11794

Galanin is a 29 amino acid peptide isolated from porcine intestine and sequenced by Tatemoto et al (1983). It has been demonstrated in the peripheral and central nervous sytem (Rokaeus et al, 1984) and very detailed accounts of the distribution of galanin-like immunoreactivity (GAL-LI) in the rat central nervous system have been published recently (Skofitsch and Jacobowitz, 1985; Melander et al, 1986). These studies demonstrated numerous GAL-LI cell bodies in cerebral cortex, hypothalamus, locus coeruleus and in brainstem raphe nuclei but not in primary motor neurons. In reviewing normal rat material prepared with a GAL antibody (Peninsula Labs) we found apparent GAL-LI in the neurons of all cranial motor nuclei innervating striated muscle in colchicine pretreated brains. This was not evident without colchicine pretreatment.

To investigate this further, adult rats were anesthetized and subjected to unilateral section of either the 7th or 12th cranial nerve or the sciatic nerve. The animals were sacrificed after a three to four day survival under deep anesthesia and sections were prepared for immunohistochemistry using the ABC method (Hsu et al, 1981) with antisera to GAL or the calcitonin gene related peptide (CGRP). CGRP has been demonstrated previously in both spinal and cranial motor neurons (Gibson et al, 1984; Skofitsch and Jacobowitz, 1985; Kawai et al, 1985). In the control material from this study, CGRP-LI is present in spinal and cranial motor neurons without either colchicine pretreatment or nerve section. No GAL-LI is evident in control sections. After 7th or 12th nerve section, CGRP-LI is greatly increased in motor neurons of the appropriate nucleus extending from individual neuronal perikarya to demonstrate extensive dendritic arborizations. Similarly, after 7th nerve section, a large number of facial motor neurons show GAL-LI and, after 12th nerve section, a large number of hypoglossal nucleus neurons show GAL-LI. No increase in GAL-LI is observed in lumbosacral spinal motor neurons after sciatic nerve section.

These observations indicate that motor neurons of cranial nerve nuclei contain both galanin and CGRP whereas spinal motor neurons contain only CGRP. The significance of this differential co-localization of peptides in cholinergic motor neurons remains to be elucidated. Supported by NIH grant NS-16304.

83.11 COEXISTENCE OF NEUROPEPTIDES IN LUMBAR DORSAL ROOT GANGLIA OF THE CAT. M.G. Johnson, K.E. Miller, and V.S. Seybold, Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

Substance P (SP), vasoactive intestinal peptide (VIP), somatostatin 15-28 (SS), and cholecystokinin octapeptide (CCK-8) have been reported to coexist in perikarya of dorsal root ganglia (DRG) in the cat. In addition, SP and calcitonin gene-related peptide (cGRP) have been reported to coexist in varicosities in the spinal cord of cat where they are presumed to be of primary afferent origin. The purpose of this study was to quantify the extent of coexistence of SP with cGRP, SS, or CCK-8 in dorsal root ganglion cells of the cat. Four male cats (1.5-2.5kg) were cannulated intrathecally and colchicine was administered (500μg in 200μl) at spinal segments ranging from L5-S3. After 48 h., animals were anesthetized and transcardially perfused with fresh Zamboni's fixative. DRG L5 or L6 were removed, and cryostat sections (5-10μm) of these tissues were stained using a technique for the simultaneous visualization of 2 antigens by immunofluorescence. Immunoreactive perikarya were counted in each tissue section using the presence of a nucleus as the criterion for including a cell in each population of immunoreactive cells. Population totals were determined for the number of immunoreactive cells from the same ganglion level of each cat. The percent of coexistence was then calculated for combinations of 2 peptides using the following equation:

$$\frac{\text{number of cells stained for peptides B + A}}{\text{number of cells stained for peptide A}} \times 100 = \text{\% of cells containing peptide A that also contain peptide B}$$

Finally, a mean±SEM of each percentage was determined. We report that 79.8±0.3% of SP cells contained cGRP, while 35.6±8.1% of cGRP cells contained SP (n=910). For combinations of SP and CCK-8, 42.5±8.7% of SP cells contained CCK-8, while 53.0±2.3% of CCK-8 cells contained SP (n=494). For combinations of SP and SS, 25.6±2.7% of SP cells contained SS, while 76.8±7.3% of SS cells contained SP (n=296). A few leu-enkephalin immunoreactive cells were found in some ganglia, and they were always also immunoreactive for SP. No perikarya were immunoreactive for VIP, oxytocin, vasopressin, or dynorphin A (1-8) at the levels examined. These data confirm that neuropeptides coexist in the lumbar DRG of the cat, but indicate that in no case, is the extent of coexistence of SP with cGRP, CCK-8, or SS complete. These data are useful in classifying populations of primary afferent perikarya, and such classification of neurons may ultimately lead to predictions regarding their functional properties. Furthermore, localization of peptides in DRG perikarya will be the groundwork for studies aimed at the role of peptides as neuromodulators in spinal cord circuitry. This work supported by NS 17702.

83.12 LOCUS COERULEUS (LC) NEURONS PRODUCE, BUT DO NOT TRANSPORT, NEUROPEPTIDE TYROSINE (NPY). E.L.Gustafson and R.Y. Moore, Depts. of Neurology, Neurobiology and Psychology, SUNY at Stony Brook, Stony Brook, NY 11794

The LC is comprised of noradrenaline (NA)- producing neurons that project widely over the neuraxis with major terminal fields in the cerebral cortex, thalamus, cerebellum and spinal cord (Moore and Card, 1984). The projections of LC neurons are somewhat topographically organized (Loughlin et al, 1986) and, since only a portion of the neurons contain both NA and NPY (Everitt et al, 1984), the topography of LC projections could be determined by transmitter content. To investigate this problem further, the following studies were carried out. First, the exact number and location of NPY-immunoreactive (NPY+) neurons in the rat LC was determined in colchicine-pretreated brains. NPY+ neurons are present throughout the LC, with a slight preferential location in rostial and dorsal parts of the nucleus, and are 48% of the total neuronal population. Second, the number and distribution of neurons in LC and the distribution of axonal plexus demonstrated by NPY antisera was compared to that shown by antisera raised to the C-flanking peptide of the NPY precursor (CPON; Allen et al, 1985). CPON-like immunoreactive neuronal perikarya in LC and axonal plexuses in areas innervated by LC are identical to those shown by NPY antisera indicating that LC neurons express NPY messages. Third, the number of retrogradely-labeled, NPY+ neurons in LC was analyzed after large injections of fluorogold into neocortex, thalamus or cerebellum. The results are expressed as the percent of total LC neurons that are retrogradely-labeled and NPY+ for each injected area: neocortex, 60%-42%; thalamus, 46%-18%; cerebellum 23%-10%. The latter are of particular interest as thalamus contains little NPY and cerebellum none. Fourth, intraventricular 6-hydroxydopamine treatment markedly reduced DBH immunoreactivity in areas that receive an LC innervation. However, in areas that also have a dense NPY+ plexus, particularly the neocortex, the NPY+ innervation is unaffected. Further, 6-hydroxydopamine abolishes retrograde transport of tracer from cortex to LC.

These data indicate that NPY content is not determinant of the projection patterns of LC neurons. They can be interpreted three ways. First, NPY and NA could be differentially distributed in LC neuron axons such that collaterals to brainstem contain NPY whereas those to forebrain contain only NA. Second, NPY may be present in such low amounts in LC neuron axons that it is not demonstrable with the antisera used. Third, and this seems most likely, NPY is produced in some LC neuron perikarya but is not transported to the axon or its terminals. This would indicate a local neuronal function. Supported by NIH grant NS-16304.

83.13 LOCALIZATION OF DESCENDING METHIONINE-ENKEPHALIN- AND SUBSTANCE P-IMMUNOREACTIVE NEURONS FROM THE MEDULLA IN THE CAT. V. Krishna Reddy*, Simon J. Fung, Robert M. Bowker, and Charles D. Barnes, Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

Within recent years, immunocytochemical studies have revealed the presence of many different peptide-containing neurons located in the medullary raphe and gigantocellular nuclei. Two of these peptides -- methionine-enkephalin and substance P -- are known to colocalize with many of the serotonergic cells in the raphe nuclei (Glazer et al., 1981; Leger et al., 1986; Lovik et al., 1983). In the present study, the locations of the spinally projecting neurons containing either SP- or M-ENK-like immunoreactivity are shown by employing a combination of axonal transport methods and peptide immunocytochemistry. In anesthetized cats, the enzyme horseradish peroxidase (HRP) was injected bilaterally into the lumbar or cervical enlargements, followed by colchicine administration into the lateral ventricle 48 hours later. The animals were then routinely perfused after a 24-36 hour survival period and the brains were prepared for frozen sectioning (25-30 μm). The brainstem sections were first reacted for HRP using diaminobenzidene and heavy metal intensification and then incubated in antisera raised to either SP or M-ENK (dilutions 1:6000). The sections were processed by peroxidase immunocytochemistry. The resulting sequence revealed brown-stained neurons containing black HRP granules in double-labeled cells.

Following the tissue processing, many spinally projecting SP-like neurons were evident in each of the three medullary raphe nuclei -- the raphe magnus, raphe obscurus, and raphe pallidus. The double-labeled cells were distributed among both immunoreactive and HRP-filled cells. In a similar manner, M-ENK-like neurons were seen to project to the spinal cord from these same nuclei. Quantitative analyses are currently in progress to ascertain the extent of these descending peptidergic pathways and how they are related to the population of 5HT neurons located in these same nuclei. The results of this study demonstrate directly that the SP-like and M-ENK-like cells originating from the medulla have extensive projections to the spinal cord in the cat. In addition, these findings will be discussed in relation to the descending 5HT populations and to the colocalization of these peptidergic neurons with 5HT cells. (Supported by NIH grants NS22321, NS20979 and NS24388.)

83.14 EDINGER-WESTPHAL NEURONS WHICH PROJECT TO SPINAL CORD CONTAIN CORTICOTROPIN RELEASING FACTOR IN THE CAT. R.Y. Chung*, P. Mason, A. Strassman, R. Maciewicz, Neurology Service, Massachusetts General Hosp., and the Neuroscience Program, Harvard Medical School, Boston MA 02114.

The Edinger-Westphal nucleus (EW) is a well-defined collection of medium sized neurons lying dorsal and anterior to the somatic divisions of the oculomotor nucleus. Although classically regarded as the source of parasympathetic preganglionic neurons that innervate the orbit, in the cat, rat, and monkey, EW neurons project to the cerebellum and to the superficial layers of the caudal trigeminal nucleus and spinal dorsal horn. Many EW neurons that project to spinal cord exhibit immunoreactivity for choline acetyltransferase as well as the peptides substance P and cholecystokinin (CCK).

Since corticotropin releasing factor (CRF) has been localized to cells in posterior hypothalamus and medial midbrain (including EW) in the rat, the present study was conducted to determine whether CRF-like immunoreactivity (CRF-LI) is also present in cat EW neurons that project to spinal levels.

In colchicine pretreated animals, a high density of cells in the ventral, medial midbrain exhibit CRF-LI; these cells are primarily concentrated within the boundaries of EW. Within EW, CRF-immunoreactive cells extend in a continuous distribution from the caudal pole of EW rostrally into the anteromedian nucleus (AM). Although most EW cells are immunoreactive for CRF-LI, no staining is observed in the oculomotor nucleus or adjacent nuclei. To determine whether EW cells with CRF-LI project to spinal cord, immunohistochemistry for CRF was combined with retrograde transport of horseradish peroxidase (HRP) injected into the cervical cord. In these cases, numerous EW and AM neurons labeled with HRP also exhibit CRF-LI. EW may therefore be one source of previously described CRF fiber and terminal staining at spinal levels.

The distribution of neurons exhibiting CRF-LI within EW and AM is similar to the population of EW cells which contain substance P and CCK. Since a large proportion of EW cells exhibit CRF-LI, it is likely that many EW neurons contain both CRF and another peptide. Since the descending pathway from EW to spinal cord terminates in superficial dorsal horn, the descending peptidergic system containing CRF in the present study may be important in the modulation of somatosensory inputs. This suggestion is supported by recent reports of analgesic effects following systemic CRF administration.

84.1 NUCLEUS RAPHE-MAGNUS (NRM) CONTRIBUTION TO STIMULATION-PRODUCED ANALGESIA (SPA) I. MEASUREMENT OF THRESHOLD VARIABILITY.
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Electrical stimulation in or near the nucleus raphe magnus (NRM) suppresses spinal nociceptive reflexes such as the tail flick reflex (TF). If a fixed stimulation current is used to determine the liminal current (I_t) required to suppress the TF, the results are erratic. Thus, a method which rapidly and more reliably determines I_t is needed to enable further study of NRM modulation of spinal reflexes. In an attempt to provide such a method, we used trains of pulses of steadily decreasing intensity.

Rats were maintained in a lightly anesthetized state using i.v. infusion of methohexital which provides stable TF latencies (3.5-5.0 s) while eliminating signs of discomfort. A computer controlled the stimulation parameters and provided a 10 s period of constant intensity stimulation (50 Hz monopolar) followed by pulses of steadily decreasing intensity. Tail movement was detected by a strain-gauge, and the stimulus current at which the tail flicked was taken as the I_t. The starting current and rate of amplitude decline were varied to determine what effect these parameters had on I_t. I_t measurements were made in 50μM vertical steps through the NRM or repeatedly at single sites.

In the region of lowest I_t the mean was 4.4μA (S.D.-3.7 N=315). At each of these low I_t sites in NRM, variation was large. Individual values (inμA) for a typical series were (0(no flick), >15 (above starting current), 3.8, 5, 5.4, 12.8, >15μA). Slower ramps or higher starting currents did not raise mean I_t. Possible explanations for the wide variation in I_t among individual trials include the precise region of heated skin, the state of spinal neurons or external influences on NRM neurons.

This method enables rapid determination of SPA threshold. An estimate of the number of neurons involved in TF suppression can be obtained from our previous determination (Hentall et. al, J. Neurophysiol. 51:978-985, 1984) of NRM average neuronal density ($\approx$4000/mm^3) and threshold distance relations ($\approx$600/mm^2). Using these values, the number of NRM cell bodies activated by this minimum I_t (4.4μA) is approximately 11.5 Because approximately 20% of NRM neurons are off-cells (suspected TF inhibitors) the number of off-cells held active by stimulation at 4.4μA would be 2.5. Thus, electrical activation of a small number of NRM neurons appears to be responsible for TF suppression.

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84.2 PUTATIVE PAIN MODULATING NEURONS IN THE LATERAL MESENCEPHALIC AND PONTINE RETICULAR FORMATION OF THE RAT.
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Neurons whose firing rate increases (on-), decreases (off-) or remains unchanged (neutral) just prior to a tail withdrawal from noxious heat have been identified in the rostral ventral medulla (RVM) and, more recently, in the periaqueductal grey (PAG), both areas of the brainstem which, when stimulated produce a profound behavioural analgesia. Electrical stimulation of the reticular formation lateral to the PAG also produces a behavioural analgesia, the lowest threshold sites being the nucleus cuneiformis and pontine nucleus parabrachialis dorsalis. The following study was carried out to confirm that these regions of the lateral mesencephalic and pontine reticular formation are sensitive sites for stimulation-produced analgesia (SPA) and to investigate the possibility that on- and off-cells may also be present within these areas.

Single unit recording and testing for SPA (10 μA, 400 μs pulses at 50 Hz) were carried out in the caudal mesencephalic and rostral pontine reticular formation of the lightly barbiturate-anesthetized rat. SPA could be obtained at 10 μA or less from the nucleus cuneiformis, the nucleus parabrachialis and the ventral pontine reticular formation. On-(46 of 168 characterized cells) and off-cells (n=6) were found only in regions showing low-threshold SPA. As in the RVM, on- and off-cells in these more rostral areas had large receptive fields and their changes in activity preceded the tail flick response by several hundred milliseconds. However, these cells differed from those found in the RVM in that only a small minority (approximately 32%) of the cells encountered showed tail-flick related activity, and tail-flick related cells showed levels of spontaneous activity lower than in RVM. Furthermore, in contrast to RVM, spontaneous activity in these areas appeared to be independent of the depth of anaesthesia. These cells also did not have obvious periodic cycles of activity.

Thus, on- and off-cells have been characterised in another area of the brainstem sensitive for SPA, providing support for the involvement of these areas in pain modulation.

This work was supported by PHS grants DA01949 and NS21445.

84.3 NUCLEUS RAPHE MAGNUS (NRM) CONTRIBUTION TO STIMULATION-PRODUCED ANALGESIA II. CORRELATION OF CELL TYPE WITH SPA THRESHOLD
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The nucleus raphe magnus (NRM) has a well documented role in the modulation of spinal nociceptive reflexes such as the tail flick reflex (TF). Recently, neurons in this region have been classified as on-, off- and neutral cells, according to their response during the TF. On-cells are excited and off-cells are inhibited just before and during the TF. Manipulations which excite the off-cell such as electrical stimulation of periaqueductal grey or NRM and morphine administration, suppress nociceptive reflexes. Conversely, increased on-cell activity is associated with shorter TF latencies. Since electrical stimulation of the NRM inhibits the TF at low currents (presumably by holding a very small number of off-cells active), the proximity of the stimulating electrode to an off-cell might be expected to affect the precise electrical threshold (I_t) for reflex suppression.

As in our adjacent study, I_t was found by delivering computer generated pulse trains of decreasing intensity to the NRM of lightly anesthetized rats and measuring the current at which the TF occurred. The NRM stimulating electrode was also used to determine cell types during vertical penetrations. A mean I_t was determined for each cell from an average of 7 measurements. Only sites with one identifiable cell type were used. Trials with I_t above the initial (maximum) current were excluded. The mean I_t was 4.6μA (S.E.=0.68) near off-cells (n=11), 6.5μA(±1.00) near on-cells (n=16) and 6.0μA(±0.89) near neutral cells (n=17).

On- and off-cells were found to have a slightly different, although overlapping, spatial distribution, thus analysis of covariation was unable to show that cell type, rather than location was correlated with I_t. However, studies of individual neurons were suggestive of this. Maps were made of mean spike height and mean I_t around 12 individual cells. Despite great variability on individual trials, 5 of 6 off-cells showed clearly decreased I_t with increased spike height, and one cell showed the reverse. No correlation between spike height and I_t was seen for 6 on-cells.

These data suggest that each activated neuron has a significant effect on the TF. Off-cells appear to suppress these reflexes and on-cells seem to facilitate them. The fact that electrical stimulation in this region nearly always inhibits the tail flick suggests a more powerful role for the off-cell than for the on-cell in spinal nociceptive modulation.
Supported by PHS grant DA01949.

84.4 Iontophoresis of the GABA antagonist bicuculline blocks off-cell pause: Evidence for GABA-mediated control of putative pain modulating neurons in the rostral ventromedial medulla.
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Off-cells are putative pain modulating neurons in the rostral ventromedial medulla (RVM) of the rat; it has been proposed that they are the RVM output neuron that inhibits nociceptive transmission (Fields & Heinricher, Phil. Trans. R. Soc. Lond. B, 308:388, 1985). Off-cells pause just prior to the tail flick response (TF) elicited by noxious heat, and they are activated by morphine in doses sufficient to produce antinociception. Off-cell activation by morphine is probably indirect, via inhibition of an inhibitory interneuron, since all known direct cellular effects of opiates are inhibitory. One likely candidate for the substance released by the hypothesized interneuron is GABA. GABA is found in the RVM, and microinjection studies indicate that GABA plays a significant role in the nociceptive modulating functions of the region. In the present study we have used single unit recording and iontophoresis of the GABA antagonist bicuculline to investigate the role of GABA in control of off-cell activity.

Male Sprague-Dawley rats were maintained in a lightly anesthetized state by a continuous infusion of methohexital at a rate which prevented signs of discomfort and allowed a stable TF latency of 4-5 s. Single unit activity in RVM was recorded using the center barrel of a 5-barrel glass pipette. Drug barrels contained bicuculline methiodide (BIC), GABA, strychnine (STR) or glycine. One barrel, filled with 3M NaCl, was used for automatic current compensation. Both spontaneous firing and TF-related changes in activity were monitored.

BIC (10 nA, 5 min), but not STR (10-20 nA, 5-15 min) blocked the TF-related pause of all off-cells tested. In most cases, BIC also increased the spontaneous firing rate. Within 1-2 min of initiating BIC ejection, most off-cells became continuously active, with a significant increase in peak firing rate.

A second class of TF-related neurons has been identified in the RVM. These cells, on-cells, show a sudden burst of activity which begins just prior to the TF, and their activity is suppressed by morphine. Unlike off-cells, on-cells did not show consistent changes in activity related to iontophoresis of BIC.

These results demonstrate the importance of GABA in control of the activity of the off-cells. They provide support for the hypothesis that a GABA-containing interneuron mediates the off-cell pause, and are consistent with the notion that opiates act in the RVM by disinhibiting off-cells.

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84.5 ALTERATIONS OF RESPONSE PROPERTIES OF SPINOCERVICAL AND SPINOTHALAMIC TRACT CELLS BY BRAIN STEM STIMULATION. C.M. Owens*, P.J. Foreman* and W.D. Willis. Dept. of Anat. & Neurosci. and Marine Biomed. Inst., Univ. of Texas Med. Br., Galveston, TX 77550.

Spinocervical (SCT) and spinothalamic (STT) tract cells transmit nociceptive information from the periphery to the thalamus and their activity can be modulated by descending pathways originating in medial brain stem nuclei. These cells can be classified based on their responsiveness to innocuous and noxious mechanical stimuli applied to their cutaneous receptive fields. We investigated the possibility that stimulation in the medial brain stem can alter the classification of a given tract cell by changing its responses to mechanical stimulation of the skin.

To date, 10 SCT cells have been examined in 7 cats and 2 STT cells in 2 macaque monkeys (Macaca fascicularis). The animals were anesthetized with alpha-chloralose and sodium pentobarbital and paralyzed with gallamine triethiodide. End-tidal CO_2 and body temperature were monitored and kept within normal limits. Tract cells were recorded extracellularly in the lumbosacral enlargement using carbon filament microelectrodes (impedances of 2-5 megohms). STT cells were identified by antidromic activation from the contralateral ventral posterior lateral thalamic nucleus. SCT cells were identified by antidromic activation from the of ipsilateral dorsolateral funiculus at the level of caudal C3 but not from C1 (or from C1 but with a 50% or more reduction in conduction velocity between C1 and C3). Activity was recorded in the form of peristimulus time histograms. Responses were determined to brushing and to three gradations of compressive stimuli ranging from marginally painful to frankly painful. The control responses were compared with those evoked during repetitive stimulation in the medial medulla or midbrain (333 Hz, 0.1 to 1 ms pulses, up to several hundred μA). Classification was done by discriminant analysis based on a large reference population of primate STT cells classified using k means cluster analysis. Data were either normalized across the responses of a cell ("within neuron" analysis) or for each response across the population of cells ("between neuron" analysis). There were 4 response classes for each analysis.

Repeated stimulation trials in the absence of brain stem stimulation resulted in classification of the cell into the same group in either analysis. Brain stem stimulation could result in a shift of the cell into a different class when the "within neuron" analysis was used. Preliminary observations suggest that SCT cells in the cat are much less responsive than STT cells observed in the monkey. Therefore, the "between neuron" analysis should be done based on a larger sample of SCT cells rather than by comparison with the reference set of STT cells.

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84.6 RECOVERY WITH MICRODIALYSIS OF SEROTONIN (5HT) RELEASED IN DORSAL HORN OF CAT SPINAL CORD. L.S. Sorkin, M.G. Hughes*, W.D. Willis and D.J. McAdoo. Depts. of Anatomy & Neurosciences and Human Biological Chemistry & Genetics, and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

5HT, a putative neurotransmitter implicated in descending analgesia systems, is found in synaptic endings throughout the spinal cord gray matter. To examine its release in specific areas, we developed a dialysis technique that allowed us to introduce some substances into and to retrieve others from extracellular fluid in a discrete region of the dorsal horn. Cats were anesthetized with alpha-chloralose and pentobarbital, paralyzed and artifically respired. After a lumbar laminectomy, a hollow dialysis fiber (150μm ID, 9μm wall, 9kD cutoff) was inserted transversely through the spinal cord. The fiber was coated except for a 1 mm segment that was positioned in the dorsal horn. Ringers' solution was pumped through the fiber at approximately 5μl/min. Samples were collected every 10 min. The collected fluid was assayed for 5HT and its metabolite, 5-hydroxyindole acetic acid, using HPLC with electrochemical detection. At the end of each experiment, ferric chloride followed by potassium ferrocyanide was pumped through the fiber so that a Prussian blue mark could be used to define the dialysis site. In some trials, the Ringers' solution was replaced by fluid containing 100 mM K^+ (substituted for Na^+), 100 mM K^+ and 2 mM Co^{++} (substituted for Ca^{++}), or 0.35 mg/ml parachloroamphetamine (PCA). Serotonin was sometimes, but not always, present in detectable quantities in control samples. PCA or 100 mM K^+ in the perfusate solution caused release of 5HT. However, 100 mM K^+, in the presence of zero Ca^{++} and 2 mM Co^{++} was ineffective in causing 5HT release. Additionally, the ipsilateral dorsolateral funiculus (DLF) was electrically stimulated (0.5 msec long pulses at 5 Hz) rostral to the dialysis site concurrent with sample collection during some trials. This also caused 5HT release. Often the elevated levels of 5HT outlasted the stimulation for up to 40 min.

Thus, with our dialysis system we have demonstrated calcium dependent potassium evoked release of 5HT. This coupled with DLF stimulation evoked release indicates that the 5HT we are measuring is of neural origin.

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84.7 QUANTITATIVE CHARACTERIZATION OF INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION FROM THE LATERAL RETICULAR NUCLEUS (LRN). A.J. Janss and G.F. Gebhart. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52240.

Involvement of the LRN in the caudal ventrolateral medulla in centrifugal modulation of nociceptive transmission has been demonstrated in the rat using the analgesiometric tail-flick and hot plate tests; behavioral tests are an indirect measure of nociception and cannot discriminate between inhibition of motor and sensory activity. In this study, inhibition of nociceptive spinal neurons from the LRN in rats was characterized.

Rats were anesthetized with pentobarbital (45 mg/kg) for cannulation of the femoral artery and vein and tracheotomy. They were paralyzed with pancuronium (0.4 mg/kg iv) and ventilated with a gaseous mixture of N_2O:O_2 (2:1). Anesthesia was maintained throughout the experiment by infusion of pentobarbital (5-7 mg/kg/hr iv). The spinal cord was exposed between segments C2-C3 and T12-L2 by laminectomy. Stimulation electrodes were lowered into the contralateral ventrolateral quadrant of the cervical spinal cord (VLQ), ventroposteriolateral nucleus of the thalamus (VPL), and LRN; a recording electrode was placed in the lumbar spinal dorsal horn.

All 47 units studied responded to mechanical stimuli and to noxious heating (50°C) of the skin of the footpad; rostral projections were identified by antidromic invasion with stimulation (0.5 μs, 0.5 Hz) in the VPL and VLQ. Stimulation-produced inhibition (constant cathodal current; 100 μs, 100 Hz) of neuronal responses to heat in the caudal medulla was maximal in the area of the LRN. The mean threshold intensity of LRN stimulation for inhibition of unit responses to heat was 13.0 ± 1.8 μA and the mean intensity attenuating neuronal responses to heat to 50% of control was 34.4 ± 3.3 μA. Inhibition of spontaneous activity of nociceptive neurons by LRN stimulation did not differ in magnitude from inhibition of responses to heating of the skin. Unit response to graded heating of the skin (42-50°C) was a linear, monotonic function. Stimulation in the LRN decreased the slope (42 ± 4% of control) of the stimulus response function and increased the neuronal response threshold (2.0 ± 0.7°C). Monosoduim glutamate (50 nmoles) microinjected into stimulation sites attenuated neuronal responses to heat only when microinjected into the LRN (mean = 35 ± 6% of control). Microinjections of lidocaine made into the ventrolateral medulla blocked LRN stimulation-produced inhibition without affecting neuronal spontaneous activity or responses to heating of the skin. These results clearly establish a role for the LRN in the modulation of spinal nociceptive transmission, but the LRN does not appear to be the supraspinal source of tonic modulation in the rat. Supported by NS 19912 and T32 GM07069.

84.8 CHEMICAL VS. ELECTRICAL INHIBITION OF DORSAL HORN NEURONS FROM THE BRAINSTEM IN THE RAT. S.L. Jones and G.F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

Opioid receptors and peptides have been identified in brainstem sites which also have been demonstrated to be involved in stimulation-produced descending inhibition of spinal nociceptive transmission [e.g., locus coeruleus/subcoeruleus (LC/SC), nucleus raphe magnus (NRM), periaqueductal gray (PAG)]. Morphine, which has been demonstrated to act supraspinally (as well as at the spinal level), is hypothesized to modulate spinal nociceptive transmission via activation of endogenous descending inhibitory systems.

The objectives of this study were: 1) to quantitatively examine the effects of morphine microinjections in the PAG, the LC/SC and the NRM on responses of dorsal horn units to noxious heat (50°C), and 2) to quantitatively compare morphine's effects with glutamate- and stimulation-produced inhibition of unit responses to heat in the deeply pentobarbital-anesthetized, paralyzed rat.

Morphine sulfate (MS; 10-20 μg, 0.5 μl) was microinjected into 12 sites in the midbrain PAG. Dorsal horn unit responses to noxious heat were inhibited to 64.3 ± 7.6% of control at 8/12 sites; MS had no effect on unit responses to heat at 4/12 sites in the PAG. At 8/13 sites in the pontine LC/SC, MS microinjections inhibited unit responses to noxious heat to 63.8 ± 9.9% of control; at 3/13 sites unit activity was increased to 137.9 ± 8.2% of control and at 2/13 sites MS produced no change in unit activity. The microinjection of MS into the medullary NRM produced inhibition of unit responses to noxious heat at 8/12 sites to 59.8 ± 5.9% of control and had no effect at 4/12 sites in the NRM.

Summarized below are the mean effects (± SEM) of MS and glutamate microinjections and electrical stimulation on unit responses to noxious heat for sites in the PAG, the LC/SC and the NRM where both glutamate and MS microinjections were made at the same sites (n=5). Inhibition is expressed as % of control.

	μA	Stim	MS	Glutamate
PAG	95.0 ± 5.0	16.3 ± 8.1%	62.2 ± 11.3%	60.7 ± 1.5%
LC/SC	100.0 ± 0.0	23.7 ± 7.8%	60.5 ± 12.7%	55.8 ± 11.6%
NRM	85.0 ± 10.0	45.4 ± 12.0%	53.9 ± 7.5%	55.7 ± 13.6%

These results suggest that MS can modulate spinal nociceptive transmission by activating the PAG-NRM bulbospinal relay at either the midbrain or medullary level or the pontine coeruleospinal pathway.

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84.9 PERIAQUEDUCTAL GRAY STIMULATION INDUCES GASTROINTESTINAL UPSET WHILE PRODUCING CUTANEOUS ANALGESIA. K.F. Green and D.S. Sacks. Psychology Dept., California State University at Long Beach, 90840 and Psychology Dept., Bowling Green State University, Bowling Green, Ohio, 43402.

Abundant evidence indicates that electrical stimulation of the periaqueductal gray (PAG) can block cutaneous pain. In contrast very little work has been done on the effect of PAG stimulation on pain of visceral origin, such as gastrointestinal (GI) upset induced by injected lithium chloride. The present work addressed this problem.

The subjects were 28 male Wistar rats with unilateral PAG electrodes. When stimulated, all showed consistent, profound cutaneous analgesia in the tail flick test. Four groups were defined as follows. Group LS, the experimental group, drank water sweetened with saccharin, and was then injected with lithium chloride and stimulated in the PAG for the 2-hr duration of the GI upset. On a later day this group (as were all other groups) was tested with sweet water for a conditioned flavor aversion (CFA). Group L24S, the CFA control, drank sweet water and was injected with lithium chloride; 24 hr later the PAG was stimulated for 2 hr. Group S24L, the PAG stimulation control, drank sweet water and was stimulated for 2 hr; 24 hr later lithium chloride was injected. Group N, the no-lithium no-stimulation control, simply drank sweet water.

PAG stimulation did not attenuate GI pain, as indicated by CFAs in Group LS that were as strong as CFAs in Group L24S. Indeed, PAG stimulation alone produced CFAs to sweet water in Group S24L. No CFAs were shown in Group N.

Later tests, where PAG stimulation was paired with one side of a shuttle box, indicated no aversion to an environment. Therefore the aversive consequences of PAG stimulation were due to internally referred visceral stimuli.

Similar findings of GI upset accompanying morphine or rotation stress-induced somatic analgesia have been reported. These data reinforce accounts of behavior that point to separate mechanisms for processing information in somatic and visceral milieus. It may be that manipulations that induce somatic analgesia also induce GI upset.

84.10 THE PAG: AN EXAMINATION OF THE DISTRIBUTION OF OPIOID AND NON-OPIOID SITES, THEIR INTERACTION, AND THE ROLE OF SEROTONIN. D.S. Nichols*, B. Thorn, and G.G. Berntson, Psychobiology Program, Dept. of Psychology, The Ohio State University, Columbus, Ohio 43210.

The midbrain periaqueductal gray (PAG) has been reported to support stimulation-produced analgesia (SPA) in both humans and animals. Some sites within the PAG have qualities that suggest an endogenous opiate system, characterized by naloxone-reversibility, a diminuation of the analgesic affect following repeated stimulation, and the development of cross- tolerence between two PAG sites. Stimulation of other sites within the PAG results in analgesia that is not naloxone reversible. Naloxone-reversible sites have been reported primarily in the ventral PAG , and non-naloxone-reversible sites primarily in the dorsal PAG. Stimulation of both systems appears to activate intraspinal systems, which may or may not utilize serotonin.

The present study examines the distribution of opioid and non-opioid sites within the PAG, the interaction of these sites through the production of cross-tolerence, and the ability of the serotonin antagonist, methysergide to reverse the SPA from each. Albino rats were stereotaxically implanted with two monopolar stimulating electrodes, aimed at sites varying along ventral-dorsal, medial-lateral, and anterior-posterior axes. Analgesia was produced by a 10 sec. stimulation utilizing biphasic rectangular-wave pulse pairs of 1-msec. duration at a 50 Hz frequency, with intensity ranging from 10-300 microamps. The effect of stimulation was examined with the tail-flick test. Each animal underwent a series of tests to determine SPA characteristics. If a site supported SPA, it was then tested for naloxone-reversibility (10 mg/kg naloxone), and 48 hours later for methysergide-reversibility (3 mg/kg methysergide) to assess the opioid and serotonin contributions to the SPA. If two sites within an animal supported SPA, one site was stimulated to tolerence and then stimulation was transferred to the second site to determine if cross-tolerence occurred.

240 sites were sampled, of which 80 supported SPA. Preliminary histologies support the ventral-dorsal distribution of opioid and non-opioid sites, with predominantly a midline focus. This distribution is present throughout the anterior-posterior extent of the PAG. Cross-tolerence was obtained between dorsal and ventral sites and this was bi-directional. Methysergide was found to reverse SPA from both naloxone-reversible and non-reversible sites. The findings suggest that although these systems are anatomically distinct, they do not operate independent of each other and that serotonin is a common element.

84.11 INFLUENCE OF INJECTIONS OF MORPHINE INTO THE PAG AND AMINE ANTAGONISTS INTO THE SPINAL CORD ON NOCICEPTIVE RESPONSES ORGANIZED AT DIFFERENT LEVELS OF THE CNS IN THE RAT. G. S. Borszcz, A.H. Lichtman*, & H.C. Hughes. Dept. of Psychology, Dartmouth Coll., Hanover, NH.

We have recently shown that by applying the psychophysical method of constant stimuli to a wheel turn / tail flick paradigm, thresholds of nociceptive responses organized at different levels of the CNS can be assessed simultaneously (Borszcz & Lichtman, 1986). This paradigm requires rats to turn a wheel in order to terminate shock applied to the tail. The probability of wheel turn (cephalic) and tail flick (spinal) responses at each of several current intensities was assessed, and the resulting psychometric functions were evaluated to determine nociceptive thresholds.

Using these procedures the influence of morphine sulfate microinjected into the ventrolateral periaqueductal gray (vPAG) on wheel turn and tail flick thresholds was examined. Since the antinociceptive effects of morphine microinjected into the vPAG are partially the result of activating noradrenergic and serotonergic receptors within the spinal cord, the influence of intrathecally administered amine antagonists on morphine induced antinociception was also studied. Each rat was chronically implanted with a cannula directed towards the vPAG and an intrathecal catheter whose tip rested near the lumbar enlargement of the spinal cord. After recovery, nociceptive baseline thresholds were obtained for both tail flick and wheel turn responses. Nociceptive thresholds were again assessed after: 1) microinjection of morphine sulfate into the vPAG (3*ug*/.5*ul*), 2) intrathecal injection of the amine antagonists (methysergide and phentolamine, 15*ug*/15*ul* each), and 3) combined microinjection of morphine sulfate into the vPAG and intrathecal injection of amine antagonists.

Preliminary results indicate that microinjection of morphine into the vPAG elevates wheel turn thresholds to a greater degree than tail flick thresholds. The intrathecal administration of amine antagonists had little influence on baseline threshold values for either response. When morphine microinjection was accompanied by intrathecal administration of amine antagonists, tail flick thresholds were reinstated to baseline levels while wheel turn thresholds remained significantly elevated. These results are comparable to those reported by Yaksh (1979) using the radiant heat / tail flick and hot plate / paw lick tests, and indicate that the neural mechanisms subserving cephalically versus spinally organized nociceptive responses are differentially influenced by morphine administration.

84.12 SYNAPTIC ORGANIZATION OF THE NUCLEUS RAPHE DORSALIS OF THE CAT. G. Chazal[1] and P.T. Ohara. Anatomy Department, University of California, San Francisco, San Francisco, CA 94143, USA. [1]I.N.S.E.R.M., U-6, 280 Bd. Ste Marguerite, 13009 Marseille, FRANCE.

The nucleus raphe dorsalis (NRD) is a major source of 5-HT projections to other regions of the CNS and is involved in a number of CNS functions including pain modulation. This study describes the synaptic organization of NRD and provides anatomical evidence for dendro-dendritic interactions.

Adult cats were perfused under deep barbiturate anaesthesia with 2% Paraformaldehyde/ 2% Glutaraldehyde in 0.1M phosphate buffer, pH 7.6. 50um vibratome sections of the brain stem were osmicated, dehydrated in alcohols, stained with uranyl acetate and embedded in Epon. Areas containing Nucleus Raphe Dorsalis (NRD) were trimmed from the vibratome sections and serially sectioned for electron microscopy. For all terminal types the nature of the synaptic contact was confirmed using serial section analysis.

Five different classes of terminals are identified based on the shape and packing density of the synaptic vesicles and type of contact they establish. The most common class (RDI-type) contains densely packed, round, agranular synaptic vesicles and establish asymmetrical synaptic contacts. A second class (RDII-type) also contains spherical synaptic vesicles but establishes symmetrical synaptic contacts with dendrites of all sizes. Most of the terminals in these two classes contain a few dense-cored synaptic vesicles but a small sub-group contains many dense-cored vesicles. A third, less frequent, class (RSI-type) have sparsely packed spherical synaptic vesicles and the majority of these terminals have asymmetrical contacts. A fourth terminal class contains pleomorphic synaptic vesicles (P-type) and contact dendrites of all sizes and usually establish symmetrical synaptic contacts. Finally, boutons thought to be the vesicle-filled excresences of dendrites (PSD) are found. Such boutons contain pleomorphic vesicles, are presynaptic to other PSDs as well as conventional dendrites and are presynaptic to other PSDs as well as conventional dendrites and are postsynaptic to the other terminal types described.

Somata within the nucleus exhibited somatic spines but receive few synaptic contacts. Most axo-somatic terminals contain either round or pleomorphic vesicles and have postsynaptic thickenings intermediate between the symmetric and asymmetric type. (Supported by NS 23347 and INSERM-France to GC).

84.13 ORGANIZATION AND CYTOCHEMISTRY OF BRAINSTEM NEURONS WITH DIVERGENT COLLATERALS TO THE MIDBRAIN PERIAQUEDUCTAL GREY (PAG) AND THE SPINAL CORD. Kwiat, G.* and Basbaum, A.I. (SPON: N.M. Lee). Dept. of Anatomy, University of California, San Francisco 94143.

The analgesic action of electrical stimulation of the midbrain periaqueductal grey (PAG) is thought to result, in part, from activation of medullary serotonergic and noradrenergic neurons, which, in turn, project to and inhibit spinal nociceptive neurons. The analgesia may also involve activation of neurons which have divergent projections to the PAG and the spinal cord. This study used a multiple labelling approach to address the latter possibility.

First, 2 to 3 ul of a 5% solution of the retrograde tracer, True Blue, was injected bilaterally into the cervical enlargement. Three days later, a second retrograde tracer, WGA-apoHRP-Au (Basbaum and Menetrey, 1985) was microinjected into the PAG. Four days later the rats were perfused with a 4% paraformaldehyde fixative. Twenty micron cryostat sections were silver intensified (to visualize the gold) and an immunofluorescence procedure was used with antisera directed either against 5HT, or, to identify catecholamine neurons, tryosine hydroxylase (TH).

Both the nucleus raphe magnus (NRM) and adjacent reticular formation contained a large number of gold-labelled cells. Of these, about 20% also contained True Blue, ie, they project to both the PAG and spinal cord. Despite the large number of spinally projecting 5HT neurons in this region, none of the True Blue-gold double labelled cells were 5HT immunoreactive. Some 5HT immunoreactive neurons in the nucleus reticularis paragigantocellularis lateralis projected to the PAG, but these did not also project to the cord. Other cells in this region had divergent collaterals to the PAG and spinal cord but were not 5HT immunoreactive.

Triple labelled cells containing TH immunoreactivity and retrograde labels from PAG and spinal cord were found in the locus coeruleus and A5 cell group. Other TH cells in the ventrolateral medulla, nucleus of the solitary tract, and locus coeruleus projected to the PAG but were not retrogradely labelled from the spinal cord. Spinally projecting TH positive cells in the Kolliker Fuse nucleus did not project to the PAG.

These data indicate that there is a widespread pontomedullary catecholaminergic projection to the PAG. A component of this projection derives from cells with divergent collateral projections to the spinal cord. In contrast, the spinally-projecting 5HT neurons of the NRM do not collateralize to the PAG. Further studies of the cytochemistry of the raphe neurons which do collateralize to PAG and cord, and which may contribute to the analgesic action of PAG electrical stimulation, are in progress.

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84.14 THE RELATIONSHIP OF ENKEPHALIN AND NEUROTENSIN IMMUNOREACTIVE TERMINALS WITH PERIAQUEDUCTAL GRAY-RAPHE MAGNUS PROJECTION NEURONS. F.G. Williams* and A.J. Beitz. Dept. of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

The midbrain periaqueductal gray (PAG) is a key component of the brain's endogenous analgesia system. Several lines of evidence suggest that the neuropeptides enkephalin (ENK) and neurotensin (NT) play a role in PAG-induced analgesia. The present study was undertaken to determine if ENK and/or NT-containing synaptic terminals interact with PAG-raphe magnus projection neurons.

Wheat germ agglutinin-horseradish peroxidase (WGA-HRP, 1ug) was injected into the raphe magnus of 10 Sprague-Dawley rats (275-300 grams) under chloral hydrate anesthesia. Twenty-four to thirty-six hours later the animals were perfused with calcium-free Tyrode's solution, followed by 4% paraformaldehyde/0.4% glutaraldehyde. Brains were post-fixed for 12 hours before sectioning at 50 microns on a vibratome. WGA-HRP was visualized on the sections using tetramethylbenzidine (TMB) as described by Rye, et al. (1984). After the TMB reaction, sections were immunohistochemically stained either for NT, using rabbit antibody kindly supplied by Dr. L. Jennes, or for ENK using a rabbit antibody kindly supplied by Dr. R. Elde. Primary antibodies were visualized using Vectastain ABC kits and diaminobenzidine. Sections were processed both for light and electron microscopy.

PAG neurons retrogradely-labeled from raphe magnus were most concentrated in the caudal region of the PAG, where they are found immediately ventral to the aqueduct. Some retrogradely-labeled neurons were observed in the dorsal raphe nucleus. By light microscopy, numerous puncta immunohistochemically stained for NT and ENK were also concentrated in this region. Electron microscopy was then employed to clarify possible relationships between these immunoreactive structures and retrogradely-labeled PAG projection neurons. For both antisera, greater than 90% of the immunostained structures were terminal boutons containing both dense-core and round agranular vesicles. Immunopositive dendrites were rare and no positive perikarya were observed. Most NT and ENK-positive terminals were immediately adjacent to unlabeled dendrites characterized by a light cytoplasm. Two or more positive terminals were often observed forming symmetrical synapses with a single unlabeled dendritic profile. Both NT and ENK-positive terminals were also observed in contact with dendrites containing retrograde TMB label from raphe magnus. NT and ENK immunoreactive terminals were rarely observed on the perikarya of neurons containing retrograde label. These results suggest that both ENK and NT immunoreactive terminals interact with PAG neurons that project to raphe magnus.

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84.15 THE INFLUENCE OF PAIN AND MORPHINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. S.R. Cohen and E.D. London. Neuropharm. Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

To identify brain areas which might play a role in different types of pain and morphine-induced analgesia, rates of local cerebral glucose utilization (LCGU) were assessed in rats with and without pain which received morphine or saline. Particular attention was paid to forebrain and limbic areas to focus on the affective component of pain and morphine's effect.

The 2-deoxy-D-[1-^{14}C]glucose (DG) technique of Sokoloff et al., (J. Neurochem., 28:897, 1977) was used to measure LCGU. Rats were assigned to one of three pain groups: no pain, formalin pain, or tail-immersion pain. Rats in the formalin pain group received an injection of 0.05 ml 2.5% formalin into the plantar surface of a hindpaw 30 min before DG (100 uCi/kg, i.v.). Animals in the tail-immersion pain group had their tails dipped in a 55°C water bath at the following times after the DG injection: 2, 4, 8, 10, 14, 16, 20, 25, 30, 35 and 40 min. In addition, each rat received either morphine (8 mg/kg, s.c.) or saline 30 min before DG.

No effect of either type of pain could be discerned in any of the 48 brain regions studied. These included the brainstem nuclei implicated in pain and analgesia (e.g., dorsal raphe, raphe magnus, gigantocellular nucleus (n.), paragigantocellular n., periaqueductal gray) and somatosensory areas (ventroposterolateral thalamic n. (VPL), gracile n., somatosensory cortex), as well as limbic areas (e.g., accumbens n., septal n., habenula, amygdala).

Morphine decreased LCGU in 17 regions, including many forebrain and limbic sites. Significant decrements were observed in the lateral septal n., paratenial, centromedian, VPL, gelatinosus, mediodorsal, and paraventricular thalamic nuclei, medial prefrontal cortex, lateral hypothalamus, ventral tegmental area, stria medullaris, lateral habenula, median raphe n., interpeduncular n., mammillary bodies, and entorhinal cortex. In contrast, morphine increased LCGU in the external cuneate n.

In most areas, LCGU was the same ipsilateral and contralateral to the catheterized leg. However, LCGU was decreased in the contralateral VPL as compared to the ipsilateral VPL in all groups, possibly due to the intraoperative use of a local anesthetic (bupivacaine HCl, 1 mg).

Anatomical sites involved in interactions between morphine and pain could not be determined as the pain stimuli did not affect LCGU. Nevertheless, the decrements in LCGU produced by morphine in limbic and forebrain structures in this study are consistent with the clinical observation that much of morphine's analgesic effect is due to a reduction of the affective component of pain.

84.16 **PHARMACOLOGIC CHARACTERIZATION OF SPINAL CORD 5-HT$_1$ RECEPTOR SUBTYPES.** **F.P. Zemlan and R.M. Murphy. Lab. of Geriatric Research, and Dept. of Physiol. and Biophysics, Univ. of Cincinnati College of Medicine, Cinti, OH 45267-0555.**

Recent biochemical and behavioral studies indicate that serotonin (5-HT) may differentially affect pain transmission at the spinal level. With the recent discovery of multiple 5-HT$_1$ binding sites in the spinal cord, it is possible that the variable effects of 5-HT at the spinal level may be mediated by particular 5-HT$_1$ receptor subtypes. The present studies report our initial findings characterizing these multiple 5-HT$_1$ binding sites in spinal cord.

Saturation experiments using [^{3}H]5-HT and the selective 5-HT$_{1A}$ ligand [^{3}H]8-OHDPAT (0.10 - 45 nM) indicate the presence of both high (K_D = 0.10 nM) and low (K_D = 3-6 nM) 5-HT binding sites in spinal cord. Increasing concentrations of Mg^{2+} or Ca^{2+} (10^{-6} - 10^{-1} M) significantly increased specific high affinity [^{3}H]5-HT binding. No significant increase in binding occurred at higher ligand concentrations (>5 nM).

At lower ligand concentrations (0.3 nM), which predominantly labeled higher affinity [^{3}H]5-HT binding sites, addition of GTP decreased specific binding by 80%, while at ligand concentrations (10 nM) which predominantly labeled lower affinity sites, GTP decreased [^{3}H]5-HT by only 40%. In the presence of divalent cations GTP concentrations above 10 uM eliminated all higher affinity [^{3}H]5-HT binding sites.

Competition of 5-HT agonists and antagonists (10^{-15} - 10^{-4} M) against [^{3}H]5-HT (0.3 nM - 5 nM, 0.1 mM GTP) yielded steep inhibition curves (n = 0.98 - 1.07) for 5-HT, 5-MT metergoline and cyproheptadine. Competition curves for 8-OH-DPAT, buspirone, 5-MeODMT, mCPP, quipazine and TFMPP were shallow with Hill coefficients between 0.77 and 0.51 suggesting the presence of two binding sites for these competitors.

Selectivity of competing agonists (500 nM) for 1A and 1B receptors ([^{3}H]5-HT = 0.5 nM) was determined in the presence of 100 nM mCPP and 100 nM 8-OH-DPAT respectively. Kinetic parameter estimates indicated that 40% of spinal cord [^{3}H]5-HT binding sites were labeled in the nanomolar range by 1A selective agonists (8-OH-DPAT, buspirone and 5-MeODMT) while the remaining 60% were labeled by 1B selective agonists (mCPP, quipazine and TFMPP).

The present studies characterized two distinct 5-HT$_1$ binding sites in spinal cord. Reference to our behavioral data reported at this meeting indicate that the two 5-HT$_1$ receptor subtypes may have opposite effects on spinal pain transmission. (NS18326 and NS07840)

84.17 SELECTIVE 5-HT1A AND 5-HT1B AGONISTS DIFFERENTIALLY AFFECT SPINAL PAIN REFLEXES. E.J. Purpus*, R.M. Murphy and F.P. Zemlan (SPON: N. Sperelakis). Lab. of Geriatric Research, and Dept. of Physiol. and Biophysics, Univ. of Cincinnati Col. of Med., Cinti, OH 45267.

The present study reports the effects of selective 5-HT_{1A} and 5-HT_{1B} agonists on the sensitivity of three spinal nociceptive reflexes.

Two days after spinal transection, animals were injected i.p. at 20 minute intervals with increasing doses (0, 0.1, 0.4, 2.0, 13 mg/kg) of either a 5-HT_{1A} selective agonist (8-OHDPAT, buspirone) or a 5-HT_{1B} selective agonist (mCPP, TFMPP). The nociceptive sensitivity of the ventroflexion, dorsiflexion and lateral flexion reflexes was quantified as size of the RF area of the reflex in cm^2 (1 cm^2 = 8.1 mg).

Both the 1A agonists produced significant dose-response related increases in the sensitivity of all three spinal reflexes. The following ED_{50}'s were obtained: ventroflexion - buspirone ED_{50} = 0.15 mg/kg, 8-OHDPAT ED_{50} = 0.30 mg/kg; dorsiflexion - buspirone ED_{50} = 0.03 mg/kg, 8-OHDPAT ED_{50} = 0.019 mg/kg; and lateral flexion - buspirone ED_{50} = 0.015 mg/kg, 8-OHDPAT ED_{50} = 0.017 mg/kg.

An opposite effect of 1B agonists administration on the ventroflexion reflex was observed with a significant dose-response related decrease in sensitivity to noxious stimulation seen at all doses (mCPP ED_{50} = 1.6 mg/kg; TFMPP ED_{50} = 1.06 mg/kg).

The effects of 1B agonists administration on the dorsiflexion and lateral flexion reflexes was variable with the lowest dose (0.1 mg/kg) producing a significant increase in sensitivity followed by a significant dose-response related decrease in nociceptive sensitivity seen at the higher doses.

Selectivity of the above sensory hypersensitivity to spinal 1A receptors was assessed employing the 1A selective blocker spiperone (0.001, 0.01, 0.1 and 1.0 mg/kg). An increase in sensitivity to noxious stimulation was seen following buspirone treatment (2 mg/kg). However, subsequent administration of increasing doses of spiperone resulted in the total blockade of the buspirone effect.

The present study indicates that two days after spinal transection, the ventroflexion reflex is most sensitive at differentiating between 5-HT_{1A} and 5-HT_{1B} agonists. The results also indicate that the two 5-HT_1 receptor subtypes characterized in our receptor binding study reported at this meeting may have opposite effects on spinal pain transmission. (NS07840 and NS18326)

84.18 FUNCTIONAL LOSS OF THE 5-HT_{1B} PRESYNAPTIC RECEPTOR IN SPINAL CORD OF AGED RATS. R.M. Murphy and F.P. Zemlan. Lab. of Geriatric Research, and Dept. of Physiol. and Biophysics, Univ. of Cincinnati College of Medicine, Cinti, OH 45267-0555.

Although certain biochemical changes in the 5-HT system have been identified with the normal aging process, no known functional changes have yet to be examined. The present study reports our initial findings assessing the effects of aging on the functional integrity of the presynaptic 5-HT_{1B} receptor in spinal cord.

Lumbar spinal cord slices (0.4 mm thick) from adult (3 mos.) and aged (18 mos.) rats were incubated for 15 min at 37°C in 5 ml Krebs-Ringer buffer containing 100 nM [^{3}H]5-HT. Two spinal cord slices were transferred to each chamber and superfused with oxygenated medium containing 100 nM fluoxetine, at a rate of 0.5 ml/min. Samples were collected at 3 min intervals. After 60 min, the tissue was exposed to elevated potassium (25 mM K^+) for 6 minutes. Drugs (concentrations: 1 nM - 100 nM) were present throughout the last 20 min of basal efflux and during the 6 min exposure to the elevated K^+. The fractional efflux rate was determined as previously described (Eur. J. Pharm. 63: 179,1982).

The 5-HT_{1B} selective agonist mCPP, and 5-HT produced a significant 25-70% reduction in the K^+-evoked release of [^{3}H]5-HT in adult rats. The 5-HT_{1A} selective agonists 8-OHDPAT and 5-MeODMT produced a dose-dependent increase (5-29%) in K^+-evoked [^{3}H]5-HT release. Only at 100 nM was the increase significant for 8-OHDPAT ($p<.05$).

In aged rats, mCPP and 5-HT, in contrast to the adult rats, resulted in increased (10-50%) K^+-evoked [^{3}H]5-HT release similar to the effect produced by the 5-HT_{1A} agonists in the adult rat.

Additionally, 5-HT (100 nM) resulted in a substantial 50% increase in spontaneous release in aged rats. This is in contrast to no effect of 5-HT or the other drugs on the normal slow decline in basal [^{3}H]5-HT release in adult rats. Possible alterations in the transmembrane sodium gradient in the aged rat may account for these results.

The present study indicates the functional loss of the 5-HT_{1B} presynaptic receptor in the spinal cord of aged rats. Although more extensive studies are required before firm conclusions can be reached, the present study indicates that the serotonin system mediating spinal pain transmission may be compromised in aged rats. (NS07840)

84.19 STIMULATION-PRODUCED ANALGESIA FROM SITES IN THE VENTROLATERAL PONS IS MEDIATED BY A SPINAL ALPHA 2-ADRENERGIC SYSTEM. J.F. Miller & H.K. Proudfit, Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago. IL 60680.

Stimulation of the rat ventrolateral tegmentum (VLPT) produces a potent analgesia, as measured by the tailflick test (Miller & Proudfit, Soc.Neurosci.Abstr., 10: 201, 1984). The present experiments demonstrate that the stimulation-produced analgesia (SPA) obtained from VLPT sites is mediated by a spinal alpha 2-noradrenergic system.

Adult female Sprague-Dawley rats were implanted with both a unilateral bipolar stimulating electrode in the VLPT (AP:+0.4 mm; L:1.6 mm; V:+1.4 mm) and an intrathecal catheter ending in the lumbar subarachnoid space. Following a 4-5 day recovery period, baseline tailflick latencies (TFLs) were determined and the rats were screened for analgesic VLPT sites. Stimulation parameters were: 60 Hz, 0.1 msec square wave pulses, 50-300 uA. In the first experiment, rats in which VLPT stimulation markedly elevated TFLs then received an intrathecal microinjection of either the alpha-adrenergic antagonist phentolamine HCL (30 or 60 ug) or the beta antagonist ±-propranolol HCl (111.6 ug). Control animals received an equal volume microinjection of saline vehicle. In the second experiment, rats with analgesic VLPT placements received intrathecal microinjections of either the alpha 1-selective antagonist WR-4101 (37 ug), the alpha 2-selective antagonist yohimbine (37 ug), or saline. For all animals, the effectiveness of VLPT stimulation in elevating TFLs was again assessed 15, 30, and 60 min after drug injection.

Intrathecal administration of phentolamine was found to antagonize SPA elicited from VLPT sites in a dose-dependent fashion, with the higher dose completely abolishing the analgesic effect of VLPT stimulation. Propranolol, on the other hand, was ineffective in blocking SPA from VLPT sites. An alpha 2 receptor system is indicated, in that yohimbine, but not WR-4101, also attenuated SPA obtained with electrical stimulation of VLPT sites.

Immunohistochemical staining of a small number of brains from the first experiment for dopamine-beta-hydroxylase (DBH) showed the electrode placements to lie medial to DBH-immunoreactive neurons in the rostral pons located along the border of the lateral lemniscus.

This work was supported by USPHS Grant DA03980.

84.20 ALTERATIONS IN THE ACTIVITY OF DORSAL HORN NEURONS PRODUCED BY ELECTRICAL STIMULATION OF NEURONS IN THE VENTROLATERAL PONTINE TEGMENTUM. F.M. Clark, L.F. Fitzgerald* and H.K. Proudfit Dept. of Pharmacol., Univ. of Ill. at Chicago, Chicago, IL 60680.

Electrical stimulation of the nucleus raphe magnus (NRM) inhibits the firing of spinal cord dorsal horn neurons. Such inhibition is mediated by a direct raphespinal serotonergic projection from the NRM and by a bulbospinal noradrenergic projection from unidentified catecholamine-containing neurons. We have recently demonstrated that electrical stimulation of the ventrolateral pontine tegmentum (VLPT) produces antinociception which is mediated by noradrenergic neurons. These data indicate that the catecholamine bulbospinal neurons in the VLPT may be those neurons activated by electrical stimulation of the NRM. The present study was designed to characterize the effects produced by electrical stimulation of VLPT neurons on the activity of nociceptive dorsal horn neurons.

Female Sprague Dawley rats (250-350 g) were anesthetized with urethane (1.2 g/kg) and body temperature was maintained at 37°C. The right internal carotid artery was cannulated to monitor blood pressure and a laminectomy exposed the lumbar spinal cord. A bipolar stimulating electrode was stereotaxically lowered into the VLPT and recordings were made from dorsal horn neurons with glass microelectrodes. Stimulus parameters were 0.1msec square wave pulses varying in frequency from 100 to 300 Hz. and in intensity from 25 to 400 microamps.

A total of 74 units responsive to mechanical stimulation were isolated. All recorded units were located in the spinal cord dorsal horn in laminae I-VI and their cutaneous receptive fields were ipsilateral and confined to the right lower limb. All units were responsive to either brush and/or pinch with toothed forceps. Once a unit was characterized, the effect of electrical stimulation within the VLPT on the unit's firing characteristics was examined. Systematic electrode penetrations were made through the VLPT just medial to the rubrospinal tract. Of the 44 cells tested, the response to noxious stimulation was increased in 18 cells, inhibited in 17 cells and 9 cells were not affected. All units affected were either responsive to noxious stimulation alone or were responsive to both innocuous and noxious stimuli. Excitation and inhibition were elicited from similar locations in the VLPT.

The results of these studies indicate that stimulation in the VLPT produces both excitation and inhibition of dorsal horn neurons. These findings are consistent with behavioral studies which indicate that both antinociceptive and aversive effects can be elicited by electrical stimulation of various sites in the VLPT. (This work was supported by USPHS Grant DA03980).

84.21 EFFECTS OF NUCLEUS RAPHE MAGNUS STIMULATION ON NOCICEPTION AFTER INTRATHECAL INJECTION OF 5'-N-ETHYLCARBOXAMIDE ADENOSINE. Aran, S. and Proudfit, H.K. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Recently, we have demonstrated that intrathecal injection of norepinephrine (NE) and 5'-N- ethylcarboxamide adenosine (NECA), an adenosine analog, interact synergistically to produce antinociception. The present studies were undertaken to determine whether the release of endogenous NE produced by electrical stimulation of neurons in the nucleus raphe magnus (NRM) also interacts synergistically with intrathecally-injected NECA.

Male Sprague Dawley derived rats (350-400 gm) were implanted with an intrathecal catheter and a bipolar stimulating electrode. The tip of the stimulating electrode was located near the dorsal margin of the NRM. One week after surgery baseline nociception was determined using the tail flick test. After determination of baseline tail flick latencies (TFLs), a stimulus-response curve was generated for electrical stimulation of the NRM (square wave pulses: 100 usec, 50, 100, and 150 uA, 60 Hz) applied for 60 sec. NRM stimulation produced statistically significant increases in TFLs using current intensities of 100 and 150 uA. Animals were then given an intrathecal injection of a subeffective dose of NECA (0.3ug/15ul). TFLs were determined again 30 and 60 min after drug injection and stimulus-response curves were constructed at both time points. This dose of NECA, in the absence of prior NRM stimulation, did not produce any significant changes in TFLs (separate control group). However, TFLs were increased significantly by NECA when it was injected after NRM stimulation. Although NRM stimulation produced significant antinociception before the injection of NECA, such stimulation failed to further increase TFLs above the elevated TFL values observed after the injection of NECA.

To determine whether NE was involved in producing the antinociception observed following NECA and NRM stimulation the alpha-noradrenergic antagonist phentolamine (PHE) was injected intrathecally. The elevated baseline TFL produced by NECA, which was observed following NRM stimulation, was reversed by PHE.

These data suggest that endogenous NE, released in the spinal cord by electrical stimulation of NRM neurons, potentiates the antinociceptive effects of the adenosine agonist NECA. (This work was supported by USPHS Grant DA 03980).

84.22 EVIDENCE FOR THE CHOLINERGIC MEDIATION OF THE ANTINOCICEPTION INDUCED BY ELECTRICAL STIMULATION OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS IN RATS. M.A. McCartney and H.K. Proudfit. Dept of Pharmacology, University of Illinois at Chicago, Chicago, IL 60612.

The nucleus raphe magnus (NRM) and adjacent magnocellular reticular formation have been shown to mediate antinociception. Raphe spinal neurons in the NRM appear to be activated by cholinergic neurons since injection of the cholinergic agonist carbachol into the NRM produces antinociception which can be reversed by the cholinergic muscarinic antagonist atropine. There is also anatomical and electrophysiological evidence for a cholinergic projection to the NRM region from the pedunculopontine tegmental nucleus (PPTg)/ nucleus cuneiformis area. The purpose of our studies was to provide behavioral evidence that the cholinergic projection from the PPTg to the NRM mediates antinociception.

Bipolar stimulating electrodes were implanted into the PPTg area of male Sprague Dawley-derived rats. Guide cannulas for subsequent microinjections were placed in the area of the NRM in a separate group of animals. Upon recovery, baseline nociception was measured using the tail flick test. The PPTg area was stimulated (60 Hz, 25-250 uA, 100 uS square wave pulses) and tail flick response latencies were obtained, both during and 60 sec after the 60 sec period of stimulation. Atropine sulfate was injected, either subcutaneously (1 mg/kg) or directly into the NRM (5 ug in 0.5 ul saline) to determine whether the stimulation-induced antinociception was mediated by cholinergic receptors. Electrical stimulation and testing were repeated after the injection of atropine.

A significant increase in tail flick latency (309%) was observed during the 60 sec stimulation and return to baseline values occurred within 60 sec after the cessation of stimulation. This hypoalgesia was reversed by systemic atropine administration. Atropine administration alone had no effect on baseline tail flick latencies. In addition, preliminary studies indicate that microinjection of atropine into the NRM also reversed the stimulation-induced antinociception.

These studies provide evidence for a cholinergic projection from the PPTg to the NRM that mediates antinociception in rats. This work was supported by USPHS Grant DA 03980.

COMPARATIVE NEUROANATOMY: AMPHIBIANS, REPTILES AND BIRDS

85.1 IMMUNOREACTIVITY OF SOMATOSTATIN, SEROTONIN, SUBSTANCE P, AND ENKEPHALIN IN THE MESENCEPHALON OF RANA PIPIENS. DS Adli, WLR Cruce, and RH Ho. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Department of Anatomy, Ohio State University, Columbus, OH 43210.

Contrasting results regarding the localization of somatostatin (SOM), serotonin (5HT), substance P (SP), and enkephalin (ENK) in the frog CNS have been reported. To investigate this matter, we used the PAP method of Sternberger to localize these neurochemicals in adjacent sections obtained from the mesencephalon of a Ranid frog. Its mesencephalon is made up of (i) the optic tectum, dorsally, (ii) the tegmentum, ventrally, and (iii) the torus semicircularis, in between the two former structures.

Adult northern leopard frogs were fixed by perfusion with Zamboni's solution. Coronal and horizontal sections were cut at a thickness of 30-50 um on a vibratome. The sections were then processed for the localization of somatostatin like (SLI), substance P like (SPLI), serotonin like (5HTLI), and enkephalin like (ELI) immunoreactivities. As controls, adjacent sections were incubated in specific preabsorbed primary antibody serum.

Each neurochemical has a laminar distribution pattern in the optic tectum. Immunoreactivities for all four can be found not only in the region dorsal to layer 6, but also in layers 5 and 3. An intense ELI is seen as a layer close to the dorsal surface. Positively stained cell bodies in layer 6 (ENK, 5HT, and SOM cells) and in layer 4 (SOM cells) have processes projecting towards the dorsal surface of the tectum. In the torus semicircularis where SPLI, 5HTLI, and ELI are present, we only detect SP stained cells.

In the tegmentum, the entire gray (encompassing all four of Potter's fields) is rich with immunoreactivities to ENK, SP, 5HT, and SOM. The densest SPLI in the gray area is in the periventricular region. Cells in the anterodorsal field (AD) and nucleus profundus mesencephali are heavily labelled with ENK containing terminals. ENK cells are found in the anteroventral field (AV) and the ventral posteroventral field (PVV). Within this tegemental region, the ventromedial AV and the dorsolateral part of PV contained SOM cells, as did the dorsal part of nucleus interpeduncularis (NIP). NIP, itself, is rich with 5HTLI, SPLI, and SLI. 5HTLI and SPLI are also present in nucleus isthmi (NI), with the cell plate of NI being heavily labelled for 5HT. Other heavily labelled 5HT cells are located in the dorsal raphe and laterally in the stratum album. Some lightly labelled 5HT cells are also observed medial to red nucleus.

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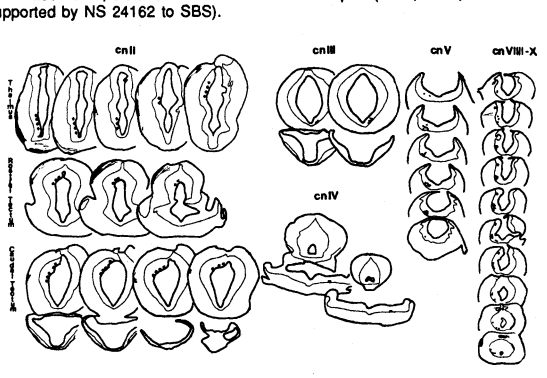
We thank Dr. Elde for the 5HT and ENK antibodies.

85.2 CRANIAL NERVE NUCLEI OF THE SALAMANDER *NOTOPTHALMUS (TRITURUS) VIRIDESCENS*. M.C. Anderson*, B.M. Davis and S.B. Simpson, Jr. (SPON: W. L. Muhlach).Biological Sciences, Univ. of Illinois at Chicago, Chicago, Il,60680

Regeneration of the salamander spinal cord has been studied for over a hundred years because of all adult (i.e. post-developmental) vertebrates, salamander shows the most robust recovery following major spinal lesions. Despite its long history as a model for regeneration we still do not know the source of regenerated spinal axons and neurons. Recently our laboratory has begun examining these issues to provide a foundation for future experiments at the cellular and molecular level. Our first experiments examined the regenerated bulbospinal projection to the lumbar spinal cord following a complete thoracic transection. While significant regeneration of bulbospinal axons was seen (in some case > 50%), it was difficult to identify the various regions of the salamander brain which were participating in the regeneration process. Unlike other vertebrates the salamander brain contains no discrete nuclei or identifiable landmarks. All somata lie in layers next to the brain ventricles with no apparent organization. The cells are surrounded by white matter which varies in thickness. Since no brain atlases exist, HRP was used to backfill cranial nerves. In this way cranial nerve motor nuclei and sensory terminations were identified as a first step in providing an atlas for the salamander brain. These results show that despite the lack of apparent organization the cranial motor nuclei are present in discrete groups. The overall pattern of cranial sensory and motor input suggests that *triturus* contains nuclei homologous to other vertebrates brain nuclei. CN=cranial nerve, __ = HRP filled somata, __ = putative sensory terminals, fine lines = HRP filled fibers, distance between sections =100µm. (MCA, BMD, SBS supported by NS 24162 to SBS).



85.3 MUSCULOTOPIC ORGANIZATION OF THE HYPOGLOSSAL NUCLEUS IN THE GRASS FROG (Rana pipiens). A. Sokoloff, Biological Anthropology, Harvard University, Cambridge, MA 02138.

Recent tracer studies in the rat and cat have demonstrated that specific tongue muscles are innervated by discrete groups of neurons in the hypoglossal nucleus (HGN). Neurons innervating the styloglossus and hyoglossus muscles are located in dorsal or dorsolateral HGN regions, neurons innervating the genioglossus muscle are located in ventral HGN regions, and neurons innervating the geniohyoid muscle are located in a cell column lying ventral to the main body of the HGN. The tongue of the grass frog differs structurally and functionally from the tongue of mammals. To determine the extent of musculotopy in the ranid HGN and to provide a comparative basis for understanding evolutionary changes in HGN organization, the innervation of tongue musculature in the grass frog was investigated.

Discrete injections of the retrograde tracer WGA-HRP were made into tongue and geniohyoid muscles of twenty six frogs. Tongue, brainstem and cervical spinal cord were removed, sectioned and processed for peroxidase with tetramethyl benzidine. Injections into the hyoglossus muscle label neurons in dorsal HGN regions. Labeled neurons are present from caudal HGN levels to the rostral pole of the HGN but are not observed in the caudal pole of the HGN. Injections into the genioglossus basalis muscle label neurons in ventral and lateral nucleus regions at caudal and middle HGN levels. Injections into the genioglossus medialis muscle label neurons in dorsal regions at caudal levels, throughout the nucleus at middle HGN levels, and in ventral regions at rostral HGN levels but do not label neurons in the rostral pole of the HGN. Injections into the geniohyoid muscle label neurons in a column lying ventral to the main body of the HGN. Neurons labeled with geniohyoid injections are present from middle to rostral nucleus levels.

These results demonstrate that the musculotopic organization of the HGN of Rana pipiens is similar to the general pattern of HGN organization described for mammals. Such similarity suggests the possibility that the spatial organization of HGN motoneurons has been conserved in evolution. In light of the structural and functional differences in the tongues of ranids and mammals, conservation of HGN organization suggests that evolutionary divergence of tongue function might be due more to alterations in the muscular architecture of the tongue than to changes in the pattern of HGN organization.

Supported by NSF Graduate Fellowship.

85.4 FMRFamide-IMMUNOREACTIVE NEURONS IN THE TERMINAL NERVE AND BRAIN OF AMPHIBIANS EXHIBIT SEASONAL DIFFERENCES. L.E. Muske and F.L. Morre. Zoology Department, Oregon State Univ., Corvallis, OR 97331

The terminal nerve (TN), a ganglionated nerve associated with the olfactory system, is present in most vertebrate classes. Neurons immunoreactive (ir) to gonadotropin-releasing hormone (LHRH) have been identified in the TN of teleosts, elasmobranchs, mammals and amphibians. Immunoreactivity to the molluscan tetrapeptide FMRFamide and to other neuroactive substances has been found in the TN of fishes, but except for LHRH-ir, there is no immunocytochemical information on the TN in other taxa. We used immunocytochemical techniques and a variety of antibodies to examine the brains and olfactory systems of three representative amphibians. Immunoreactivity was visualized using avidin/biotin immunoperoxidase (Vector Labs) or immunofluorescent techniques.

In the tree frog Hyla regilla, antisera to FMRFamide (Dockray LI35, Bishop/O'Donohue 231) labeled TN fibers throughout distal olfactory and vomeronasal nerves, and TN fibers and perikarya extending from the proximal olfactory nerves to the anterior commissure. The distribution of FMRFamide-ir closely resembles that of LHRH. Double labeling studies indicate, however, that FMRFamide- and LHRH-ir TN neurons comprise separate populations. In Rana pipiens, a few FMRFamide-ir fibers, possibly belonging to the TN, were found in the olfactory nerve. FMRFamide-ir was never found in the olfactory system or TN of the urodele Taricha granulosa.

Immunoreactivity to Substance P (SP), pancreatic polypeptides and tyrosine hydroxylase (TH) has been identified in the TN of fishes. Antisera to these substances (SP: Immunonuclear Corp.; NPY: Polak 1101, Terenius 102B; TH: Tank/Weiner 16) produced labeling in the brain, but not in the TN of amphibians.

In all three species, anti-FMRFamide also labeled periventricular cell bodies in the caudal preoptic area (POA) and dense fiber network throughout the brain. In Hyla and Taricha, FMRFamide-ir neurons exhibited differential staining associated with the seasonal cycle: Staining in the POA was more intense, and more TN neurons were visualized in breeding, compared with non-breeding individuals. The presence of FMRFamide-ir in the TN, a structure that has been implicated in reproductive behaivor, and the seasonally-related differences in FMRFamide-ir label in amphibians, suggest a possible role for this substance in reproduction or related behaviors.

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85.5 ASCENDING PROJECTIONS OF THE TORUS SEMICIRCULARIS IN RANID FROGS. T. J. Neary. Anatomy Dept., Creighton University, Omaha, NE 68178.

Wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) was applied into the midbrain auditory center (torus semicircularis) of bullfrogs (Rana catesbeiana) under tricaine anesthesia. The frogs survived 4 days and WGA-HRP was visualized using tetramethyl benzidine. After WGA-HRP applications in the medial or auditory torus, labelled ascending fibers were followed ipsilaterally in two bundles, one passing through the hilus of the torus ("hilar pathway") and the other coursing just medial to the ventral optic tract ("ventrolateral (VL) pathway"). Fibers continuously left the hilar pathway laterally to course through and apparently terminate in tectal laminae 3 and 5. Several of these fibers passed through the tectum to enter and terminate in the pretoral grey. The hilar pathway ended in a periventricular system of fibers running in the cell-free zone between the dorsal and ventral thalamus. The VL pathway ascended through the mesencephalon and eventually joined the lateral forebrain bundle (LFB). Fibers from the VL pathway contributed to light terminal fields in the following thalamic nuclei: posterior, ventromedial (VM), ventrolateral (ventral part, VLv) and anterior. They also formed a light terminal field laterally adjacent to the posterior nucleus and possibly another in the posteroventral part of the lateral nucleus. VL pathway fibers terminated heavily in the ventromedial third to one-half of the central thalamic nucleus and moderately in the rest of the central nucleus and anterior division of the lateral nucleus (La). A few toral fibers continued with the LFB to enter the striatum, while a few others left the LFB to join the medial forebrain bundle and enter the caudal septum. Contralateral toral projections were observed to the anterior and central nuclei, and La in the thalamus, and to the pretoral grey, optic tectum, and opposite torus in the midbrain.

All applications in the lateral, somatosensory torus have, so far, spread into the optic tectum. The pattern of projections seen in these cases was similar to that described for medial toral applications with the following major differences: 1) projections to the neuropil adjacent to La, the nucleus of Bellonci, the corpus geniculatum, and the lateral pretectum were present and these can be attributed to tectal involvement (Scalia, 1976); 2) projections to the medial part of the central nucleus were reduced; 3) projections to VM and VLv were increased. This last difference is notable in view of the presumed somatosensory projection to VM and VLv from the obex region (Neary and Wilczynski, 1977) for it suggests a possible convergence of somatosensory information from the obex and midbrain upon these two ventral thalamic nuclei.

Supported by NSF Grant BNS-7924699 and BRSG grants to Creighton University.

85.6 MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL EVIDENCE FOR A RETINOPETAL PROJECTION IN ANURAN AMPHIBIANS (RANA CATESBIANA AND XENOPUS LAEVIS). H. Uchiyama*, T.A. Reh° and W.K. Stell, Dept. Anat. and Lions' Sight Centre, and °Dept. Med. Physiol., Univ. Calgary, Calgary, Alberta, Canada T2N 4N1

Retinopetal neurons (neurons efferent from the brain to retina) have been shown in most vertebrates by means of modern neuroanatomical tract tracing methods. However, in anuran amphibians, their existence is controversial. Although some researchers have suggested their presence by physiological data, Scalia and Teitelbaum (1978) denied it in frogs and toads judging from failure of brain neurons to label retrogradely after intraocular HRP injection. In this study, we describe centrifugal fibers from the brain to the retina and tentatively identify the cells of origin by means of immunocytochemical and HRP tracing methods.

Both tadpoles and adults of Rana catesbiana and Xenopus laevis were used. For immunocytochemistry, we used three antisera; anti-FMRFamide (Fa) (O'Donohue 231), anti-LHRH (Sherwood GF4) and anti-substance P (SP)(INC lot 8352022). This SP antiserum has been shown to recognize N-terminal sequence around amino acid (3-7) of SP after preabsorption with SP fragment (7-11) (Stell et al., 1987). The pattern of immunoreactivity with this preabsobed antiserum was almost identical with that of Fa in this study.

We observed identical patterns of Fa-immunoreactive (ir) fibers in the optic nerve and retina in both Rana and Xenopus adults and tadpoles. In the retina, these fibers run in the optic nerve fiber layer, pass through the ganglion cell layer and inner plexiform layer (IPL), run in the junctional layer between the IPL and inner nuclear layer (INL), and terminate at the innermost sublayer of the INL. We found inconsistently a few weakly Fa-ir amacrine cells, which were not stained with the anti-SP serum, in contrast to the Fa-ir fibers. We observed no LHRH-ir fibers in the retina or optic nerve of either tadpoles or adults. Near the entrance to the brain the Fa-ir fibers in the optic nerve make a tight bundle which can be traced rostrally in the ipsilateral preoptic area to the septo-preoptic junctional area. Seven days after optic nerve crush, the Fa-ir fibers disappeared, distal but not proximal to the crush. Fa-ir perikarya were distributed similarly in both Rana and Xenopus brain. They were seen in (a) diagonal band of Brocha, (b) median septal nucleus (MSN), (c) bed nucleus of anterior commissure (bnAC) of Hoffman, (d) anterior preoptic area, and (e) caudal portion of periventricular preoptic nucleus (cPPN). In adults, the distribution pattern of LHRH-ir perikarya overlapped with that of Fa-ir perikarya in most nuclei. However, large bipolar or multipolar neurons in bnAC and granular neurons in cPPN, like the fibers in the retina and optic nerve, were immunoreactive for Fa and SP(3-7), but not LHRH.

These immunocytochemical data may suggest that neurons in bnAC are the origin of Fa-ir centrifugal fibers to the retina. We were not able to label these neurons (or any others) retrogradely by injecting markers intraocularly. But by HRP injection into the septum and anterior preoptic area of adult Rana, we labeled fibers that run caudally in the preoptic area and enter the ipsilateral optic nerve. This may confirm the Fa-ir retinopetal pathway suggested by the immunocytochemical data.

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85.7 COEXISTENCE OF SUBSTANCE P WITH LEU-ENKEPHALIN OR CALCITONIN GENE - RELATED PEPTIDE IMMUNOREACTIVITIES IN THE AMPHIBIAN CNS.

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Co-localization studies for neuroactive substances were undertaken as a new tool for comparative analysis of the vertebrate CNS.

Employing symultaneous double immunofluorescence staining (for primary antibodies raised in different species), the distribution of Substance P (SP) immunoreactivity (IR) was compared with other reaction patterns for neuropeptides (in particular for Leu-Enkephalin - Leu-Enk - and Calcitonin Gene-Related Peptide - CGRP -) in the untreated CNS of two representatives of Amphibia (Triturus cristatus, Urodela; Rana esculenta, Anura).

Leu-Enk-IR appeared co-distributed with SP-IR in several areas of the amphibian brain and in particular in the dorsal and tuberal hypothalamus, preoptic area, striatum, rhomboencephalon. Moreover, the coexistence of the two immunoreactivities was observed in some fibres and neurons in the preoptic area, tuberal and dorsal hypothalamus. CGRP-IR was co-localized with SP-IR in the primary sensory afferent fibres of the spinal cord and of some cranial nerves. In the outer zone of the neurohypophysial median eminence and in the posterior hypothalamus (frog), CGRP-IR and SP-IR appeared co-distributed, but not coexisting.

Computer graphic representation and semiquantitative evaluation of coexistence were performed by interactive image analysis.

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85.8 THE ACCESSORY OPTIC SYSTEM IN A LIZARD.

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The accessory optic system of Anolis carolinensis was investigated using both HRP histochemistry and the Golgi technique. The afferents to accessory optic nucleus of the basal optic root (nBOR) were determined by the retrograde transport of HRP from the nucleus. The retinal afferents to nBOR consisted of large diameter axons up to 3um in diameter. Other afferents included the ventrolateral portion of the corpus striatum, the anterior portions of the corpus geniculatum lateralis ventralis and in the pretectal region the nucleus lentiformis mesencephali and the nucleus geniculatum pretectalis. The efferent projections of nBOR were to the contralateral nBOR, the nucleus lentiformis mesencephali as well as bilateral projections to the oculomotor nuclei, the nucleus interstitialis, the cerebellar cortex and the inferior olivary nucleus.

The cells in nBOR were extremely variable in size and shape. Cell counts through the nucleus indicate an average of 2400 cells in nBOR. Of these cells 35% were confirmed as neurons by HRP histochemistry. The remaining population appear to be glia or interneurons. Retrograde labelling indicated that the largest neurons 10-20um in diameter and the medium elongated neurons 10 by 5um contributed the the projections to the cerebellum, inferior olivary nucleus and commissural connections. The cells projecting to the oculomotor and interstitial nuclei were all of the elongate type. The dendritic fields of nBOR neurons were small and restricted to the nucleus. The large neurons often had dendrites no more then 20um in length.

Comparing the present results with our earlier analysis of the nucleus of the basal optic root in Rana pipiens we find an increase in the number of cells and the diversity of cell types. The nBOR of lizard also shows smaller dendritic fields then that frogs. The connectivity of the two systems is remarkably similar. The major differences appear to be the connections with the striatum and cerebellum in Anolis. Finally, in Rana pipiens the oculomotor neurons send dendrites directly into nBOR. In Anolis, the oculomotor neurons dendritic field is smaller (like that of nBOR neurons) and does not enter nBOR.

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85.9 MIDBRAIN AND SPINAL PROJECTING NEURONS OF REPTILIAN DORSAL COLUMN NUCLEUS.

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Reptiles, like mammals, possess a prominent dorsal column nucleus (DCN). In mammals, the DCN is a heterogenous area in terms of its input, output, and intrinsic organization. In Caiman crocodilus, the only non-mammalian amniote in whom the neural circuitry of the dorsal column system has been investigated from periphery to the telencephalon, the DCN projects to three main targets: the spinal cord, the cerebellum, and the midbrain somatosensory recipient area. We investigated the relay cell populations in the DCN of Caiman that project to two of these regions: the spinal cord and the midbrain somatosensory recipient area.

The origin of spinal cord projecting DCN neurons was determined by application of horseradish peroxidase (HRP) crystals to cut fibers after myelotomy in 7 animals undergoing a 1 to 3 level cervical laminectomy. The source of midbrain projecting DCN cells was investigated by HRP injections into the midbrain somatosensory recipient area by a transtectal approach in 11 animals. In each experiment, tissue was processed by standard neurohistochemical techniques using tetramethylbenzidine as the chromogen.

Retrogradely labeled DCN neurons observed after spinal injections were located ipsilaterally in the posterior one-half of the DCN, immediately ventral to the main body of the DCN, as determined by the latter's input from the dorsal funiculus. In favorable preparations apical dendrites were visualized extending into the main DCN in close relationship with incoming dorsal funicular axons. On the other hand, retrogradely labeled DCN cells observed after HRP injections of the midbrain somatosensory recipient area were located contralaterally in the posterior one-half of the main body of the DCN. Favorable retrograde DCN labeling demonstrated a variety of cell shapes and dendritic morphology. No DCN cells in the rostral half of the nucleus were observed after either spinal or midbrain injections.

The results of these 2 experiments suggest that DCN relay cells that project to the spinal cord and midbrain somatosensory recipient area originate from separate neuronal populations. Furthermore, the locus of these 2 separate DCN relay cell populations in Caiman are similar in location to spinal and mesencephalic projecting DCN cells in cat. These latter findings coupled with previous studies on the neural circuitry of ascending somatosensory systems in Caiman suggest that not only are these somatosensory neuronal circuits and nuclear groups phylogenetically ancient, but that individual relay cell populations within the DCN have arisen early in evolution.

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85.10 QUANTITATIVE REGIONAL VARIATIONS OF NEUROTRANSMITTER MARKERS IN AMNIOTE TELENCEPHALON.

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Neurotransmitter histochemistry has been used in comparative neurology to establish identity or diversity among presumedly homologous brain areas. Following the concept of chemical neuroanatomy,we have undertaken a study on the levels of neurotransmitter markers in telencephalic regions of turtle,pigeon and rat. Brain samples were microdissected from fresh telencephalic slices and the levels of cholinergic,GABAergic and excitatory amino acidic markers were assayed. Independently from the expected variability in absolute levels of neurotransmitter markers in the different species,some common patterns could be noticed. In basal telencephalon,highest GABAergic levels were measured in the paleostriatum followed by the nucleus accumbens and neostriatum;cholinergic activity was highest in the neostriatum,the difference with the paleostriatum being small in turtle and pigeon and large in rat;the medial septum-diagonal band complex consistently showed higher cholinergic and GABAergic levels than the lateral septum;high affinity D-3H aspartate uptake was higher in the neostriatum than in the paleostriatum,the difference increasing enormously in rat. Among cortical and cortical-equivalent areas,D-3H aspartate uptake was higher than in basal telencephalon with the exception of the rat neostriatum;the dorsal ventricular ridge (DVR) of turtle and pigeon showed uptake levels close to those of cortical structures;GABAergic activity was 20-40% of the level in the paleostriatum. Cholinergic activity in cortical and cortical-equivalent areas showed a large variability:it was 11-17% of the neostriatal value in turtle cortices,19% in the rat neocortex,26% in the rat archicortex and 47% in the paleocortex,30% in the avian Wulst,51-55% in the turtle DVR and from 3 to 30% in different areas of the pigeon DVR. The variability in the relative levels of neurotransmitter markers may reflect modifications of neuronal connectivity and/or intrinsic neuronal populations. For instance,the dramatic increase of uptake in the mammalian neostriatum is likely due to the massive glutamatergic input from the neocortex; on the other hand the increased difference between cholinergic levels in the neo and paleostriatum may be related to the increase of cholinergic interneurons in mammalian neostriatum,possibly associated with loss of cholinergic neurons (either intrinsic or extrinsic in the paleostriatum. Ultimately,these evolutionary patterns may result from plastic brain responses to different selective pressure.

85.11 COMPARATIVE ASPECTS OF THE TELENCEPHALIC CORTEX IN LIZARDS: PUTATIVE HOMOLOGIES WITH THE MAMMALIAN HIPPOCAMPUS.
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The cerebral cortex of lizards consists of medial, dorsomedial, dorsal and lateral areas, comprising layers 1 (molecular layer), 2 (principal cell layer), and 3 (polymorphous cell layer).Main cortical afferents are from the septum, thalamus, mamillary bodies and raphe nuclei; the lateral cortex is reached by a projection from the olfactory bulb. The raphe projection is serotonergic and terminates mainly in layers 1 and 3. Cortical efferents course to the septum, thalamus and nucleus accumbens striati. Intracortical fibers pass from the lateral, dorsal and dorsomedial fields to the medial area, which in turn projects to the dorsomedial and dorsal cortices. The latter pathway is characterized by the high content of zinc in its preterminal boutons. Cells in the dorsomedial area and in the adjacent part of the dorsal cortex send fibers to layer 1 of the contralateral medial and dorsomedial areas. Nearly all cells in layers 1 and 3 are non-pyramidal and contain the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Some GABA-positive neurons are found also in layer 2. GABA-immunoreactive presynaptic boutons make symmetric synaptic contacts mainly with the somata and apical dendrites of the principal cells in layer 2.

Together with earlier published data on the cytoarchitecture, cytology and histochemistry of the cerebral cortex of lizards, our results on the cortical lamination, distribution of cell types and patterns of extrinsic and intrinsic fiber connections coincide surprisingly well with the corresponding data that have been reported for the hippocampal formation of the mammalian brain, and support earlier suggestions that areas of the telencephalic cortex may be homologous to areas of the hippocampal formation.

In detail, we find similarities between: the lateral cortex of lizards, and the entorhinal cortex of mammals; the medial cortex of lizards, and the area dentata of mammals; the dorsomedial cortex plus adjacent parts of the dorsal cortex, and the Ammon's horn of mammals.

85.12 AN IMMUNOCYTOCHEMICAL STUDY OF THE OPTIC TECTUM IN RATTLESNAKE.
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The optic tectum in the rattlesnake serves as the principal neural structure for integrating information from visual and infrared modalities. Its layered architecture, the substrate for multisensory integration, has been described, but mainly with conventional cell and fiber stains. With such stains, the tectum is subdivided into SO (stratum opticum: 50-75um thick), SFGSa,b,c (stratum fibrosum et griseum superficiale: 150-200um), SGC (stratum griseum centrale: 100-150um), and SAC (stratum album centrale: 400-500um). Recording sites yielding visual responses predominate in SO and SFGS; those yielding multisensory responses are concentrated in SGC, and those yielding infrared responses predominate in SAC.

We reexamined tectal organization for evidence of further subdivisions, using histochemical and immunoperoxidase techniques. We found immunoreactivity for several substances, in a pattern corresponding only approximately with classical Nissl-based lamination. GAD (glutamic acid decarboxylase) and AChE (acetylcholinesterase) reactivity are concentrated in 3 bands occupying the 250-350 um immediately below the SO: a band dense for GAD and light for AChE, one moderate for GAD and dense for AChE (this includes GAD reactive horizontal processes and small cell bodies), and one moderate for AChE and light for GAD. These 3 bands, exhibiting some complementarity between AChE and GAD, lie within the 4 cytoarchitectonic subdivisions of SFGS and SGC. The central band (dense AChE, moderate GAD) occurs near the border of SFGS and SGC, and also exhibits immunoreactivity that is weak for L-Enk (leu-enkephalin), moderate for neuropeptide Y (NPY), dense for 5-HT (serotonin), and dense for substance P. Substance P-like reactivity extends further into SFGS and SGC than do 5-HT, L-Enk, and NPY. In SAC, our results indicate a different, more diffuse organization. This zone is traversed by varicose fibers showing immunoreactivity for 5-HT, substance P, L-Enk, met-enkephalin, and (weakly) neuropeptide Y, as well as reactivity for AChE. None of these show obvious sublaminar segregation.

In addition to its layered chemoarchitecture, the SFGS/SGC border region exhibits some tangential zonal organization, which is most evident in AChE material. Concentrations of AChE occur in discontinuous islands 50-100um across (ca. 100um center-to-center). Thus, the middle layers (lower SFGS and upper SGC, corresponding to multisensory responses) show significant chemical diversification. Unlike species such as pigeon or frog, however, the rattlesnake seems to have only a modest degree of segregation of different peptide/neurotransmitter candidates in SFGS and SGC; most of those screened to date are compressed into a 100-150um wide zone that straddles the SFGS/SGC border. (supported by NSF BNS 8015839)

85.13 SUPRASPINAL CONNECTIONS OF A NECK MOTOR NUCLEUS (SSp) IN THE PIGEON.
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The pigeon's collimotor system mediates both the control of head stabilization and the transport of a prehensile effector organ: the beak. We have identified supraspinal connections of neurons in the most rostral (C-1) extension of the neck motor column, the nucleus supraspinalis (SSp), following WGA-HRP injections covering this area.

Retrogradely labeled cells were observed (**1**) in various parts of the vestibular nuclei, (**2**) in the abducens nucleus and the medial reticular formation medially and ventrally adjoining this nucleus, (**3**) in the pre-/perihypoglossal region and (**4**) in the interstitial nucleus of Cajal. Vestibular nuclei containing clusters of retrogradely labeled cells included (**i**) the main (lateral) part of the tangential nucleus and the entire dorsolateral nucleus (VDL) contralaterally, (**ii**) the rostral part of the descending nucleus and the adjacent part of the medial nucleus (VeD/VeM: the "accessory" region) bilaterally, and (**iii**) the rostromedial part of the superior nucleus (VeS: group "A" of Wold) ipsilaterally. Scattered labeled cells were found in the lateral aspects of VeS and in the more caudal portions of VeD.

The distribution of anterogradely transported label observed after WGA-HRP injections into these putative premotor regions suggests that the vestibular region is the major source of axosomatic projections upon SSp. With the exception of the (lateral) tangential nucleus (the avian interstitial nucleus of the vestibular nerve), all vestibulomotor clusters projecting to SSp receive direct cerebellar corticovestibular projections (Arends, *Soc. Neurosci. Abs.*,1985; **11**: 692). With the exception of VDL, these vestibular regions also project to the midbrain oculomotor complex, presumably by way of collaterals. The remaining corticovestibular target nuclei —the infracerebellar nucleus and the medial part of the tangential nucleus— project exclusively upon the oculomotor complex and do not contribute afferents to SSp.

Although no retrogradely labeled neurons were observed among the giant cells of the lateral vestibular nucleus (VeL) following HRP injections covering SSp, collateral projections upon SSp were seen to emerge from ipsilateral lateral vestibulospinal tract (LVST) fibers after WGA-HRP injections involving VDL, VeL and extending into the subjacent border region of VeS, VeD and VeM. This finding indicates that some of the ipsilaterally projecting vestibulocollic neurons do so by way of the LVST rather than via the MVST, which contains the bulk of the vestibulospinal fibers from areas other than VeL.

The limited caudal extent (C1-2) of the bulk of direct vestibulocollic connections other than those originating in VeL (Cabot et al., *Progr. Brain Res.*,1982, **57**: 79-108 [pigeon]; Gross & Oppenheim, *J.comp.Neurol.*,1985, **232**: 162-179 [chicken]), taken together with their collateral projections upon the oculomotor system, suggest that SSp plays a major role in head stabilization subserving gaze control.

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85.14 STEREOTAXIC MAPPING OF VISUAL AND MONOAMINERGIC PATHWAYS IN THE HOUSE SPARROW BRAIN.
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The distribution of primary visual pathways, indoleaminergic and catecholaminergic cells and fibers were studied within the framework of a stereotaxic analysis of the house sparrow (Passer domesticus) brain. Adult sparrows (3 males, 3 females) were anaesthetized and received intraocular injections of 5 ug cholera toxin conjugated with horseradish peroxidase (CTHRP) in 5 ul 1% DMSO. After 48 hrs, sparrows were anaesthetized again and placed in a stereotaxic instrument while a series of bilateral needle tracks were placed in the brain. Sparrows were then perfused transcardially with a modified Zamboni's fixative. Brains were processed for CTHRP histochemistry, and immunohistochemistry localization of serotonin (5HT-LI) and tyrosine hydroxylase (TH-LI)cells and fibers.

Major visual projections were observed in the visual suprachiasmatic nucleus (vSCN),nucleus lateralis anterior (LA), ventral lateral geniculate (GLv) and the dorsolateral nuclei (DL) in the diencephalon, and in the mesencephalic lentiform nucleus (LM), ectomammilary nucleus (EM) and optic tectum (Te) in the mesencephalon. Large numbers of small retrogradely labeled cells were also observed in the isthmooptic nucleus (IO).

Diencephalic 5HT-LI cells were observed in the organum periventricularum (PVO), but most cells were observed in mesencephalic and metencephalic nuclei, primarily in the nucleus centralis superior (CS), tegmentum ventralis (TV), and nucleus annularis (NA). Scattered cells were observed in area ventralis (AVT), tegmentum pedunculopontis(TPc) and the substantia griseum centralis (GCT). Dense fiber distributions were observed throughout the telencephalon with the conspicuous exception of nucleus basalis (B). In the diencephalon, fibers were widely distributed but concentrated in the organum vasculosum lamina terminalis (OVLT), vSCN, nucleus paraventricularum magnocellularis (PVM), GLv and median eminence (ME). Fibers were also present in several layers of the Te.

TH-LI cells were widely distributed in the telencephalic lateral septal nuclei (LS), diencephalic preoptic area (PA), lateral hypothalamic area (LH), periventricular preoptic nucleus (PPM), posteriomedial nucleus (PMH) and nucleus tuberis (NT). Mesencephalic TH-LI cells could be divided into a dorsal tegmental group and a ventral tegmental group. The dorsal group included the GCT and locus coeruleus (LC). The ventral tegmental group was more extensive with cells in the AVT, TPc, nucleus pontis reticularis (RPO) and nucleus pontis lateralis (PL). Major TH-LI fiber distributions included the lo bus paraolfactorius (LPO), septum, paleostriatum, OVLT, PVM, PPM, ME and cerebellum.

These data will be analyzed and compared to existing immunohistochemical analyses of dopamine-B-hydroxylase and phenylethanolamine N-methyltransferase distributions. Supported by NSF 85-19660

85.15 EFFERENT PROJECTIONS OF THE PARABRACHIAL NUCLEI IN THE PIGEON. J.M. Wild, J.J.A. Arends and H.P.Zeigler. Department of Anatomy, University of Auckland, Auckland, New Zealand, and Biopsychology Program, Hunter College, New York City, N.Y., U.S.A.

The avian central viscerosensory system comprises the nucleus of the solitary tract (nTS) and a variety of brainstem, diencephalic and basal telencephalic nuclear targets of nTS, in particular the parabrachial nuclear complex (PB) of the dorsolateral pons (Wild, J.M. et al., Soc. Neurosci. Abstr., 11:1309, 1985). In order to determine the subsequent projections of PB, injections of either wheatgerm-agglutinin conjugated horseradish peroxidase (WGA-HRP) or tritiated amino acids were made into PB and surrounding regions and the projections charted following conventional histochemistry or autoradiography. Caudal to PB an extensive terminal field was observed throughout the medullary parvocellular reticular formation (Rpc) and the dorsal part of the central nucleus (Cnd) including the region of the nucleus ambiguus. A particularly dense and localized terminal field occupied the whole extent of the hypoglossal nucleus (Wild, J.M. and Arends, J.J.A., Brain Res., 407:191, 1987) but projections to nTS and DMN X were inconspicuous. Rostral to PB terminal fields were observed within many (but not all) of the adjacent coerulean nuclei (Kitt, C.A. and Brauth, S.E., J. comp. Neurol., 247:69, 1986)eg strata cellulare externus and internus (SCE, SCI) of the hypothalamus, dorsal thalamus, "ventral paleostriatum" (VP) and nucleus accumbens (Ac), and archistriatum (A). WGA-HRP injections into these and other regions of the forebrain and brainstem retrogradely labelled PB neurons and suggested the following pattern of projections from PB subnuclei: The ventrolateral PB subnucleus provides the major descending projection to the ventrolateral medulla and exclusively to nXII. The dorsolateral, dorsal and medial subnuclei largely project rostrally to SCI, SCE and rostral PVM of the hypothalamus, various dorsal thalamic nuclei, VP, Ac, the nucleus of the anterior pallial commissure (nCPa), and A. Since VP, Ac and nCPa also receive projections from nTS, and since VP also projects to PB and nTS, these three nuclei may form part of the bird's "visceral forebrain". In particular, some components of VP seem more appropriately comparable with the bed nucleus of the stria terminalis of mammals. In summary, the avian PB, like its mammalian counterpart, is a major center for the integration and distribution of visceral, and possibly gustatory, information.

PRESYNAPTIC MECHANISMS I

86.1 EVIDENCE THAT ACETYLCHOLINE RELEASE IS MEDIATED BY A PRESYNAPTIC MEMBRANE PROTEIN. Morel N, Israel M.*, Manaranche R.* and Lesbats B.*
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A protein (the mediatophore) which mediates the calcium dependent release of acetylcholine from proteoliposomes has been purified from the presynaptic plasma membrane. Removal of associated lipids inactivated the protein which became water soluble ; this permitted to evaluate its Stokes radius (53 A), its sedimentation coefficient (9.8 S) and hence an approximate molecular weight of 210 KDa. Electrophoretic methods showed that the protein is made of 17 KDa subunits, not linked by disulfide bonds. When observed after negative staining, it has an average diameter of 8 nm and seems pentameric (Israel et al, 1986, PNAS 83, 9226).

Proteoliposomes equipped with purified mediatophore showed a calcium dependent activation and a calcium dependent "fatigue" of ACh release similar to that of synaptosomes (Israel et al, 1987, J.Neurochem.in press).

The drug Cetiedil which inhibits acetylcholine release from intact tissue and synaptosomes by acting after the calcium entry step, was able to block with the same Ki the release mediated by the purified mediatophore (Morot-Gaudry Talarmain Y. et al, 1987, J. Neurochem., in press).

An antibody to the purified protein was raised in rabbits. By indirect immunofluorescence, it stained nerve terminals at motor end plates in Torpedo and rat.

86.2 **CONCENTRATIONS OF SYNAPTIC VESICLES AND CALMODULIN IN PRESYNAPTIC NERVE TERMINALS OF ELECTRIC RAY ELECTRIC ORGAN.** G. P. Miljanich and T. I. Prigozy*. Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089.

For both in vitro reconstitution and detailed modeling of various presynaptic functions, it is necessary to determine the relative and molar concentrations of the requisite presynaptic components. Toward that end, the molar concentrations of synaptic vesicles and the calcium-binding regulatory protein, calmodulin, have been determined in electric organ nerve terminals.

Two previously characterized monoclonal antibodies, which bind specifically to epitopes on two different integral proteins in the synaptic vesicle membrane (Carlson and Kelly, 1983; Buckley and Kelly, 1985), were used to assay the vesicle content of immunopurified electric organ synaptosomes (Miljanich, Brasier, and Kelly, 1982). With highly purified electric organ synaptic vesicles serving as a standard, synaptic vesicle protein was found to represent 19.4 $\pm$ 2.5% of the total nerve terminal protein. Scanning densitometry of electrophoretically separated nerve terminal proteins was employed to determine the calmodulin content of this nerve terminal preparation. Using affinity purified electric organ calmodulin as a standard, and measuring the integrated difference between densitometric scans of electrophoretograms run in the presence and in the absence of calcium, calmodulin was found to represent 7.1 $\pm$ 0.9% of the total nerve terminal protein. Applying the known protein particle weight of synaptic vesicles and the molecular weight of calmodulin in conjunction with the percentages of total synaptosomal protein for the two components, the molar ratio of calmodulin to synaptic vesicles was calculated to be approximately 90 to 1. This ratio includes an indeterminant number of vesicle membrane "equivalents" which are incorporated in the presynaptic plasma membrane and which are also detected by the anti-synaptic vesicle antibodies.

Morphometric analysis of micrographs of intact electric organ revealed that at least 11% of the total volume of nerve terminals is synaptic vesicle volume. Assuming that immunopurified synaptosomes are representative of intact nerve terminals, and using the above volume percentage in conjunction with the known volume of synaptic vesicles, the effective concentration of synaptic vesicles in electric organ nerve terminals was estimated to be at least 0.5uM. With this concentration and the calmodulin:vesicle molar ratio (and assuming that the fraction of vesicle membrane equivalents inserted into the presynaptic plasma membrane is negligible), the average concentration of calmodulin in the terminal is roughly 45uM.

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86.3 SYNAPTOPHYSIN-CONTAINING VESICLES FROM RAT BRAIN AND PC 12 CELLS: A COMPARISON. H. Rehm*, B. Wiedenmann*, and C.-M. Becker* (SPON: W. Huttner). ZMBH and Department of internal medicine, University of Heidelberg, FRG.

Synaptophysin was initially discovered as a major Ca^2 - binding membrane protein of synaptic vesicles from brain (Wiedenmann, B., and Franke, W. Cell,. 41:1017, 1985; Jahn et al. PNAS, 82:4137-4141, 1985; Rehm et al. EMBO Journal, 5:535-541, 1986). Subsequently synaptophysin containing vesicles were also found in endocrine organs like the islets of Langerhans and the adrenal medulla or in tumors derived from these organs like the PC 12 cell line (Wiedenmann et al. PNAS, 83:3500, 1986; Navone et al. J. Cell Biol., 103:2511-1527, 1986)

The function of synaptophysin is unknown. The PC 12 cell line could be used for experiments designed to address this question. We therefore investigated whether the synaptophysin containing vesicles from brain and PC 12 cells are of similar type. To facilitate this analysis we have developed a simple dot immunoassay which allows a quick, specific and sensitive determination of synaptophysin. Synaptophysin containing vesicles from brain and PC 12 cells were compared by physical methods like density gradient centrifugation on Percoll and size exclusion chromatography on Sephacryl S-500 and controlled pore glass columns. For further analysis an immunoprecipitation method was established which allowed a preliminary investigation of the protein composition of these vesicles.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 317) and the Bundesministerium für Forschung und Technologie (BCT 381-5).

86.4 PRODUCTION OF PROTEIN III-SPECIFIC ANTIBODIES. M.D. Browning and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University. New York, N.Y. 10021.

Acid extracts of all rat brain regions contain four prominent phosphoproteins: synapsin Ia (M_r 85,000), synapsin Ib (M_r 80,000), protein IIIa (M_r 74,000) and protein IIIb (M_r 55,000). We have previously shown that these four proteins possess significant strutural homology and that all four proteins appear to be co-localized to presynaptic terminals where they are associated with synaptic vesicles. However, previous studies of proteins IIIa and IIIb (collectively referred to as protein III) were hampered by the fact that all polyclonal antibodies raised against protein III exhibited significant cross-reactivity towards synapsin I. In an effort to obtain protein III-specific antibodies, we raised monoclonal antibodies against protein IIIb which had been purified by preparative SDS-PAGE. In some cases, protein III was proteolysed and the synapsin I-like fragments were removed by immunoprecipitaion with synapsin I antibodies and the remaining fragments were conjugated to thyroglobulin for immunization. We have been successful in obtaining a collection of monoclonal antibodies which exhibit a high degree of specificity for protein III in Western blots. One antibody (CII.21) has been utilized to develop a radioimmunoassay (RIA) for protein III that is sensitive to as few as 10 fmol of protein III. In this assay, synapsin I exhibits less than 1% cross-reactivity. We have used this assay in conjunction with an RIA specific for synapsin I to determine the amounts of synapsin I and protein III in various brain regions. In all brain regions examined, except for the olfactory bulb, the molar ratio of synapsin I to protein III was approximately 2:1. However in the peripheral nervous system, the concentration of protein III was higher than that of synapsin I. For example, in the superior cervical ganglion, the molar ratio of synapsin I to protein III was approximately 1:4. We have also succeeded in using antibody CII.21 to affinity purifiy protein III with a 50-fold greater yield than had been obtained with previously used procedures.

86.5 STIMULATION-DEPENDENT PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN RAT CORPUS STRIATAL SYNAPTOSOMES. J.W. Haycock. Department of Biochemistry, Louisiana State University Medical Center, New Orleans, LA 70119.

Stimulation of intact peripheral noradrenergic and adrenergic tissues produces an increase in tyrosine hydroxylase phosphorylation, an activation of tyrosine hydroxylase, and an acceleration of catecholamine biosynthesis. In vitro, several protein kinases can phosphorylate and activate tyrosine hydroxylase purified from pheochromocytoma. Such observations have led to the hypothesis that the phosphorylation of tyrosine hydroxylase underlies the stimulation-dependent regulation of catecholamine biosynthesis. Although tyrosine hydroxylase purified from rat corpus striatum can be phosphorylated and activated by at least one protein kinase, evidence for the phosphorylation of tyrosine hydroxylase in intact brain and/or dopaminergic systems has been lacking. The present studies demonstrate that (1.) tyrosine hydroxylase is phosphorylated in dopaminergic nerve terminals from rat corpus striatum and (2.) agents which increase dopamine release and dopamine biosynthesis also increase the phosphorylation of tyrosine hydroxylase.

In the present studies, rat corpus striatal tissue was dissected and homogenized in sucrose. Synaptosomes were prepared by standard centrifugation techniques, and the synaptosomal preparation was preincubated with $^{32}PO_4$. Aliquots of the synaptosomal preparation were treated with secretagogues (elevated K+, veratridine, A23187), and the tissue was then solubilized with sodium dodecyl sulfate (SDS). Tyrosine hydroxylase was immunoprecipitated with an antibody which recognizes denatured tyrosine hydroxylase, and the immunoprecipitates were subjected to SDS-PAGE and autoradiography.

^{32}P was incorporated into tyrosine hydroxylase in a time-dependent manner, and each of the secretagogues increased the phosphorylation of tyrosine hydroxylase. Unlike systems such as adrenal chromaffin cells, PC12 cells or superior cervical ganglia, synaptosomes present no cell body associated tyrosine hydroxylase. As such, these data indicate that stimulation-dependent regulatory processes exist in brain dopaminergic nerve terminals to alter the phosphorylation state of tyrosine hydroxylase. Furthermore, these data raise the possibility that brain and/or dopaminergic tyrosine hydroxylase is regulated by processes similar to those previously described for peripheral adrenergic/noradrenergic systems. Studies comparing the site-specificity of tyrosine hydroxylase phosphorylation in striatal synaptosomes and adrenal chromaffin cells are currently being undertaken.

86.6 A SUBTYPE OF NEURONAL PROTEIN KINASE C ACTIVITY WITH UNIQUE SUBSTRATE SPECIFICITY. Phillip J. Robinson, C. K. Black* and W. Lovenberg*. Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

Protein kinase C (PKC) is the Ca^{++} - activated, and phospholipid-dependent protein kinase found in high concentrations in brain. PKC is further stimulated by diacylglycerol (DAG), which increases its affinity for Ca^{++} and phospholipids. In addition, PKC can be activated by tumor-promoting phorbol esters, such as phorbol-12-myristate-13 acetate (PMA), which compete for the same binding site. Recently multiple forms of PKC have been recognised, and although it appears likely, it has not previously been demonstrated that these enzymes possess different substrate specificities. To address this question we have examined the effects of activators and inhibitors of PKC on the phosphorylation of endogenous substrates in rat brain. A cytosolic fraction from rat cortical synaptosomes was the source of both PKC and endogenous substrates. The proteins were labelled by incubation for 60 sec in the presence of [γ-^{32}P]ATP, 30mM tris/HCl pH 7.4, 1mM Mg^{++}, 1mM EGTA, 1mg/ml protein and the activators of specific protein kinases. Phosphoproteins were detected by polyacrylamide gel electrophoresis and autoradiography. In the presence of Ca^{++} and phosphatidyl- serine (Ca^{++}/PS) the phosphorylation of a number of specific proteins was stimulated, notably a 96,000 dalton protein termed P96 and another known substrate for PKC, termed P83. Neither phosphoprotein was phosphorylated by cAMP- or calmodulin-dependent protein kinase. However, in the presence of Ca^{++}, PMA stimulated the phosphorylation of P83, but not of P96, suggesting a limited substrate specificity for PMA-stimulated PKC. PMA activation also occurred in the absence of Ca^{++} (1mM EGTA). These distinct PKC activities were termed PKC1 (for PMA-stimulated) and PKC2 (for Ca^{++}/PS-stimulated) and could be further distinguished with two PKC inhibitors, sphingosine (SPH) and palmitoylcarnitine (PC). SPH more selectively inhibited PKC1 activity towards P83 (IC_{50} 26μM) than PKC2 activity towards P96 (IC_{50} 270μM). In contrast, PC was more selective towards PKC2 labelling of P96 (IC_{50} 96μM) than PKC1 labelling of P83 (IC_{50} 530μM). This is consistent with the known ability of SPH to compete for the phorbol ester binding site and of PC to compete with phospholipid for binding to PKC. Although these data do not yet prove that PKC1 and PKC2 represent distinct isoenzymes of PKC, they show that different activators and inhibitors of PKC can modulate two distinct substrate specificities of the enzyme. We propose that the multiple effects of phorbol esters in intact cells may be mediated only through a single form of PKC activity.

86.7 INHIBITION OF VOLTAGE-GATED K CHANNELS IN SYNAPTOSOMES BY sn-1,2-DIOCTANOYLGLYCEROL, AN ACTIVATOR OF PROTEIN KINASE C. K.A. Colby* and M.P. Blaustein, Department of Physiology, University of Maryland School of Medicine, Baltimore, MD. 21201

The effects of sn-1,2-dioctanoylglycerol (diC8), an analogue of diacylglycerol which activates calcium/phospholipid-dependent protein kinase (C kinase), were tested on K channel function in rat brain synaptosomes. At least four distinct classes of K channels can be measured in synaptosomes with ^{86}Rb efflux techniques (Bartschat & Blaustein, 1985, J. Physiol. 361:419). One type of channel maintains resting K conductance. There are three voltage-regulated K channels: A voltage-gated, rapidly inactivating channel; a voltage-gated, non-inactivating channel; and a calcium-gated channel. Incubation of synaptosomes prepared from rat hippocampus with diC8 does not affect resting K conductance. However, diC8 dramatically inhibits ^{86}Rb efflux stimulated by depolarizing concentrations of K^+ (see Table). This effect is due to inhibition of efflux through voltage-gated K channels (ΔK). DiC8 does not affect ^{86}Rb efflux through calcium-gated K channels (ΔCa). Time course experiments suggest that diC8 partially inhibits a rapidly inactivating K channel which may correspond to the "A" channel, and markedly inhibits a non-inactivating, voltage-gated K channel which may correspond to the delayed rectifier. The increase in neurotransmitter release which accompanies activation of C kinase (e.g. Malenka et al., 1987, Brain Res. 403:198) may result from inhibition of these voltage-gated K channels. (Supported by NIH grant NS-16106).

TABLE I. Inhibition of ^{86}Rb Efflux from Synaptosomes by DiC8

Hippocampal synaptosomes were preloaded with ^{86}Rb and incubated with 100 μM diC8 for 6 min. ^{86}Rb efflux was measured for 5 sec. as previously described. Resting efflux is the Rb efflux under nondepolarizing conditions (5 mM K) The K-stimulated efflux (ΔK) equals efflux into 50 mM K minus resting efflux. Ca-dependent efflux (ΔCa) is that which occurs into 50 mM K + 1 mM Ca, minus the efflux into 50 mM K alone. The means of 4 replicate measurements (±SE) from one experiment are shown. Similar results were obtained in two other experiments.

Efflux Component	^{86}Rb efflux/5 sec (% of Total ^{86}Rb Content)	Percent Inhibition
Resting efflux	10.84 ± 0.15	-
K-stimulated (ΔK)	11.66 ± 0.91	-
Δ K + diC8	6.26 ± 1.16	46%
Ca-dependent (ΔCa)	4.30 ± 1.38	-
Δ Ca + diC8	4.40 ± 1.17	0

86.8 MODULATION OF SPONTANEOUS SYNAPTIC CURRENTS IN CULTURED HIPPOCAMPAL NEURONS BY PHORBOL ESTERS AND BAY K 8644. D.M. Finch and M.B. Jackson. Departments of Neurology and Biology and Brain Research Institute, University of California, Los Angeles, CA 90024.

We performed patch clamp recordings in the whole cell mode from cultured embryonic mouse hippocampal neurons. The cells (in TTX-containing bath) showed spontaneous inward currents, with a rise time of 1-2 msec, decay time of 5-10 msec, and an amplitude that varied from just greater than the noise level (1-2 pA) to more than 40 pA. Recordings under current clamp showed that these currents corresponded to spontaneous depolarizations of up to several mV. The frequency of the spontaneous currents was usually less than 0.1/sec. The currents were blocked by bathing the cells in 1 mM of the glutamate receptor antagonist gamma-D-Glutamylglycine. Therefore, the source of the currents is thought to reflect spontaneous release of excitatory, glutamatergic transmitter substance by terminals contacting the cells from which recordings were obtained. Spontaneous outward currents (possibly due to spontaneous release of inhibitory transmitter substance) were seen only rarely, and were not blocked by gamma-D-Glutamylglycine. The frequency of the spontaneous inward currents was increased in high potassium bath (16 mM), but did not obviously depend upon holding potential. Incubating cells in tetanus toxin, which has been shown to block synaptic transmission, decreased or blocked the currents. These observations provided more evidence that the currents reflected activity of presynaptic terminals.

Application of micromolar concentrations of phorbol ester 12,13 dibutyrate (PDB, a protein kinase C activator) increased the frequency of the spontaneous inward currents. The latency of this effect varied from a few seconds to a few minutes. Local application (via puffer pipets) or bath application were both effective. The increase in the rate of the spontaneous currents was only slowly reversible after removal of PDB. Application of vehicle alone (0.1% DMSO) had no consistent effect. Local application of the calcium channel agonist BAY K 8644 also increased the frequency of the spontaneous inward currents, in about one third of the cells, indicating a modulating or mediating role for calcium. However, the currents were not abolished by bathing the cells in a solution containing 0 mM calcium, 5 mM EGTA (to chelate residual calcium), and 2 mM cobalt (to block external calcium channels). This suggests that internal release of calcium, perhaps from mitochondria or endoplasmic reticulum, may trigger these events.

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86.9 α-LATROTOXIN RAISES CYTOPLASMIC IONIZED CALCIUM IN XENOPUS OOCYTES INJECTED WITH RAT BRAIN mRNA. J.A. Umbach, A. Grasso, and C.B. Gundersen. Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024 and Institute of Cell Biology, Rome, Italy.

When Xenopus oocytes are injected with poly(A)$^+$mRNA from rat brain, they express a variety of receptors, channels and transport proteins in their plasma membranes. We hypothesized that this expression system might be of value toward further characterizing the binding site on nerve cell membranes for the polypeptide neurotoxin, α-latrotoxin. This spider venom toxin binds with high affinity and considerable specificity to an acceptor protein associated with the nerve endings of a wide variety of vertebrates and invertebrates. Within 7d of injecting Xenopus oocytes with 30-50ng of rat brain mRNA (isolated by chloroform-phenol extraction and oligo dT cellulose chromatography), two-electrode voltage clamp revealed the appearance of oscillatory chloride currents evoked by the bath application of α-latrotoxin (10-25nM). This chloride conductance appeared to be identical to the conductance activated by application of serotonin, glutamate or acetylcholine to these cells. Previous work has indicated that this chloride channel is activated by a rise of cytoplasmic ionized calcium. We used calcium-selective microelectrodes to confirm that α-latrotoxin (10-20nM) produced a significant change (from a resting level of 60-100nM to values above 700nM) of oocyte ionized calcium. (These measurements also showed that the chloride channels of the oocyte membrance were activated when cytoplasmic ionized calcium reached 650nM.) The α-latrotoxin effect was largely dependent on external calcium and was not reversed by washing. By contrast, the toxin had no effect on cytoplasmic calcium (or membrane currents) of uninjected oocytes or on oocytes injected with non-neuronal preparations of mRNA (eg., rat muscle). Moreover, if oocytes that had been injected with rat brain mRNA were pretreated with concanavalin A (5ug/ml), a lectin that is known to block α-latrotoxin action in other systems, no membrane currents were detected upon toxin application (up to 40nM α-latrotoxin). These results indicated that Xenopus oocytes can be induced to express on their surface the effector site for a highly selective presynaptic neurotoxin.

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86.10 ANESTHETICS ALTER INTRACELLULAR IONIZED CALCIUM CONCENTRATIONS IN SYNAPTOSOMES. L.C. Daniell and R.A. Harris. Dept. of Pharmacology, V.A. Med. Ctr. and Alcohol Res. Ctr., Univ. of Colorado Health Sci. Ctr., Denver, CO. 80262.

Previous work from this laboratory showed that ethanol (350-700 mM) increases resting intracellular ionized calcium concentrations (Ca_i) in mouse whole brain synaptosomes. Studies have also shown that various anesthetic agents enhance calcium-dependent potassium conductances and reduce calcium currents in neurons, indicating that calcium-dependent mechanisms are altered by anesthetics in neuronal tissue.

The effects of anesthetic agents selected from various chemical classes (n-alkanols, halothane, diethylether and pentobarbital) on Ca_i were assessed in synaptosomes isolated from mouse whole brains. Fura-2, a fluorescent calcium indicator, was used to quantitate Ca_i. Fura-2-loaded synaptosomes were incubated with drugs at 35°C in low calcium buffer containing approximately 70 uM calcium for 10 min prior to measurement of Ca_i. Measurements were corrected for autofluorescence and leak of Fura-2 from synaptosomes.

Resting Ca_i was approximately 280 nM following incubation of synaptosomes for 10 min. Resting Ca_i was significantly increased by n-alkanols (butanol, pentanol and hexanol), halothane (3-6 mM) and diethylether (100 mM). In contrast, pentobarbital (0.01-1 mM) did not alter resting Ca_i. Depolarization of synaptosomes by the addition of KCl (final concentration, 50 mM) significantly increased Ca_i by approximately 60 nM and this value of Ca_i was further increased by prior incubation with n-alkanols (pentanol and hexanol) or halothane. Pentobarbital (0.01-1 mM) and diethylether (10-50 mM) reduced KCl-stimulated values of Ca_i. Subtraction of resting Ca_i from KCl-stimulated values of Ca_i showed that KCl-stimulated increases in Ca_i were reduced by n-alkanols, pentobarbital and diethylether but unchanged by halothane.

These results show that anesthetics differentially alter Ca_i in brain tissue. These effects of anesthetics may have important consequences for neurotransmission, ion channel regulation and other calcium-dependent processes in brain tissue. Supported by the VA and AA06399 and AA03527.

86.11 EFFECTS OF ACUTE THALLIUM ADMINISTRATION ON TRANSMITTER RELEASE AND Ca INFLUX. W.D. Atchison, J. Thornburg, U. Joshi* and A.C. Barton, Dept. Pharmacol./Toxicol., Neuroscience Program and Center for Environ. Toxicol., Michigan State Univ., E. Lansing, MI 48824.

Thallium (Tl^+) like its neighboring elements in the periodic chart, Pb and Hg, is neurotoxic. However, while the effects of the inorganic divalent cations on synaptic transmission are well known, the effects of the monovalent Tl^+ are unknown. The goal of this project was to determine whether Tl^+ causes suppression of evoked release and stimulation of spontaneous release of transmitter as do inorganic divalent cations such as Pb or Hg. Effects of acute administration of micromolar concentrations of Tl^+ on synaptic transmission were measured electrophysiologically at the neuromuscular junction of the rat and neurochemically as determinations of ^{45}Ca influx in K^+-depolarized forebrain synaptosomes from the rat. Neurochemical studies of $^{45}Ca^{2+}$ influx into forebrain synaptosomes depolarized with elevated $[K^+]$ were used to determine if the Tl^+-induced suppression of synaptic transmission was related to diminished entry of Ca^{2+} into the axon terminal following depolarization. After a latent period, Tl^+ gradually reduced the amplitude of nerve-evoked endplate potentials (EPPs) and occasionally blocked them completely. The depressant effects of Tl^+ were more pronounced as the concentration was increased. When total block of the EPP occurred, it could usually be reversed by washing the preparation with Tl^+-free solutions. Tl^+-induced block of the EPP was sometimes preceded by a brief period during which EPP amplitude was increased. Block of the EPP was due at least in part to prejunctional effects of Tl^+, as mean quantal content was reduced in Tl^+-poisoned preparations. Tl^+ caused a biphasic effect on miniature endplate potential (MEPP) amplitude; initially mean MEPP amplitude was increased by Tl^+, but subsequently MEPP amplitude also declined. Decreased MEPP amplitude was not caused by depolarization of the postjunctional muscle membrane by Tl^+, because endplate membrane potential was not altered by exposure to Tl^+ for periods of up to 90 min. The frequency of occurrence of MEPPs was increased by Tl^+. This effect was not blocked by increasing the Mg^{2+} concentration nor by increasing the K^+ concentration. Studies of $^{45}Ca^{2+}$ influx into isolated synaptosomes depolarized by K^+ indicated that Tl^+ did not suppress depolarization-dependent uptake of Ca^{2+} into the nerve terminal; total uptake of $^{45}Ca^{2+}$ during 10 sec of depolarization was not reduced by Tl^+, nor was either the "fast" nor "slow" component of uptake reduced. Results of the present study suggest that acute application of Tl^+ suppresses synaptic transmission by both presynaptic and postsynaptic mechanisms, but the presynaptic component probably is not related to block of Ca^{2+} entry into the axon terminal. (Supported by BRSG funds from the College of Veterinary Medicine and by NIH grant ES03299. Dr. Joshi was supported by funds from the Center for Environmental Toxicology.)

86.12 THE PATTERN OF DEPOLARIZATION-INDUCED CHANGES IN INTRASYNAPTOSOMAL FREE Ca^{++} MEASURED WITH FURA-2 CORRESPONDS TO THE PATTERN OF ACETYLCHOLINE RELEASE INDUCED BY KCl BUT NOT VERATRIDINE. Kenneth A. Stauderman*, Paul Lundy*#, Phillip J. Robinson*, Walter Lovenberg*. (SPON: David J. Jones) Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215, # Defense Research Establishment Suffield, Ralston, Alberta, Canada.

Recent advances in both the techniques for preparing synaptosomes and the instrumentation for measuring fluorescence changes of the Ca^{++} sensitive dye fura-2 have improved the speed and quality of measurements of intracellular free Ca^{++} ($[Ca^{++}]_i$) in purified synaptosomes. In parallel with a continuous chemiluminescent assay for acetylcholine (ACh) release, (Israel and Lebats, J. Neurochem, 39: 248-250, 1982) we compared at pH 7.4 the time course of depolarization-induced ACh release with depolarization-induced changes in $[Ca^{++}]_i$, using rat striatal synaptosomes loaded with fura-2. Purified synaptosomes, prepared using a percoll-gradient, were loaded with 5μM fura-2/AM by a modification of the procedure by Ashley et al. (Biochem. J, 219: 149-158, 1984). Fluorescence was measured by a dual-excitation spectrophotometer (Deltascan I, Photon Technology Int.) with excitation alternating between 340 and 380 nm thirty times a second. The fluorescence ratios (340/380) thus obtained were used to calculate the absolute $[Ca^{++}]_i$ which was expressed in nM concentrations ± SEM. Striatal synaptosomes had a resting $[Ca^{++}]_i$ of 137 ± 9.4 nM. Depolarization with 40 mM KCl produced two responses: a rapid increase in $[Ca^{++}]_i$ that peaked at 416 ± 21 nM within 3 seconds of the onset, followed by a gradual decline in $[Ca^{++}]_i$ until a new steady-state was reached at about 260 nM. Both responses were dependent on extracellular Ca^{++}. On the other hand, depolarization with 1μM veratridine caused only a slow rise in $[Ca^{++}]_i$ that equilibrated at 430 nM 2 minutes after application. Similar to the $[Ca^{++}]_i$ response, 40 mM KCl-induced ACh release consisted of two phases: a fast phase that peaked at 1-3 seconds, and a slow phase that peaked at 60 seconds. Both phases were dependent on extracellular Ca^{++}. Surprisingly, this pattern of ACh release was also observed in response to veratridine. Thus, KCl and veratridine produce similar patterns of ACh release, but only the KCl response corresponds to the observed changes in $[Ca^{++}]_i$. These results indicate there is not always a direct relationship between average $[Ca^{++}]_i$ (measured with fura-2) and ACh release.

86.13 EFFECTS OF BACITRACIN ON IN VITRO 3H-SEROTONIN RELEASE AND CALCIUM-45 UPTAKE IN RAT HYPOTHALAMUS. M. S. Saporito and R. O. Warwick.* Dept. of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Philadelphia, PA. 19104.

Serotonin (5-HT) release from nerve terminals is a calcium (Ca) dependent event which is regulated by a presynaptic autoreceptor (5-HT1b). This autoreceptor regulates voltage dependent Ca channels which couple Ca influx with 5-HT release (Gothert, N-S Arch. Pharmacol. 314:223, 1980). The peptide antibiotic bacitracin (BCN) is used as a peptidase inhibitor in radioligand binding assays. We have incorporated BCN into superfusion experiments designed to study peptide effects on 5-HT release in vitro. Since BCN also decreases binding affinity of 5-HT for 5-HT1 receptors in the rat hippocampus (Rostene et al., J. Neurosci. 3:2414, 1983) and can chelate Ca, it may alter calcium associated events such as 5-HT release and autoreceptor function.

In this study we assessed the ability of BCN to modify 5-HT release and autoreceptor activity in rat hypothalamic (HYP) slices. Additionally, we examined the effects of BCN on 3H-5-HT and 45Ca uptake in a crude synaptosomal fraction (P2) prepared from rat HYP. Superfused rat HYP slices preloaded with 3H-5-HT were stimulated with two 6 min periods of 25 mM K+. Stimulated release was calculated as the ratio of peak 2 to peak 1 (S2/S1). BCN (2 ug/ml) and/or 5-methoxytryptamine (5-MEOT; 1 uM) were included in the superfusion buffer for the second stimulation period.

Control S2/S1 ratios were 0.80 ± 0.05. 5-MEOT decreased the S2/S1 ratio by 20%. BCN had no effect alone, but enhanced 5-MEOT activity by 25%.

Stimulated 45Ca uptake was assessed by the method of Leslie et al. (Brain Res. 325:99, 1985). Following a 12 min preincubation period, synaptosomes were depolarized with 30 mM K+ for 3 sec in the presence of 60 uM Ca45. Uptake of Ca45 in nondepolarizing buffer (5 mM K+) was subtracted to yield stimulated uptake.

Control stimulated uptake was 576 ± 60 pmoles/mg protein which was significantly ($p < 0.05$) decreased by 55% with BCN (2 ug/ml). BCN had no effect on nonstimulated uptake. Uptake of 3H-5-HT (50 nM) by P2 fraction of rat HYP was not altered by 2 ug/ml BCN.

These data suggest that BCN enhances autoreceptor activity by blocking voltage operated Ca2+ channels involved in coupling Ca influx with 5-HT release.

86.14 [^{3}H]GABA RELEASE FROM STRIATAL SLICES CAN OCCUR VIA CALCIUM-INSENSITIVE, REVERSE TRANSPORT. Sandor Bernath* and Michael J. Zigmond, Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

The role of Ca^{2+} in mediating transmitter release is generally well established. However, its involvement in the release of GABA is controversial. To examine this issue, slices (350 um) were prepared from rat striatum, preincubated with [^{3}H]GABA (0.1 uM) in the presence of ß-alanine (1 mM), an inhibitor of GABA uptake into glia, and then superfused at 37°C with Krebs buffer containing aminooxyacetic acid (0.1 mM), an inhibitor of GABA transaminase. Total tritium efflux was used as an index of GABA release. Electrical stimulation (2 Hz, 20 mA, 3.0 msec pulse, 360 shocks) elevated resting release approximately 2-fold. Reducing external Ca^{2+} failed to abolish stimulus- induced tritium overflow, although the precise impact of altering Ca^{2+} was a function of external Ca^{2+} concentration and the period of superfusion. Thus, if external Ca^{2+} was lowered to 0.1 mM twenty min prior to depolarization, tritium overflow occurred at 49% of the control rate, while if Ca^{2+} was omitted and 1 mM EGTA added, tritium overflow occurred at or slightly above the control rate. Moreover, if slices were exposed at a Ca^{2+}-free medium plus EGTA throughout a 70-min period of superfusion overflow increased 3-fold. Reducing Ca^{2+} increased spontaneous tritium efflux under all conditions. Tetrodotoxin (5 uM) abolished the overflow of tritium during depolarization in both normal and Ca^{2+}-free conditions. Nipecotic acid (0.1 to 1mM), an inhibitor of neuronal and glial GABA uptake, enhanced both the spontaneous efflux and evoked overflow of tritium from control slices, indicating the important role of GABA uptake in removing GABA from extracellular fluid. Nipecotic acid also increased spontaneous release when external Ca^{2+} was reduced or totally removed. However, under these conditions the electrically-evoked overflow was greatly reduced. In contrast, adding ß-alanine (1 mM) to the superfusion medium increased basal efflux and depolarization-induced overflow under all conditions. These results suggest that the electrically-evoked release of [^{3}H]GABA from striatal slices is of neuronal origin and depends on voltage-dependent sodium channels, but can occur in the absence of Ca^{2+}. They further suggest that Ca^{2+}-independent release depends on the high affinity GABA transport system in nerve terminals. (Supported in part by USPHS grant NS19608. S.B. is on leave from the Institute of Experimental Medicine, Hungarian Academy of Science, Budapest, Hungary.)

86.15 CALCIUM-DEPENDENT POTENTIALS IN LIZARD MOTOR NERVE TERMINALS. E.F. Barrett and K. Morita*. Dept. of Physiology & Biophysics, Univ. of Miami Med. Sch., P.O. Box 016430, Miami, Fla. 33101.

A microelectrode was inserted into motor axons innervating the ceratomandibularis muscle of lizards (Anolis sagrei), within 1 mm of the motor terminals. Preparations were pretreated for 1 hour in 10 mM tetraethylammonium (TEA) to increase the duration of the action potential and the resting input resistance of the axon. Action potentials were evoked by stimulating the nerve trunk via a suction electrode; muscle contractions were blocked with 30-60 μM carbachol or by cutting the muscle fibers. In physiological saline containing 2 mM Ca, 2 mM Mg and 10 mM TEA, action potentials recorded near the motor terminals were followed by this sequence of afterpotentials: (1) a depolarizing plateau (D1) with an amplitude of about 30 mV, terminated after about 40 msec by a rapid partial repolarization, (2) a smaller depolarization (D2) that decayed over a time course of several hundred msec, and (3) a hyperpolarization of about 5 mV that decayed over a time course of seconds. These afterpotentials were not seen in axons separated from their terminals. These terminal-specific afterpotentials disappeared when the preparation was perfused with low [Ca]-high [Mg] solutions or with 1 mM Ni or Mn, and were enhanced when bath [Ca] was increased. The duration of D1 increased following addition of 1 mM 4-aminopyridine or 1 mM Ba, or after [TEA] was increased to 25 mM. The D1 plateau potential thus appears to be due to calcium influx into the motor terminals. The slow hyperpolarizing afterpotential was increased by K-free solutions or 1 mM caffeine; it was reduced when bath Ca was replaced by 2 mM Ba, but was not abolished by ouabain. Thus the slow hyperpolarizing afterpotential appears to be mediated by a Ca-sensitive K efflux. The configuration of these terminal-specific afterpotentials changed during repetitive stimulation, in a manner that varied with bath [Ca]. For example, an increase in stimulus frequency from 0.1 to 1 Hz transiently increased D1 duration in 1 mM Ca, but reduced D1 duration in 4-6 mM Ca. These results suggest that changes in Ca entry during successive action potentials may contribute to the changes in evoked transmitter release observed during repetitive stimulation. Supported by NIH grants NS 12404 and GM 30377.

86.16 ADRENERGIC EFFECTS ON CALCIUM-DEPENDENT POTENTIALS IN LIZARD MOTOR NERVE TERMINALS. K. Morita* and E.F. Barrett (SPON: R. Keane). Dept. of Physiology & Biophysics, Univ. of Miami Med. Sch., P.O. Box 016430, Miami, Fla. 33101.

We applied adrenergic agonists and antagonists to the lizard ceratomandibularis nerve-muscle preparation, and used an intra-axonal microelectrode to record the calcium-dependent afterpotentials originating in the motor nerve terminals (recording techniques described in preceding abstract, Barrett & Morita, 1987). All preparations were bathed in 10 mM TEA, plus 30-60 μM carbachol to prevent muscle contraction. Action potentials evoked by stimulating the proximal nerve trunk were followed by a multi-component depolarizing afterpotential and a slow hyperpolarizing afterpotential, both of which were Ca-dependent. The depolarizing afterpotential is due at least in part to Ca influx, and the slow hyperpolarizing afterpotential is due mainly to Ca-dependent K efflux (see preceding abstract). Exposure to epinephrine (10-100 μM) usually reduced the amplitude and duration of both these Ca-dependent afterpotentials in a dose-dependent and reversible manner. However, both afterpotentials were transiently enhanced during washout of the epinephrine. In some axons the afterpotentials were enhanced even during exposure to epinephrine. When epinephrine was applied in the presence of a beta-adrenergic antagonist, propranolol (1-10 μM), the afterpotentials were always inhibited, whereas when epinephrine was applied in the presence of an alpha-adrenergic antagonist, phentolamine (1-10 μM) or prazosine (1-10 μM), both afterpotentials were always enhanced. These calcium-dependent afterpotentials were also enhanced by a beta-adrenergic agonist, isoproterenol (10 μM). These results suggest that lizard motor nerve terminals possess both alpha-adrenergic receptors, which inhibit Ca entry, and beta-adrenergic receptors, which enhance Ca entry. Supported by NIH grants NS 12404 and GM 30377.

86.17 CALCIUM CURRENTS IN MAMMALIAN MOTOR NERVE TERMINALS. B.R. Hamilton and D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

Depolarization of nerve terminals by invading action potentials is known to open voltage dependent calcium channels, resulting in an inward calcium current that leads to vesicular fusion with terminal membrane and consequently, the release of neurotransmitter. Calcium currents have been measured directly in squid giant synapse and mouse motor nerve terminals. Voltage dependent calcium channel characterization has been possible in tissue culture and dissociable cell systems, as in the case of DRG cells where L, N and T type calcium channels have been identified. To date these characterizations have not been extended to motor nerve terminals. We have undertaken studies utilizing loose-patch electrodes and patch-clamp amplifiers to examine calcium currents in motor nerve terminals of rat EDL.

Incubation of the EDL preparation in 10 μM 4-(4-diethylaminostyryl)-N-methylpyridinium iodide allows viewing of end-plates with epi-fluorescence at 400X magnification and subsequent placement of electrodes. Post-synaptic responses are blocked with appropriate levels of d-tubocurarine. Initial terminal recordings are dominated by outward K^+ currents which can be blocked with TEA (1 to 10 mM) and 3,4 diaminopyridine (50 to 250 μM). Stimulation of the nerve via a suction electrode, in the presence of these blocking agents, results in an outward capacitative spike attributed to the depolarizing influx of Na^+ at the last node, followed by a slowly developing inward current, 10 to 15 pA in amplitude and 4 to 6 ms in duration. The slow inward terminal current is blocked by the known inorganic calcium channel blockers Co^{2+} (10 mM) and Cd^{2+} (1mM), thus indicating that this is most probably a Ca^{2+} current. This terminal Ca^{2+} current appears to be relatively insensitive to dihydropyridine antagonists such as nifedipine (10 to 20 μM). Double pulse protocols, though, have not revealed any significant Ca^{2+} dependent inactivation.

It has been hypothesized that N type channels are located at active zones and are responsible for transmitter release, while L type channels may be present, but away from active zones. Relative insensitivity to nifedipine suggests the presence of N type channels, while the lack of inactivation would be considered a characteristic of L type channels. Thus, it would appear likely that the slow inward current that we have identified in rat EDL motor nerve terminals is the result of Ca^{2+} ion flux through both L and N type Ca^{2+} channels. Supported by NIH grant NS13600 and the Muscular Dystrophy Association.

86.18 THREE POTASSIUM CURRENTS IN MOUSE MOTOR ENDINGS. A. Mallart, N. Tabti* and C. Bourret*. Lab. Neurobiologie Cellulaire et Moléculaire, CNRS, F-91190 Gif sur Yvette, France.

Action potentials elicited by motor nerve stimulation promote conductance changes at the presynaptic terminals which give rise to local circuit currents flowing between the terminals and the parent axon. The recordings obtained using extracellular electrodes placed on motor endings or inside the perineurium of preterminal nerve bundles showed a large outward current which was demonstrated, by its sensitivity to K channel blockers, to correspond to an increase in gK at the terminals during the repolarizing phase of the action potential (Brigant, J. L. and Mallart, A., J. Physiol. 333:619, 1982). The use of adequate channel blocking agents allowed the separation of this K current into three distinct components: i) 3,4-diaminopyridine (3,4-DAP) in bath application (200-400 μM) suppressed a large fraction of the outward current which was supposed to correspond to IK. ii) A fraction of the 3,4-DAP resistant outward current was abolished in Ca^{2+}-free media and in the presence of inorganic Ca channel blockers, TEA (5-10 mM) or charibdotoxin (CTX, 20 nM) and was, thus, identified as a Ca-dependent K current (Mallart, A., J. Physiol., 368:577, 1985). iii) In 3,4-DAP and CTX treated preparations, where IK and IK(Ca) channels were expected to be blocked, addition of TEA caused a marked enhancement of the wave form component that signals ICa, which suggests the suppression by this agent of another type of IK, 3,4-DAP resistant but TEA sensitive, not hitherto described in presynaptic terminals. Further separation of these two types of IK was achieved by using first TEA (10 mM) to suppress both the TEA sensitive component of IK and IK(Ca). This treatment induced only a moderate ICa response which was greatly enhanced by subsequent addition of 3,4-DAP (300 μM) or UO_2 (50 μM). On the basis of this pharmacological analysis one can distinguish two distinct, voltage dependent, IK components: one being sensitive to TEA and the other sensitive to 3,4-DAP and UO_2. It is not clear what is the functional significance of these three K currents. IK(Ca) should be small under physiological conditions since CTX failed to affect the presynaptic signals in standard saline. Although the relative potency of each fraction of IK remains to be determined, one can say, from the large size of the presynaptic 'K signal', that the combined action of both components is able to induce a really sharp decay phase of the presynaptic action potential.

We wish to thank C. Miller for a gift of charibdotoxin.

87.1 THREE-DIMENSIONAL ORGANIZATION OF A BIDIRECTIONAL-EXCITATORY CHEMICAL SYNAPSE. Ulrike Grünert,* and Peter A. V. Anderson. (SPON: M. J. Greenberg). Whitney Lab. and Depts. of Physiology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

Morphologically non-polarized, or bidirectional chemical synapses are common in cnidarians, and present in other phyla, including vertebrates, but only in the jellyfish Cyanea has their bidirectionality been confirmed physiologically (Anderson, P. A. V., J. Neurophysiol., 53: 821-835, 1985). In thin sections these synapses in cnidarians are characterized by very marked pre- and post-synaptic densities and by the presence of 3-4 large synaptic vesicles. Both terminals contain a similar number and the two vesicle clumps are usually directly opposed to one another. Cisternae are usually present. The scarcity of vesicles in a given thin-section of such a synapse has often been received with some scepticism, particularly since a typical post-synaptic potential might be of the order of 50 mV. This prompted us to determine the three-dimensional organization of these synapses with a view to ascertaining the total number of vesicles present at the synapses, the degree to which vesicles in the two terminals were opposed and the presence and organization of other organelles in the synapse.

Preparations of the motor nerve net of Cyanea were exposed by oxidation of the overlying epithelial layer, then fixed and embedded in the conventional way. 5 mM Cd was present during the early stages of fixation to minimize vesicle exocytosis. Serial section were then cut and the organization of the synapse determined by tracing the images from photomicrographs onto plexiglass. Four complete synapses were examined in this manner.

A typical terminal contains 40 to 50, 90 to 100 nm diameter, slightly irregularly shaped vesicles. These form a single layer against the cell membrane and are separated by electron dense material. The organization of the two terminals is the same and the vesicles form two opposing plaques. Immediately internal to the vesicles is an extensive cisternal network that covers the entire vesicle array. From reconstructions, it appears to form a single perforated sheet from which arise numerous processes, some of which are fine, others large and bulbous. Its appearance in reconstructed terminals was reminiscent of sarcoplamic reticulum. Occasionally, the cisternae appear to be connected to the vesicles by fine necks of membrane, suggesting that the vesicles bud-off from the cisternae.

The major conclusion of this work is that the organization of the bidirectional synapses in Cyanea is similar to that of most other synapses, with the exception that the cisternal network is far more extensive that is reported for other synapses. The significance of this is not known.

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87.2 MEAN SYNAPTIC LENGTHS OF LAMINAE I-IV IN THE RAT DORSAL HORN ARE NOT HOMOGENEOUS. R.E. Coggeshall, R.P. Bolender*, K. Chung, C.E. Hulsebosch and D.L. McNeill*. Marine Biomedical Institute and Dept. of Anatomy & Neurosciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550 and Dept. of Biological Structure, University of Washington School of Medicine, Seattle, WA 98195.

A knowledge of synaptic size and distribution is essential in order to understand spinal cord organization. One parameter of synaptic size we have examined is the mean length of the synaptic cleft (MLSC). In the present study the MLSC was determined for laminae I-IV in the right and left dorsal horns of normal rat spinal cord. Four 3 month old Sprague-Dawley rats were perfused and the second sacral (S2) spinal cord segment removed. Each S2 segment was cut into 3 transverse slices then embedded for transmission electron microscopy. A thin section was cut from each slice and a low power (X 1200) montage was constructed of the right and left dorsal horns. Laminae I-IV boundaries were drawn on each montage and a template consisting of an orthogonal grid with holes located 8 cm from each other was placed randomly over the montage. Pen marks were made on the montage through the holes. A high power (X 38,000) micrograph was then taken using the pen mark on the montage as the center of the micrograph. Micrographs were subsequently arranged by lamina and right or left dorsal horn. Synapses were identified in each micrograph and their lengths were measured using an acoustic plotter. The MLSC for combined right and left laminae I-IV are listed below.

Lamina	MSL + S.D. (μm)
I	.29 ± .10
II outer (o)	.28 ± .10
II inner (i)	.28 ± .10
III	.30 ± .12
IV	.30 ± .11

The MLSC of laminae IIo and IIi is significantly smaller than that of III and IV ($p < 0.05$). There is no significant difference in the MLSC of laminae I versus II-IV or between individual lamina when compared with the respective lamina on the contralateral side. In addition, there is no significant difference with respect to medial to lateral placement of the MLSC within each lamina. Serial section reconstructions confirmed that synapses in all laminae were disc shaped with widths varying between .15-.40 μm. These data will be useful in comparing the normal with experimental situations.

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87.3 SUBSTRUCTURE IN THE POSTSYNAPTIC DENSITY OF PURKINJE CELL DENDRITIC SPINES REVEALED BY RAPID FREEZING AND ETCHING. D.M.D. Landis. Depts Neurology, Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106

We have used rapid freezing, freeze-substitution fixation, and shallow etching after freeze fracture to examine the distribution and structure of proteins composing the postsynaptic density in Purkinje cell dendritic spines synapsing with parallel fibers in normal mouse cerebellar cortex. The density consists of thin protein filaments which insert on the true inner surface of the postsynaptic membrane and which bind a variety of globular structures that we interpret as cytoplasmic proteins. It has long been appreciated that the size of the postsynaptic density is different at different classes of synaptic junction, but our studies reveal the additional complexity that the structure of the postsynaptic density varies as different Purkinje spines are compared to one another.

Slabs of cerebellar cortex were quickly excised and the pial surface frozen by impact against a copper block cooled by liquid helium. In tissue prepared by freeze fracture and then etching to remove 20-30nm of water prior to rotary replication with platinum alloy, the number and packing density of the globular proteins varies from region to region within a single density, and is even more variable when different junctions are compared. While actin-like microfilaments and spectrin-like filaments are juxtaposed to the postsynaptic density, they do not appear to be continuous with the constituent filaments of the density. We did not detect neurofilaments or microtubules in the postsynaptic density.

The fine filaments of the postsynaptic density appear to form a supporting framework for the various globular proteins, and to position them in the region of the spine which has the greatest concentration of ionized calcium entering with the synaptic current and the greatest extent of postsynaptic depolarization. The heterogeneity of the globular proteins in the postsynaptic density indicates that constituent proteins vary from density to density and are not uniformly distributed across a single density. Present evidence suggests that the nature of the neurotransmitter and the mechanism of excitatory action are the same at all parallel fiber synapses with Purkinje cell spines. Perhaps the variations in structure of the postsynaptic density at different spines reflect different histories of synaptic activity, resulting in qualitative and quantitative changes in the protein composition of the density.

87.4 DISTRIBUTION OF CONTRACTILE AND CYTOSKELETAL PROTEINS IN THE DENTATE MOLECULAR LAYER. M. Morales* and E. Fifkova (SPON: J. A. Markham). Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Contractile and cytoskeletal proteins may be involved in important neuronal functions. In our previous work (Fifkova and Delay, J. Cell Biol., 95:345, 1982), we have shown that actin filaments are distributed through the neuron with the highest density in dendritic spines. Actin filaments are anchored to the spine apparatus and determine the characteristic shape of the spine. They are also responsible for the absence of cytoplasmic organelles from the spine. Labeling of actin filaments with the S-1 fragment of myosin necessitated membrane permeation with saponin. Since the permeated membrane may affect the organization of actin filaments, it seemed important to verify actin organization with another method which does not affect the integrity of the plasma membrane. Such a method involves immunogold labeled antiactin antibodies in a postembedding procedure which leaves the plasma membrane intact. Actin filaments are well preserved because the protocol uses low concentrations of glutaraldehyde (0.5%) and instead of OsO_4 uranylacetate as a secondary fixative during dehydration. The tissue was embedded in Lowicryl K4M, which does not require absolute dehydration and may be done at low temperatures, both of which help to retain antigenicity. The pattern of distribution of actin filaments in spines with the immunogold labeling in dendritic spines was similar to that we observed with the S-1 fragment of myosin. As with the latter method, actin is distributed across the neuron with the highest density in dendritic spines. Gold particles were clearly associated with the postsynaptic density (PSD), with the plasma membrane and membranes of the spine apparatus. Thus, with two different methods, we have confirmed the density and distribution of actin filaments in dendritic spines. Since the postembedding technique allows us to use more than one antibody in the same tissue, we stained alternate grids with the antitubulin and antiplatelet myosin antibody. The antitubulin antibody showed the expected heavy labeling of microtubules in dendrites, axons and perikarya. Labeling could be also seen in axon terminals, in their center or periphery, but not at the active synaptic zone. This observation confirms that of Cumming et al. (Neurosci. Lett., 37:215, 1983). The antitubulin antibody failed to localize tubulin in spines and the PSD. The antimyosin antibody was distributed in spines along actin filaments in a concentration far below that of actin, which agrees with observations in other nonmuscle cells. Myosin labeling was absent from the PSD, however, it was seen under the plasma membrane and membranes of the spine apparatus. Sparse myosin labeling could be seen in axon terminals and dendrites. Taken together, these observations localize actin and myosin as the main contractile proteins in dendritic spines. Supported by NIA grant AG04806. The antibodies were the gift of Dr. J. M. Scholey, Department of MCD Biology , University of Colorado.

87.5 DENDRITIC SPINES ARE SUSCEPTIBLE TO STRUCTURAL ALTERATIONS INDUCED BY DEGENERATION OF THEIR PRESYNAPTIC AFFERENTS.

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The most common approach used to study thalamocortical (TC) connectivity involves the labeling of the thalamocortical afferents by lesion-induced degeneration. However, despite the plethora of information regarding the morphology of degenerating axon terminals and of their postsynaptic elements, no systematic morphological analysis is available regarding the possible effects of the degeneration of TC projections on the morphology of their cortical targets, which are mainly dendritic spines. The aim of the present study was to investigate which specific structural alterations, if any, occur in the dendritic spines of cortical neurons, following the degeneration of presynaptic TC axon terminals. TC afferents to SmI cortex of adult male CD-1 mice were lesioned electrolytically. Four days post-lesion, the brains were fixed with aldehydes and processed by the Golgi gold-toning method. Dendritic spines belonging to layer IV spiny stellate neurons were examined in series of thin sections. Structural features were compared for spines synapsing with degenerated TC terminals, and with unidentified intact axon terminals, on the same dendritic segments. Results showed that the heads of denervated spines are significantly wider (+11%, p=0.03) and less variable in width (-6%, p=0.03) than the heads of spines whose innervation is intact. In addition, the synaptic junctions of denervated spines typically are concave with respect to the spine head, whereas the majority of those belonging to innervated spines are convex (significance, p=0.01). Structural features typifying transneuronal degeneration were not detected within the spiny dendrites. A body of data exists which indicates that the structural differences observed in this study are not related to the identity of the presynaptic element, but instead result directly from denervation, and so it is concluded that changes in the structure of dendritic spines can occur soon after synaptic inactivity is induced. Supported by N.I.H. # NS 20149-04.

87.6 SV2 AND P38/SYNAPTOPHYSIN CO-PURIFY ON SMALL SYNAPTIC VESICLES FROM MAMMALIAN BRAIN. E. Floor, Dept. of Anatomy, Univ. of Wisconsin Medical School, Madison, WI 53706.

A transmembrane glycoprotein, SV2, of Mr ~100,000 has been identified by means of a monoclonal antibody, 10H3, isolated by K. Buckley and R.B. Kelly (J. Cell Biol. 100, 1284) and shown to be on purified synaptic vesicles from elasmobranch fish and to be present in mammalian neurons and endocrine cells. Another synaptic vesicle-specific protein, p38/synaptophysin, was shown to be a major protein on mammalian synaptic vesicles by Jahn et al. (PNAS 82, 4137), and Wiedenmann and Franke (Cell 41, 1017). These two proteins were localized to mammalian synaptic vesicles largely by immunocytochemical methods. In the present experiments, SV2 and p38/synaptophysin levels during purification of synaptic vesicles from rat brain (Soc. Neurosci. Abstr. 11, 644) were measured by radioimmunoassay (RIA) with antibodies 10H3 and R10, respectively. R10 is a rabbit antiserum against purified synaptic vesicles that recognizes predominantly a 39K protein on Western immunoblots that was shown to be p38/synaptophysin. The RIA results were confirmed qualitatively by Western immunoblot analysis.

During synaptic vesicle purification, single peaks of SV2 and p38/synaptophysin immunoactivities were found after equilibrium density sedimentation on 10-30% Nycodenz gradients and after sizing chromatography on Sephacryl S-1000 and controlled-pore glass bead CPG-3000 columns. The peaks of the two proteins corresponded after each of these separation techniques, and both proteins were shown to be associated with vesicles ~40 nm in diameter by chromatography on a calibrated CPG-3000 column. The overall purifications of these synaptic vesicle proteins after the CPG-3000 chromatographic step were 80-fold for p38/synaptophysin and 50-fold for SV2 with respect to their concentrations per total protein in the crude homogenate. The amount of SV2 in column fractions containing larger synaptic vesicles (~100 nm) after the Sephacryl S-1000 chromatographic step was <2% of that in ~40 nm vesicles, while ~15% of p38/synaptophysin was in larger vesicles at the same purification step. The reason for this difference in levels of the two proteins is not clear. These results do not exclude the possibility that one or both proteins are present on larger synaptic vesicles. However, these results indicate that most of the SV2 and also p38/synaptophysin in rat brain are selectively localized on small synaptic vesicles.

I thank Kathy Buckley for 10H3 antibody. Supported by NIH Grant NS24890.

87.7 CLONING AND SEQUENCE ANALYSIS OF cDNA ENCODING A MAJOR SYNAPTIC VESICLE PROTEIN, p38 OR SYNAPTOPHYSIN.

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Synaptic vesicles in all parts of the nervous system share three integral membrane proteins: p65 (Matthew et al.,1981), SV 2 (Buckley and Kelly, 1985), and p38 (Wiedenmann and Franke, 1985; Jahn et al.,1985). The most abundant of these three proteins, p38, has recently been proposed as a major Ca^{++}binding protein of the synaptic vesicle (Rehm et al.,1986). To extend the study of these vesicle proteins we have recently isolated cDNA clones encoding the open reading frame and untranslated regions of the mRNA for p38 from a lamda gt11 rat brain cDNA library. The cDNA clones were initially identified by screening with a polyclonal serum to rat brain synaptic vesicles. Positive phage were further screened by using fusion proteins adsorbed to nitrocellulose filters to affinity purify monospecific antibodies from the original heterogeneous serum. Inserts from clones which selected for antibodies binding to p38 in western blots were subcloned into plasmid vectors and used to generate cDNA probes for Northern blot analysis. A major transcript of 2.5 kb was expressed specifically in brain and endocrine tissue but not in liver, spleen, or salivary gland, consistent with the tissue-specific expression of the protein detected by antibody techniques. Three overlapping clones were sequenced by standard dideoxy chain termination in M13. Approximately 85% of the cDNA was identified and sequenced in this manner. The remaining sequence at the 5' end of the mRNA was obtained by screening a second lamda gt11 library with a 5' cDNA probe. The open reading frame consists of 1100 bp that encode a protein of M_r=36,000. Since the unglycosylated protein has been reported to be 34,000, we assume the increase in molecular weight represents the addition of a signal peptide. The remainder of the transcript consists of 1.4 kb of 3' untranslated RNA and the poly A tail. The protein shares no sequence homology with other known (albeit predominantly cytoplasmic) Ca^{++}binding proteins, and may represent a new class of integral membrane proteins with Ca^{++} binding activity. The availability of a cDNA clone for this synaptic vesicle protein should facilitate studies of both its function in transmitter release as well as the regulation of a synapse-specific protein during development and regeneration of nerve terminals.

Matthew et al.,1981, J.Cell Biol. 91:257-269
Buckley and Kelly,1985, J.Cell Biol. 100:1284-1294
Wiedenmann and Franke,1985, Cell 41:1017-1028
Jahn et al.,1985, PNAS 82:4137-4141
Rehm et al.,1986, EMBO J. 5:535-541

87.8 IDENTIFICATION OF A NOVEL SYNAPSIN I-LIKE PROTEIN IN DROSOPHILA.

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Synapsin I is a major substrate for cAMP- and Ca^{2+}-dependent protein kinases, which is associated with synaptic vesicles and is implicated in regulating transmitter release. Synapsin I has been extensively characterized in mammalian brain, where it is a doublet of MW 80 and 86 kDa. We have set out to identify and characterize synapsin I in Drosophila melanogaster, since this organism offers the possibility of genetic dissection of synapsin I structure and function. In addition, Drosophila mutants exist which are defective in second messenger cascades and in neuronal and behavioral plasticity, and these mutants could shed light on physiological roles of synapsin I. For detection of synapsin I in Drosophila, we have employed antibodies against the mammalian protein (e.g., Goelz et al J. Neurochem. 45: 63 (1985)). In Drosophila, a single major protein (synapsin I-like immunoreactive protein, SLIP) of MW 150±15 kDa cross reacted with 5 out of 7 monoclonal antibodies and with all the serum antibodies raised against mammalian synapsin I. We have compared several properties of SLIP to those of mammalian synapsin I. Zn^{2+}/acid extraction (pH 3), which is a method used in purification of mammalian synapsin I, extracted ca. 90% of the Drosophila protein. After immunoprecipitation, SLIP was phosphorylated by purified mammalian cAMP-dependent protein kinase. Immunoreactivity to the mammalian anti-synapsin I antibodies as visualized by cytochemistry was high in Drosophila nervous system. High concentrations of SLIP were also detected in the Drosophila Schneider L_2 cell line, which originated from embryonic cell dissociates. Using an antibody affinity column, we were able to purify SLIP to apparent homogeneity from adult flies. We have also detected synapsin I immunoreactivity in the moths, Manduca and Cecropia, and in the locust. The MW of the relevant polypeptides were 70 and 160 kDa (Manduca), 80, 140 and 190 kDa (Cecropia), and 4 proteins in the range of 155-225 kDa (Locusta). It thus appears that polypeptides which share antigenic epitopes and additional properties with mammalian synapsin I, but differ from the latter in their MW, exist in Drosophila as well as in other insects. The effects of Drosophila mutations in the cAMP cascade on the level and phosphorylation of SLIP are currently being investigated. (Supported in part by the Rockefeller University - Weizmann Institute Research Fund).

87.9 CHARACTERIZATION AND LOCALIZATION OF G_s AND G_o PROTEIN MOLECULES IN APLYSIA NEURONS. G.J. Chin, S.S. Vogel*, S.M. Mumby* and J.H. Schwartz. Howard Hughes Med. Inst., Columbia Univ. Col. of Phys. & Surg., New York, NY 10032 and Dept. of Pharmacology, Univ. of Texas Health Sciences Center, Dallas, TX 75235.

There is biochemical (Critz, Harper & Byrne, Neur. Lett. 64:145, 1986) and neurophysiological (Brezina, Eckert & Erxleben, J. Physiol., 1987) evidence for the transducers G_s and G_i in Aplysia. We now can detect two molecular forms of G protein molecules in nervous tissue. By Western blot analysis, a 100,000 x g membrane fraction from Aplysia neural components contained immunoreactive polypeptides that are similar in molecular weight to those of bovine brain. We used affinity-purified polyclonal antisera generated against synthetic peptides corresponding to bovine brain G protein sequences. These antisera specifically recognize 1) the α-subunit of G_s 2) the α-subunit of G_o 3) the common β subunit of the G protein family or 4) the α-subunits of G_i, G_o, and G_s (Mumby et al., PNAS 83:265, 1986). A M_r 45,800 protein was recognized by the G_{common} (α) antiserum and by two antisera directed against $G_s(\alpha)$, one against a sequence near the N-terminus and the other against a sequence near the C-terminus. A M_r 40,600 protein was recognized by both the $G_{common}(\alpha)$ and the $G_o(\alpha)$ antiserum. The $G(\beta)$ antiserum recognized a protein of M_r 36,300. We demonstrated specificity by using the original synthetic peptides to block the reactions. Aplysia $G_i(\alpha)$ was not detected, possibly because it was not resolved from $G_o(\alpha)$; alternatively it may not be present in sufficient quantity.

The subcellular distribution of Aplysia G proteins was examined using the synaptosome fraction developed by Chin, Shapiro & Schwartz (Soc. Neurosci. Abs. 11:29, 1985). Quantitation of Western blot immunostaining by densitometry revealed that the subunits recognized by the $G_o(\alpha)$, $G(\beta)$, and $G_{common}(\alpha)$ antisera are enriched 4-fold in synaptosomes. The [^{32}P]ADP-ribosylated pertussis toxin substrate with M_r 40,000 is enriched 2-fold. We are extending these studies by examining the distribution of immunoreactivity in complete sequential tissue sections of ganglia. Localization of G proteins to specific neurons is also being determined by Western blotting of dissected identified cell bodies. In parallel studies, we are also studying the localization of the effector enzymes (protein kinases and phospholipases) of the transduction pathways.

87.10 IDENTIFICATION OF NOVEL SYNAPTOSOMAL PROTEIN KINASES AND THEIR ENDOGENOUS SUBSTRATES. U.K. Misra*, O.B. McDonald* and N. Sahyoun* (Spon: Will W. Pugh) Dept. of Molecular Biology, Wellcome Research Labs., Research Triangle Park, NC 27709.

Rat forebrain synaptosomes contain one or more protein kinases whose activity is unmasked after partial purification of a Triton X-100 extract on DEAE-cellulose columns. Marked phosphorylation occurs on two endogenous polypeptides with Mr values of 78 and 50K and with the corresponding pI values of 5.3 and 5.6. Phosphate incorporation is mostly labile to KOH hydrolysis.

The phosphorylation reaction is not dependent on known protein kinase activators such as Mn^{2+}, Ca^{2+}, calmodulin, phosphatidylserine, phorbol esters, and cyclic AMP. Mg^{2+} is required at relatively low concentrations with an EC_{50} of about 0.5 mM. Phosphorylation of exogenous substrates revealed that protamine, but not histones or casein, is effectively phosphorylated. Gel-permeation analysis on AcA-34 columns results in partial separation between phospholipid/Ca^{2+}-dependent histone phosphorylation and protamine phosphorylation. The synaptosomal protein kinase(s) was further characterized by separation of the proteins from the DEAE-cellulose peak fraction on non-denaturing electrophoretic gels in the presence of Triton X-100. The protein kinase reaction was conducted in the gel itself resulting in the phosphorylation of two major components. These polypeptides may be protein kinases themselves displaying autophosphorylation or may form molecular complexes with a protein kinase.

Thus, synaptosomal protein kinases described here appear to be distinct from protein kinase C, calmodulin-dependent protein kinases, cyclic AMP-dependent protein kinases and casein kinases, and may represent a novel pathway in synaptic signalling.

87.11 ETHANOL-DEPENDENT STIMULATION OF SOLUBLE PIP-KINASE ACTIVITY IN RAT BRAIN. E.B. Stubbs, Jr.* and G.Y. Sun. Biochem. Dept. and Sinclair Research Farm, Univ. of Missouri, Columbia, MO 65203.

The primary CNS depressive effects of ethanol are considered to result from the purturbation of neural membrane biophysical and biochemical properties. Particularly affected are those enzymes which actively participate in neurotransmission. Through their involvement in signal transduction, polyphosphoinositides are considered as putative participants in neuromodulation. Capitalizing on the bimodal distribution of both phosphoinositide kinases between membrane and cytosol, we examined the effects of ethanol on both PI- and PIP-kinase activities present in purified synaptosomal and cytosolic fractions isolated from rat brain. In vitro phosphorylation of inositol lipid substrates progressed linearly in the presence of buffered medium (pH 7.4) containing Na_2ATP (0.5 mM), $MgCl_2$ (10 mM) and EGTA (1 mM) when assayed at 30°C for 60s. Under these conditions, cytosolic PIP-kinase phosphorylated exogenously added PIP at an initial rate of 24 pmol/min/mg. In comparison, purified synaptosomes, when assayed under similar conditions, phosphorylated exogenous PIP at an initial rate that was 3-fold greater than that in cytosol. Ethanol, at concentrations between 0-500 mM, enhanced cytosolic PIP-kinase activity in a dose dependent manner with maximal catalysis (2-fold) occuring between 300-500 mM ethanol. Interestingly, synaptosomal PIP-kinase activity remained uneffected by the same concentration range of ethanol. Likewise, neither cytosolic nor synaptosomal PI-kinase activities were effected by ethanol. These results demonstrate modulatory effects of ethanol on cytosolic PIP-kinase which are abolished when this enzyme is present in its membrane-associated state. (Supported in part by NS20836 and AA06661 from NIH)

87.12 IN SITU PROTEIN PHOSPHORYLATION IN CULTURED PYRAMIDAL NEURONS. W.K.Scholz and P.T.Kelly. Univ.Texas HSC, Houston, TX 77025

In situ studies using hippocampal neurons were undertaken to better understand the role of protein phosphorylation in neuronal function, especially synaptic transmission. Primary cultures of pyramidal neurons were established from 18E rat hippocampi. The time course of neuronal differentiation and synapse formation in homogeneous pyramidal cultures was similar to that observed in vivo. We have shown that Ca^{++}/calmodulin-dependent protein kinase II expression, subcellular distribution, activity, and substrate phosphorylation in differentiating pyramidal cultures display developmental changes similar to that observed in newborn rat brain. We have examined cAMP- and Ca^{++}/phospholipid-dependent protein kinase activities and endogenous substrates using in vitro phosphorylation in subcellular fractions from cultured pyramidal neurons. The potential role of each kinase in signal transduction was examined by modulating in situ protein phosphorylation using membrane permeable analogues of second messengers and conditions that affect membrane depolarization properties followed by analysis of phosphoproteins using 1-D and 2-D gels and peptide mapping. Phosphoproteins labeled in situ were then compared with endogenous substrates phosphorylated in vitro under conditions optimal for Ca^{++}/CaM-, Ca^{++}/phospholipid- or cAMP- dependent protein kinases. Pyramidal neurons cultured for 18 days were prelabeled with $^{32}P_i$ and then treated with Krebs-Ringer-HEPES buffer (KRH) alone or KRH containing 8-bromo-cAMP, 12-o-tetradecanoylphorbol 13-acetate (TPA) or tetrodotoxin (TTX). Cultures were also prelabeled and treated with Ca^{++}-free KRH/15 uM EGTA. Following the different in situ labeling conditions particulate and cytosolic fractions were prepared from each culture. Proteins which showed significant increases in ^{32}P-incorporation following treatment with 8-bromo-cAMP had M_rs of 270, 74, 52, 50-54, 37, 32 and 22K. Protein phosphorylation dependent on 8-bromo-cAMP treatment was also increased in Ca^{++}-free media. TPA stimulated the phosphorylation of 120, 87, 57, 48, 47, 46, 18 and 14K polypeptides. TTX treatment, used to block spontaneous neuronal activity, increased the labeling of 270, 87, 56, 48 and 18K proteins which were also phosphorylated under other conditions (above) and 120, 37, 33, 22 and 21K proteins which were unique to TTX treatment. Homogeneous astrocyte cultures were examined under the same conditions. Analysis of all proteins whose apparent phosphorylation was stimulated in situ, revealed only 4 that were also phosphorylated in vitro (M_r = 87, 48, 46 and 74K). The apparent subcellular distribution of in situ labeled phosphoproteins was different than those phosphorylated in vitro and suggests that certain treatments may modulate the intracellular translocation of substrates and/or kinases. Whereas both neuronal and astrocyte phosphoproteins showed different in situ labeling patterns that were altered by incubation conditions, most of the labeled proteins were cell-type specific.

87.13 INFLUENCE OF SYNAPTOSOMAL CHOLESTEROL ON DOPAMINE UPTAKE. Patricia A. Maguire* and Mary J. Druse-Manteuffel. Department of Biochemistry, Loyola University Stritch School of Medicine, Maywood, IL 60153.

During aging and development, there is an increase in the cholesterol/phospholipid ratio (c/p) of synaptic membranes, a decrease in membrane fluidity and alterations in the components of several neurotransmitter systems. Since many membrane-associated functions are profoundly influenced by membrane fluidity, it is possible that at least some of the age-related changes of the neurotransmitter systems are due to alterations in the c/p and fluidity of the synaptic membranes.

In an attempt to investigate this hypothesis, we examined the influence of the c/p on a membrane-associated function of the dopaminergic system. The cholesterol content of synaptosomal membranes was altered using a lipid transfer protein (North, P. and Fleischer, S., Methods Enzymol. 98: 599, 1983) which facilitates the rapid transport of cholesterol between liposomes and synaptosomal membranes. Synaptosomal uptake of 0-5000 nM dopamine was determined in the presence and absence of 10 μM of nomifensine. Fluorescence polarization was used to assess the fluidity of the hydrophobic core (DPH) and surface (ANS) of the synaptic membranes.

Alterations in the c/p were accompanied by changes in dopamine uptake and by changes in relative microviscosity at both the surface and core of the membrane. Many of these alterations were found at c/p within the range of values found during development and aging.

The data obtained using DPH and ANS appear to fit a sigmoidal relationship, with inflection points at c/p within 10% of the value for unmodified membranes. At 37 °C, a 10% increase above the control c/p produced a 75% increase in the relative microviscosity at the membrane core, while a 10% decrease produced a 30% decrease in microviscosity.

Within the physiological range of c/p (control value ± 30%), the K_m increased ∿3-fold, or decreased ∿50% depending on whether the c/p was increased or decreased, respectively. The V_{max} for dopamine uptake showed a similar relationship to c/p. As the synaptosomal c/p increased above 0.50, there was an approximately 35% decrease in the V_{max}. The first order rate constant (V_{max}/K_m) decreased with increasing c/p.

The results of the present studies demonstrate that the transmembrane process of dopamine uptake may be inhibited by elevated synaptic c/p and increased microviscosity. These results may be relevant to the changes in dopamine uptake observed with aged animals.

87PO CHARACTERISTICS OF A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM ADULT CANINE HIPPOCAMPUS (H). P. Siekevitz and K. Wu*, Lab. Cell Biol., Rockefeller University, New York, N.Y. 10021

PSDs were isolated in a manner similar to that of cerebral cortex (CTX) PSDs (Wu et al., Mol. Brain Res. 1: 167, 1986) with a yield of 0.3 mg protein/gm wet weight. Electron micrographs and SDS-gel electrophoresis showed that the fraction was similar to CTX-PSDs except that the H-PSD had proportionally higher fodrin, calmodulin, and the major 51 KD protein content but lower actin content than the CTX-PSDs. The H-PSD have one specific GABA binding site, with a Kd of 89 nM (CTX-PSD=62) and a Bmax of 12.3 pmoles/mg (CTX-PSD=17.8). They also have a specific glutamate binding site, with a Kd of 640 nM (CTX-PSD=418) and a Bmax of 36.9 pmoles/mg (CTX-PSD=28.9). EGTA (0.2 mM), Ca^{2+} (0.5 mM) plus EGTA (0.2 mM), or Mg (1 mM) had no effect on this binding. Glutamate binding to H-PSD and CTX-PSD were similarly inhibited by 10 μM AP5 (66% and 61%), by 20 μM NMDA (61% and 52%), and by 5 μM quisqualate (41% and 38%), while AP4 gave no inhibition in both cases. Contrary to Kessler et al. (J. Neurosci. 6: 355, 1986) pre-incubation of our H-synaptic membrane (H-SM) or H-PSD preparation with 10 mM glutamate showed no effect on subsequent Cl^--dependent or Cl^--independent glutamate binding. The H-PSD bound the Ca^{2+} channel blocker, nitrendipine, with a Kd of 179 pM (CTX-PSD=207) and a Bmax of 216 fmoles/mg (CTX-PSD=196). Calmodulin was necessary for this binding, similar to CTX-PSD, since 10 μM R24571 inhibited it by 95%, and since EGTA-pretreated H-PSD lost 95% of the binding, and the addition of calmodulin plus 1.5 mM Ca^{2+} restored 90% of the binding. The Ca^{2+}/calmodulin-dependent and cAMP-dependent phosphorylations of H-SM and H-PSD proteins were the same as those of CTX-SM and CTX-PSD proteins. The data so far suggest that H-PSD may be useful in studies on the postulated involvement of the hippocampus in learning or memory.

SYNAPTIC STRUCTURE AND FUNCTION II

88.1 SYNAPTIC TRANSMISSION AT THE ONSET OF MORPHOLOGICALLY MIXED SYNAPSES. K.D. Peusner and C. Giaume*. Lab. de Neurobiologie Cellulaire, Institut Pasteur, 75724 Paris Cédex 15, France.

The tangential vestibular nucleus (TN) of the chicken is composed essentially of two types of neurons, principal cells (PC, 80%) and elongate cells (EC, 20%). The EC are innervated solely by vestibular fibers of small diameter, but the PC receive two types of vestibular afferents, the colossal vestibular fibers, which form the spoon endings (SE), and finer vestibular fibers. Ultrastructural quantitative data have shown that the SE form numerous chemical synapses on the PC in 15-16 day embryos (dE), but few gap junctions which first appear at this age. This numerical relation reverses at hatching time (Peusner, 1984). Thus, in an in vitro electrophysiological study, we asked (1) what are the membrane properties of the two neuron types in TN and (2) what is the mode of transmission between SE and PC when gap junctions first appear in 15-16 dE?

The two neuron types can be distinguished by their membrane responses to intracellular current application and their synaptic potentials evoked by vestibular stimulation. These identifications were confirmed by intracellular injections of Lucifer Yellow. Using KCl electrodes, EC have a membrane potential of -56 ± 10 mV (n=11) and an input resistance of 38 ± 18 MΩ (n=7); EC exhibit an inward rectification to hyperpolarization and repetitive discharge of action potentials when depolarized. The EC respond to vestibular stimulation with a monophasic EPSP (1.8 ± 0.3 msec latency) which increases in a graded way as a function of stimulus intensity. In contrast, PC (-56 ± 7 mV, n=14; and 25 ± 7 MΩ, n=7) demonstrate a linear current-voltage relationship for negative currents, and respond with at most one or two spikes to positive currents. Typically, on synaptic depolarization, PC show two responses with distinctive latencies and thresholds. The first response is all-or-none (latency 1.7 ± 0.3 msec) and is attributed to the firing of the colossal vestibular fibers via the SE, while the second response consists of graded synaptic potentials which may be due to the activation of the slower conducting fibers.

Thus, in 15-16 dE (i.e. when gap junctions first appear), synaptic transmission at the SE is predominantly of the chemical type, given: (1) the long latency of the synaptic response; (2) a dependency of the EPSP's amplitude on membrane potential; and (3) the absence of presynaptic fluorescence after Lucifer Yellow injection into the PC. In contrast, preliminary results obtained at 5 days after hatching show that the PC respond to vestibular stimulation with a shorter latency (<1 msec), suggesting that transmission may be in part electrical at this age. Supported in part by NIH grant R01 NS18108.

88.2 THE FATE OF SYNAPTIC VESICLE MEMBRANE FOLLOWING TRANSMITTER RELEASE. K. Ikeda and J.H. Koenig. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The present study investigates the fate of synaptic vesicle membrane following transmitter release. It is presently well accepted that transmitter is released by exocytosis of synaptic vesicles, which involves the insertion of the vesicle membrane into the plasma membrane. This membrane is then retrieved by the process of endocytosis and recycled into new vesicles.

In the temperature sensitive mutant, shibire^{ts}, of Drosophila melanogaster, synaptic vesicle recycling is blocked at the pit formation stage if the temperature is raised to 29°C. This causes a gradual depletion of synaptic vesicles if transmitter release is allowed to proceed normally. One explanation for this phenomenon would be that transmitter is released by synaptic vesicle exocytosis, and that in this mutant at 29°C the vesicle membrane cannot be recovered from the plasma membrane by endocytosis for recycling, so that depletion occurs. This implies that in a depleted terminal in shi, all of the vesicle membrane has been incorporated into the plasma membrane.

The terminals of the cervical muscle in Drosophila are normally packed with vesicles, so that the total vesicle membrane far exceeds the amount of plasma membrane of the terminal. Thus, if all the vesicle membrane were inserted into the plasma membrane, a very significant increase in the perimeter of the terminal should be observed. However, in shi at 29°C, no significant increase in the perimeters of depleted cervical muscle terminals was observed, although a small number of pits were observed on the terminal membranes at this time. As long as the temperature was kept at 29°C, no significant increase in membrane was seen. Serial sectioning did not reveal any increase in membrane away from the terminal (eg., axonal membrane) to compensate for this large loss of membrane. When the temperature was lowered to 19°C, a gradual increase in the perimeter of the terminal occurred in the form of large pits and invaginations. As recycling progressed, more and more membrane was observed, first in the form of invaginations, then cisternae, and finally vesicles.

These observations suggest that vesicle membrane may not be inserted into the plasma membrane upon transmitter release, but rather may dissemble when transmitter is released and reassemble at points along the plasma membrane of the terminal away from the active zone.

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88.3 SYNAPTIC VESICLE NUMBER CORRELATED WITH EJP AMPLITUDE.
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The relationship between the number of vesicles in the terminal and the amount of transmitter released from that terminal was investigated using the temperature-sensitive endocytosis mutant of Drosophila, shibire^{ts1} (shi). In this mutant at 29°C, endocytosis is blocked at the pit formation stage. Thus, in the shi neuron at 29°C, depletion of synaptic vesicles occurs as a result of exocytosis (transmitter release) proceeding normally while endocytosis (recycling) is blocked. Using this mutant, the number of vesicles in the synapse can be controlled by bringing the temperature to 29°C and inducing transmitter release until the desired degree of depletion is reached. In this way, the number of vesicles in the synapse (measured by electronmicroscopy) was correlated with the amount of transmitter released (measured by e.j.p. amplitude).

The neuromuscular junctions of the dorsal longitudinal flight muscle (DLM) were used in this study. Intracellular recordings were made from DLM fiber #6, which has a length constant longer than the length of the muscle fiber and is therefore isopotential. This muscle fiber is multiterminally innervated by a single motor neuron. The excitatory junction potential (e.j.p.) was recorded at 29°C while stimulating at 0.5 Hz. A gradual reduction in the e.j.p. was observed, and at various e.j.p. amplitudes the muscle fiber was instantly fixed for electronmicroscopy. The average number of vesicles/synapse for many synapses on the muscle fiber was calculated and correlated with the e.j.p. amplitude for that fiber.

It was observed that as the e.j.p. amplitude decreased, the average number of vesicles in the synapse also decreased. Also, the number of synapses with no vesicles increased with decreasing e.j.p. amplitudes. Thus, a reduction in the e.j.p. amplitude correlated with a reduction in the number of vesicles in the synapses, and also correlated with a reduction in the number of functional (undepleted) synapses. These results suggest that synaptic vesicles are directly related to the release process.

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88.4 ACTIVE ZONE STRUCTURE AT LOBSTER HIGH- AND LOW-OUTPUT SYNAPSES.
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Quantal neurotransmitter release occurs at discrete sites in the axon terminal called active zones. These sites are recognizable in freeze-fracture views as clusters of large intramembrane particles (active zone particles). The location of transmitter release sites within 50 nm of the particles, suggests that these particles affect transmitter release. To determine the relationship between the number of particles at the active zone and the amount of transmitter released, active zone particles were counted in freeze-fracture views of lobster neuromuscular synapses. In the lobster, Homarus americanus, a single excitatory axon innervates the distal accessory flexor muscle. This axon releases 3 to 10 times more transmitter from high-output synapses in the most distal portion of the muscle than from low-output synapses on the most proximal portion of the muscle. The most distal and proximal muscle fiber bundles were dissected from glutaraldehyde-fixed and glycerinated muscles and freeze-fractured. Axon terminals contained between 4 and 30 synaptic contacts each containing one or two active zones. The density of synaptic contacts and the number of active zones was similar in high- and low-output terminals. Active zone particles were counted in synapses in 6 high-output and 6 low-output axon terminals from 7 animals. High-output active zones contained 20 $\pm$ 5 (n=76) intramembrane particles arranged in a cluster 140 $\pm$ 36 nm long and 69 $\pm$ 10 nm wide. Low-output active zones contained 12 $\pm$ 2 (n=44) intramembrane particles arranged in a cluster 78 $\pm$ 12 nm long and 67 $\pm$ 10 nm wide. Although the longer high-output active zones can accommodate 50% more vesicles (6 vs. 4), this difference cannot account for the 3 to 10 fold difference in the amount of transmitter released from high- and low-output synapses. The correlation between the distribution of active zone particles and the amount of transmitter released is consistent with the active zone particles being the Ca^{2+} channels which couple the action potential to neurotransmitter release. One explanation for the nonlinear relationship between the quantity of transmitter released and the number of active zone particles is that the particles govern the local intracellular Ca^{2+} concentration at the active zone following a nerve impulse. If transmitter release depends on the fourth power of the Ca^{2+} concentration, increasing the Ca^{2+} concentration by 1.7 times (20 vs. 12 channels) would lead to a nearly 8 fold increase in the amount of transmitter released (1.7^4=8.3). This difference is in good agreement with the 3 to 10 fold difference in transmitter release observed physiologically.

88.5 MORPHOLOGICAL PROXIMO-DISTAL GRADIENTS ALONG THE FROG NMJ: A SCANNING ELECTRON MICROSCOPIC STUDY.
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Compiling physiological evidence suggests the existence of proximo-distal gradients in spontaneous and evoked release probability along the frog NMJ (Robitaille and Tremblay, Br Res Rev 12, 95, 1987). Scanning electron microscopy permits to verify the existence of corresponding morphological gradients. Cutaneous pectoris muscles were fixed with 2.5% gluteraldehyde and presynaptic terminals removed by consecutive collagenase and HCl treatments. The complete endplate region of 34 NMJs were photographed at X3000 or X5000. Between 2 and 14 branches of primary cleft were observed per endplate. Postjunctional folds (PJFs) were present at the bottom of these clefts and were usually perpendicular to the long axis of the cleft. The length and the position of more than 14,000 PJFs were measured along the clefts (mean density: 2.12 PJFs/µm of cleft). For each junction, the one or two longest branches of primary cleft generally showed proximo-distal gradients in: 1) the number of PJFs/µm of cleft, 2) the average length of PJFs and 3) the total length of PJFs/µm of cleft. The longest branches (100-120µm) have a 4 times smaller total length of PJFs/um of cleft in the distal region than in the proximal one (near the motor axon entry). This gradient in the total length of PJFs reflect a similar gradient in the length of active zones along the nerve terminal since most of the folds are located in front of an active zone (AZ). This gradient may be responsible for the lower spontaneous and evoked release probability observed in the distal regions. The gradient in the average length of PJFs could be responsible for the smaller MEPP amplitude in distal regions (Robitaille et al., Neur. Lett., 74, 187, 1987). The crest of the PJFs is covered with ACh receptors sites at a density of 26,000/µm^2. It therefore takes a crest surface of 0.3 µm^2 to have enough receptor site to have a 1 to 1 relation between the number of ACh molecules (10^4) in a quantum and the number of receptor sites (Matthews-Bellinger and Salpeter, J Physiol 279, 197, 1978). The crest surface (0.3 to 0.4 µm^2) of the smaller PFJs (0.4 µm) is very close to this critical area. Therefore when a vesicle release its content near the edge of a fold or when anticholinesterases are present, the number of receptors within reach by diffusion may limit the size of the MEPP. This may be a postsynaptic mechanism partly responsible for reducing the MEPP size in the distal region. However according to the multivesicular theory (reviewed by Tremblay et al. Br Res Rev 6, 299,1983) these smaller MEPPs may also be due to limited capacity of small AZs (located in front of short PJFs) to liberate simultaneously several vesicles.

88.6 DIFFERENTIAL EFFECT OF α-LATROTOXIN ON THE RELEASE OF ACETYLCHOLINE AND OF CALCITONIN GENE-RELATED PEPTIDE AT THE FROG NEUROMUSCULAR JUNCTION.
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Within axon terminals, classical neurotransmitters are stored in, and released from, the small synaptic vesicles (SSVs) that populate the terminals, whereas peptide neurotransmitters are stored in a different class of organelles, the large dense-core vesicles (LDCVs). LDCVs, in general, represent a minor component of the overall vesicle population. Co-secretion of classical and peptide neurotransmitters occurs in a variety of experimental conditions. However, the relative amounts of the two types of transmitter secreted appear to vary depending on the stimulation conditions.

Recently, the peptide neurotransmitter calcitonin gene-related peptide (CGRP) has been localized at mammalian motor nerve endings, and we have found by light and electron microscopy immunocytochemistry that CGRP immunoreactivity is also present at the frog neuromuscular junction (FNMJ), where it appears to be localized in LCDVs. The latter findings make it possible to study the differential release of a peptide and a classical neurotransmitter at one of the most well-characterized synapses. As a first step in identifying a possible difference in the regulation of the release of CGRP and of the quantal release of acetylcholine (ACh) at the FNMJ, we have investigated whether treatment with α-latrotoxin (α-LTx), which is known to induce complete depletion of ACh-containing vesicles, also induces depletion of CGRP. Frog cutaneous nerve-muscle preparations were incubated at low temperature (2°C) in the presence or absence α-LTx (1 µg/ml). The effectiveness of α-LTx treatment in depleting the terminals of quanta of ACh was assessed electorphysiologically. The preparations were fixed and processed either for CGRP immunofluoresecence or for conventional electron microscopy.

A prominent punctate pattern of CGRP immunofluoresence was observed not only in control terminals but also in terminals treated with α-LTx. Consistent with this observation, a morphometric analysis carried out on electron micrographs of terminals cut in cross section revealed no significant decline of the number of LDCVs in α-LTx-treated terminals compared to control terminals. In contrast, α-LTx-treated terminals were almost completely depleted of SSVs. These findings indicate that, at least under certain experimental conditions, the release of SSVs and of LDCVs from FNMJ can be dissociated and suggest the existence of differences in the cellular mechanisms that lead to the exocytosis of the two types of vesicles. (Supported by MDA grants to PDC & BC).

88.7 THE INFLUENCE OF CELL-CELL CONTACT ON THE DISTRIBUTION OF SYNAPSIN I IN HIPPOCAMPAL NEURONS IN CULTURE.
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We have used light and electron microscopic immunocytochemistry to study the role of cell interactions in the localization of synapsin I, a synaptic vesicle-associated phosphoprotein, in hippocampal neurons developing in culture. Cells were prepared from 18 day old rat hippocampi by dissociation with trypsin, plated onto polylysine-treated coverslips, and maintained in serum-free medium. Under these conditions, cells elaborate extensive axonal and dendritic arbors and form numerous synapses.

In mature cultures, stained for synapsin I by immunoperoxidase, synapsin I immunoreactivity was localized in large puncta along neuronal cell bodies and dendrites. Non-neuronal cells did not contain synapsin I. Electron microscopic immunocytochemistry demonstrated that the synapsin I-containing puncta corresponded to vesicle-filled axonal profiles that were exclusively presynaptic. Double-label immunofluorescence experiments, using antibodies against MAP2 to stain somata and dendrites, demonstrated that foci of synapsin I accumulation occurred predominantly at the sites of contact between axons and either dendrites or cell bodies. This pattern of staining was already apparent after one week in culture.

At early stages of development, before cell contacts, synapsin I was expressed by neurons, but its distribution was different. At the level of resolution of light microscopy, it appeared rather diffusely distributed throughout the cell. To determine the influence of cell-cell contact on synapsin I localization, low density cultures were prepared, in which some cells develop without contacting other cells. Even after 2 weeks under such conditions, isolated cells failed to localize synapsin I into large puncta. Neighboring cells in the same cultures that had made contacts developed foci of synapsin I in their axons.

In conclusion, synapsin I is expressed in hippocampal neurons in culture even in the absence of cell-cell contact, but under such conditions synapsin I is diffusely distributed throughout the cells. It appears that the aggregation of synapsin I into large puncta within the axon follows contact between an axon and the somata or dendrites of other cells. This probably reflects the formation of synaptic specializations. (This research was supported by NIH grant NS17112 to G.B. and an MD grant to P.DC.)

88.8 REGULATION OF ACTIN ASSEMBLY IN SYNAPTOSOMES DURING DEPOLARIZATION.
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Depolarization of synaptosomes from mouse whole brain causes a reversible polymerization of actin, the major cytoskeletal protein of terminals. Filamentous actin (F-actin) levels fluctuate 2-3 fold during a 9 sec depolarization with 75mM K^+/2mM Ca^{++} Hepes buffer, a procedure that elicits transmitter release. Depolarization was terminated after 0, 3, 6, and 9 sec by addition of glutaraldehyde, followed by staining with phalloidin conjugated to the fluorophor, rhodamine. Relative changes in F-actin levels were measured by quantitating fluorescence intensity, an indicator of phalloidin bound by the synaptosomes. F-actin cycling also occurs if synaptosomes are depolarized with calcium free buffer or are treated with a calcium containing, non-depolarizing buffer. However, F-actin levels are constant in non-depolarizing, calcium free buffer. Dioctanoylglycerol (an activator of protein kinase C) enhances the polymerizing effect of depolarization.

Phalloidin, a mushroom toxin and bicyclic heptapeptide, binds specifically and in a 1:1 molar ratio with only F-actin, not monomeric actin. Rhodamine phalloidin binding to these fixed synaptosomes is saturable and is reduced 50% by omission of membrane permeabilization, 30% by treatment with pancreatic DNase I (a specific depolymerizing agent of F-actin), and 100% by competition with unlabelled phalloidin. Others have found that phalloidin binds to both long and short actin filaments in all regions of fixed cells, regardless of the particular actin binding proteins regionally present. Therefore, the cycling of staining intensity herein reported probably reflects polymerization/depolymerization rather than filament severing or fluctuation in the accessability of filaments to stain due to depolarization induced changes in actin binding proteins. Earlier reports on lysed synaptosomes indicated that depolarization causes a net reduction in actin associated with the cytoskeleton and that both stabilizing and destabilizing factors are activated (Bernstein, BW and Bamburg, JR, J. Neurosci., 5:2565-2569, 1985; Bernstein, BW and Bamburg, JR, Neurochem. Res., in press). This report on intact synaptosomes shows that the net effect of stimulating these counterbalancing factors is a fluctuation in F-actin levels.
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88.9 THE EFFECTS OF EXTRACELLULAR CALCIUM CONCENTRATIONS ON THE IN VITRO RAT HIPPOCAMPUS.
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In vivo the extracellular calcium concentration ($[Ca^{++}]_o$) is known to vary from its baseline level of 1.5 mM and in vitro brain slice studies have been done using various $[Ca^{++}]_o$. Therefore, we examined how $[Ca^{++}]_o$ affected synaptic excitability, membrane stabilization, response to paired-stimuli, and long-term potentiation (LTP) in the rat hippocampal slice. 500 µm slices were prepared and stored in an artificial CSF containing (in mM): NaCl 127, KCl 2, $MgSO_4$ 1.5, KH_2PO_4 1.1, $NaHCO_3$ 25.7, $CaCl_2$ 1.5, and glucose 10. $[Ca^{++}]_o$ was altered by perfusing slices with a solution in which only the concentration of $CaCl_2$ had been adjusted. Field potentials were recorded in the CA1 region while stimulating the Schaeffer collateral inputs.

In every slice tested, the input-output curve in 2.5 mM or 3.5 mM $[Ca^{++}]_o$ was shifted to the left of the corresponding one in 1.5 mM $[Ca^{++}]_o$. Increasing $[Ca^{++}]_o$ also reduced the tendency towards multiple spiking. Decreasing $[Ca^{++}]_o$ to 1.25 or 1.0 mM caused either no effect or a slight depressant effect on the input-output curve. Lowering $[Ca^{++}]_o$ from 1.5 to 0.75 mM led to epileptiform discharges in all slices.

Paired-pulse phenomena were studied at an interstimulus interval of 20 msec using two methods. The first method involved determining input-output curves for the population spikes in response to both the first (conditioning) and second (test) stimuli. The second method determined the threshold for action potential firing to both stimuli. The difference between the two thresholds is a sensitive and convenient measure of paired-pulse facilitation and inhibition. Using input-output curves, in 1.5 mM $[Ca^{++}]_o$ a population spike appeared in response to the test stimulus, and became maximal, before a population spike appeared in response to the conditioning stimulus, indicating facilitation. Inhibition was only seen at high stimulus intensities in 2.5 and 3.5 mM $[Ca^{++}]_o$. Only facilitation was seen using the single unit threshold method.

LTP was induced by a train of 25 stimuli (50 Hz/500 msec) at the stimulus intensity that produced a just maximal population spike. LTP was found to be very sensitive to $[Ca^{++}]_o$: maximal in 1.5 mM, absent in 1.0 mM and intermediate in 1.25 mM. LTP was present, but less prominent in $[Ca^{++}]_o$ greater than 1.5 mM. These studies show that a variety of synaptic processes are altered by changes in $[Ca^{++}]_o$ over the range in which $[Ca^{++}]_o$ varies in vivo.

88.10 HIGH CALCIUM INDUCED SPONTANEOUS ACTIVITY IN ISOLATED SPINAL CORD.
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Calcium ions have multiple effects at synaptic junctions and their predominant action varies in different tissues. We exposed the spinal cords of infant mice in vitro to varying $[Ca^{2+}]_o$ levels. The mice were anesthetized with ether and their spinal cords were removed and hemisected. Dorsal and ventral root (DR and VR) responses were recorded with suction electrodes and interstitial calcium ($[Ca^{2+}]$) and focal potential (FP) was recorded in dorsal horn (DH) with ion selective microelectrodes. Control ACSF contained 3.5 mM K^+, 1.2 mM Mg^{2+}, 1.2 mM Ca^{2+}. Lowering $[Ca^{2+}]_o$ resulted in depression of the DR reflex, of the segmental VR reflex, and of slow DR potentials (DRP). Raising $[Ca^{2+}]_o$ to 1.8 mM resulted in enhanced reflex transmission. Further elevation of $[Ca^{2+}]_o$ caused selective increase of polysynaptic discharge. In some spinal cords at 2.4 mM and in all others at 3.6 mM $[Ca^{2+}]_o$ spontaneous irregularly recurring potential waves appeared, the amplitude and frequency of which increased further at 4.8 mM $[Ca^{2+}]_o$. The spontaneous waves were similar to evoked "slow" DR and VR responses and were reminiscent of paroxysmal discharges induced by penicillin in spinal cords in situ. They occurred in largest number in DR, but synchronized discharges were also frequent in VR and in FP of DH. The largest of the paroxysmal events approached in amplitude the maximal responses evoked by DR stimulation indicating high degree of synchronization among neurons. The NMDA antagonist APV (100µM) decreased the frequency and amplitude of the spontaneous discharges with little effect on synaptic transmission. After restoring normal bath $[Ca^{2+}]$, the paroxysmal discharges subsided more slowly than did tissue $[Ca^{2+}]_o$. The explanation of this type of paroxysmal activity may be that excess $[Ca^{2+}]_o$ may overload outward transport and thus gain access to cytoplasm; then inducing irregularly recurring inward current in interneurons with profusely branching terminal axon arbors, which then act as pacemakers to depolarize DR fiber terminals, resulting in primary afferent depolarization and simultaneously the release of excitatory transmitter onto postsynaptic cells including motoneurons. The slow subsidence of paroxysmal waves may indicate slow unloading of excess $[Ca^{2+}]_i$.

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88.11 AFTEREFFECTS OF EXPOSURE TO VERY HIGH $[K^+]$ ON SYNAPTIC FUNCTION IN HIPPOCAMPAL TISSUE SLICES. K. Kawasaki*, G. Czéh* and G.G. Somjen (SPON: S.J. Schiff). Dept. of Physiology, Duke Univ. Med. Ctr., Durham, NC 27710.

This study explores whether Leão's spreading depression (LD) of extended duration causes lasting damage to neuronal function. Slices were cut from hippocampi of young rats and maintained in an interface chamber aerated with 95% O_2, 5% CO_2 and perfused with artificial cerebrospinal fluid (ACSF) at 36°C, containing 1.2 mM each of Ca^{2+} and Mg^{2+}, and 3.5 mM K^+, flowing at 2 ml/min. Interstitial potassium ($[K^+]_o$) and extracellular potentials evoked by stimulation of the Schaffer collateral-commissural fibers were recorded in st. radiatum and st. pyramidale of CA1 sector with ion selective microelectrodes. LD was provoked by high-$[K^+]$ ACSF (133 mM K^+) flowing from tubing positioned over the slice, at 0.6 ml/min, for periods ranging from 10 sec to several min. High $[K^+]$ exposure in excess of 25 sec reliably provoked LD, recognized by a sudden negative shift of extracellular potential and elevation of tissue $[K^+]_o$ above 20 mM. Potential and $[K^+]_o$ returned to control level after a variable time following cessation of high $[K^+]$ application. LD duration was defined as the time from onset of $[K^+]_o$ elevation until 1/2 decay of $[K^+]_o$ (return to 50% of maximal level). Synaptic recovery was tested for 60 or more min following high $[K^+]$-ACSF administration. After brief periods of LD, synaptic transmission either returned to control level, or showed hyperexcitability of CA1 pyramidal cells. Hyperexcitability was manifested by increased orthodromic population spike amplitude relative to unchanged or depressed fEPSP, and multiple population spikes evoked by single afferent volleys. After more prolonged periods of LD, synaptic transmission was impaired. In many cases synaptic transmission at first recovered from LD (with or without signs of hyperexcitability), but then failed. The amplitude of the recovered population spike (expressed as % of control, measured 60 min after high $[K^+]$ administration) was inversely correlated with the duration of LD (range 80 sec to 10 min). The results indicate that LD can cause lasting damage to synaptic function. The aftereffects of LD in well-oxygenated slices resemble those of oxygen withdrawal.
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88.12 HYPOXIC DEPOLARIZATION AND LOSS OF SYNAPTIC FUNCTION IN CA1 AND DENTATE GYRUS OF HIPPOCAMPAL TISSUE SLICES. M. Balestrino and G.G. Somjen. Dept. of Physiology, Duke University Medical Center, Durham, NC 27710.

It has been reported that after transient oxygen withdrawal, synaptic transmission is more likely to recover in dentate gyrus (DG) than in CA1 sector of hippocampal tissue slices (Kass and Lipton, J. Physiol.378:313, 1986; Aitken and Schiff, Neurosci. Lett. 67:92, 1986); and that spreading depression (SD)-like hypoxic depolarization and the associated calcium loading of cells is a factor in irreversible loss of synaptic function after transient hypoxia (Balestrino and Somjen, Brain Res.385:219, 1986). We now compared the SD-like potential shifts, and the changes of extracellular calcium ($[Ca^{2+}]_o$) in CA1 and DG regions. Responses were evoked in CA1 by stimulating Schaffer-commissural fibers, and in DG by stimulating perforant path. Evoked potentials and $[Ca^{2+}]_o$ were recorded with extracellular ion selective microelectrodes. Oxygen was withdrawn from slices for variable times. In CA1, synaptic transmission was blocked sooner and tended to recover less often than in DG. During hypoxia, SD-like sustained potential shifts with associated decrease of $[Ca^{2+}]_o$ occurred in most slices in both DG and in CA1, and voltage and $[Ca^{2+}]_o$ returned to baseline upon reoxygenation. In 4 out of 19 slices, no SD-like sudden potential shift was recorded in DG, only a slow, gradual negative shift. The degree of post-hypoxic recovery of orthodromic population spike amplitude was inversely correlated with the duration of SD-like hypoxic depolarization. This correlation held for both DG and CA1, but even for equal time spent in SD, synaptic function recovered better in DG than in CA1. The magnitude of SD-related decrease of $[Ca^{2+}]_o$ was not different in DG from CA1. Addition of $CoCl_2$ (2 mM) to the bathing solution before and during hypoxia did not prevent the SD-related decrease of $[Ca^{2+}]_o$. We conclude that, even though SD-like depolarization is a factor in hypoxic neuron damage, the greater resistance of DG to hypoxia is not fully explained by a difference in susceptibility to SD-like depolarization.
(Supported by grant NS 18670)

88.13 PERSISTENT DECREASE IN INHIBITION AFTER RECURRENT HIPPOCAMPAL SEIZURES. J. Kapur, J.L. Stringer, E.W. Lothman. Department of Neurology, University of Virginia, Charlottesville, VA 22908.

It has been shown that GABA-mediated inhibition in the hippocampus decreases during electrically-induced seizures. Neither the duration of this alteration nor the effects of repetitive seizures on inhibition have been elucidated. These questions were examined in this study.

Adult albino rats were anesthetized with urethane. A stimulating electrode was positioned in one hippocampus and extracellular field potentials were recorded from the stratum pyramidale of the contralateral CA1. Stimuli (0.1 Hz, 0.4 msec) of increasing intensity were used to generate input-output curves for population spikes (PS). Inhibition was measured as the ratio of the amplitude of the second (test) PS to the amplitude of the first (conditioning) PS in a paired-pulse paradigm in which two equal intensity pulses were delivered at different (20-500 msec) interpulse intervals (IPI). Variation of inhibition as a function of the first PS amplitude was removed by appropriate adjustments of stimulus intensity. Input-output curves and inhibition vs. IPI curves were stable for 9 hrs.

Recurrent seizures were produced every 5 min for 6 hrs by 50 Hz, 10 sec stimulus trains of 1 msec pulses. There was a significant decrease in inhibition that persisted for 2 hrs after the last seizure (at which time the experiment was terminated). Typically there was also a rightward shift of the input-output curve. One seizure produced by delivering a single tetanus (50 Hz, 10 sec of 1 msec biphasic pulses) was followed by a decrease in inhibition 5-15 min later. The change in inhibition was smaller after the single seizure than after recurrent seizures and had returned to control values by 60 min. After a single seizure, the input-output curve was shifted to the left and remained so for at least 60 min. A less intense tetanus (50 Hz, 0.4 msec pulses for 400 msec) caused a leftward shift of the input-output curve but did not elicit a seizure and did not alter inhibition.

These results indicate that seizures cause a diminution of GABAergic inhibition in the CA1 region of the hippocampus that persists in the postictal period. After a single seizure, this diminution lasts less than an hour; after recurrent seizures, a greater diminution in inhibition persists for at least 2 hrs. The alterations in paired pulse inhibition can be dissociated from any particular change in input-output curves. We suggest that such a decrease of GABAergic inhibition may be involved in the development of various epileptic conditions such as kindling or status epilepticus.

88.14 CORRELATION OF DYE AND ELECTROTONIC COUPLING BETWEEN HIPPOCAMPAL NEURONS IN CULTURE. M. O'Beirne and B.A. MacVicar. Neuroscience Research Group, Univ. of Calgary, Calgary, Alberta, T2N 4N1, Canada

Dye coupling has been demonstrated in the in vivo hippocampus, in the hippocampal slice preparation and in dissociated culture of hippocampal neurons. Although electrotonic coupling has also been demonstrated in the slice and in tissue culture there is no study correlating the two in the hippocampus. These experiments have been designed to determine the extent of electrotonic coupling between dye-coupled hippocampal neurons in dissociated culture.

Hippocampi were removed from 18 day gestational rat fetuses and mechanically dissociated through a fire polished pasteur pipette. The cells were plated on polylysine coated glass coverslips and grown in DMEM + 10% HS at 37°C for up to 4 weeks. Neurons were impaled with glass microelectrodes filled with 5-carboxyfluoroscene (5-CFL). During the injection of 5-CFL, neurons were observed using a SIT video camera so that a low intensity of illumination could be used to visualize the fluorescent dye. If the cell was dye coupled to another, the second stained cell was simultaneously impaled with a microelectrode filled with 0.15 M KCL.

In 3/3 cases where neurons were dye coupled, the cells were also electrotonically coupled (with coupling ratios of .1, .2, .7). When released extracellularly into the dish, dye was never picked up by cells. Staining was observed only if dye was injected with current into the impaled cell. Stained cells quickly lost dye when damaged and mechanical movement of single stained cells did not cause artifactual dye coupling. Electrotonic coupling was never observed between non dye coupled cells. Stable recordings were obtained from neurons as long as the epifluorescence illumination was of low intensity. When the intensity was increased, action potentials broadened and neurites beaded and released dye. These events did not induce either dye or electrotonic coupling. These experiments demonstrate that dye-coupling indicates the presence of electrotonic coupling. Supported by MRC of Canada, AHFMR, and the Sloan Foundation.

88.15 COMPARATIVE SENSITIVITY ANALYSIS OF PARAMETERS FOR SYNAPTIC INPUT-OUTPUT FUNCTION

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For synapses, various structural and functional changes have been postulated or correlated with memory mechanisms. When a synapse is viewed as a sequence of signal processing steps, it can be described as a dynamic system with time functions. Using a computational model which describes presynaptic terminal shape, transmitter release, postsynaptic receptor site, and the cable equation for dendritic spines, a comparison of parameters is made as to their net result on postsynaptic response potential. Due to the nonlinear functions, the resultant equations are solved numerically.

With repeated use of a synapse, structural changes have been reported in the literature which correspond to parameters changes in the geometry on which the signals are carried. These changes may be the underlying mechanisms of memory. Initial calculations show that an increase in the number of postsynaptic receptor sites (hence maximum available postsynaptic conductance) and an increase in transmitter synthesis rate are more influential than volume changes of pre or postsynatic regions. Rate of change of transmitter release rate versus rate of growth of receptor sites determine which has more influence on overall synaptic transmission.

AUDITORY SYSTEM II

89.1 THE EFFECTS OF 8TH NERVE TRANSECTION ON THE NEURONAL AREA OF NUCLEUS MAGNOCELLULARIS IN THE RED-EARED TURTLE, CHRYSEMYS SCRIPTA ELEGANS. R.H. BROWNER, M. NIRENBERG AND B. WACHTEL. Dept. of Anatomy, New York Medical College, Valhalla, N.Y. 10595.

Turtles were anesthetized with sodium brevital (0.7mg/Kg) and placed on stereotaxic apparatus with continued anesthetized by a combination of oxygen (200ml/min), nitrous oxide (150ml/min), and halothane (1%) through a gas anesthesia machine. The skin and temporal muscle were removed by cauterization and the temporal bone over the ear was drilled away to expose the inner ear and 8th nerve. Under an operating microscope the right 8th nerve was cut with a fine micro-scissors with minimal disruption of the local blood supply. The wound was irrigated with thrombin and the bony opening was closed with dental cement. The area over the cement cap was filled with gelfoam and the skin closed with wound clips and dental cement. Three groups of turtles were allowed to survive along with a control group. They were 30,40, and 183 postoperative. Each group had 3 animals. At the appropriate survival time the animals were overdosed with nembutal and the animals were transcardically perfused first with Reptilian Ringers and then with Susa fixative without mercuric chloride. The brains were dehydrated in ethanol, cleared in benzene and embedded in Paraplast Plus. Serial transverse sections were made at 15um intervals through the brain stem. The sections were stained with Gallocyanin and coverslipped with Permount. Individual cell areas and perimeters were ,easired on a F. Haer Morphometry System. Individual cell areas was measured for the nucleus magnocellularis on every 4th section. The means derived from those values were used to estimate the variance of the neuronal area of the cell in the nucleus. The population variance was determined from differences among individual means of the cells with the F distribution using a one way analysis of variance. Based on these results an error term was obtained and used in a posthoc comparison according to the Neuman Keuls method. Data indicated significant differences between control, 30 and 40 day survivors; 183 and 30 and 40; 30 day and all groups; and 40 day and all groups. Control and 183 survivors had not significant differences. There was a decrease in mean neuronal area between 30 (203uM^2) and 40 (169.7uM^2) day survivors with a continued increase in 183 (219.4uM^2) day almost to control (227.9uM^2) levels. The numbers of neurons in the nucleus magnocellularis were compared between control and the different survival groups and there were no significant differences.

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89.2 EVOKED AUDITORY RESPONSE ACTIVITY OF *HYLA GRATIOSA* - COMPARISON WITH OTHER ANURAN SPECIES AND REGULATING FACTORS. K.M. Mudry. Department of Biomedical Engineering, The Univ. Akron, Akron, OH 44325.

At an evoked potential level the central nucleus of the posterior dorsal thalamus in frogs has response characteristics that implicate this area in the processing of behaviorally important components of species-specific sounds. The response properties of *Hyla gratiosa*, the barking treefrog, were recently studied. A pronounced nonlinear facilitation to combination tones of 300 + 2000 Hz was found. When corrected for temperature effects, this spectral combination agrees with the frequencies required by the animal to detect its species-specific sounds.

Including this new species, three species of frogs, namely, *Hyla gratiosa, Hyla cinerea,* and *Rana catesbeiana* ,with known acoustic behavioral responses and whose calls and recognition of these calls involve the detection of a bimodal spectrum have now been studied. The thalamic evoked response characteristics of each species is distinct and is closely correlated to the spectral characteristics of each of the species' calls. Specific combination tones of 300 + 2000 Hz, 500 + 3000 Hz and both 200 + 1200 Hz and 600 + 1200 Hz produce good facilitation in respectively, *Hyla gratiosa, Hyla cinerea* and *Rana catesbeiana.* An inhibitory effect of a third tone with a frequency between the two facilitatory tones is found in both *H. gratiosa* and *H. cinerea.* A dependence on rise time of the stimulus has been found with *R. catesbeiana* showing a maximal response to sounds with slow onsets and the hylid species to sounds with rapid onsets. A good response to single tones only occurs at high stimulus levels. The facilitation found in the thalamic area is not found in the VIIIth nerve whole nerve response and in the torus semicircularis, the midbrain auditory area.

The responses in the thalamic area are very difficult to study because of non-auditory influences on this center. The area exhibits a long refractory period and the temperature of the animal affects the two-tone tuning and sensitivity. In some animals no evoked potentials can be recorded from this region. The reproductive state is also likely to influence the responses of this center.

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89.3 NEURAL BASIS OF TEMPORAL PATTERN RECOGNITION IN THE FROG'S AUDITORY SYSTEM. J. C. Hall* and A. S. Feng. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

The spectral content and temporal pattern of frog mating calls are essential features for species recognition. The role of the frog's auditory system in the analysis and recognition of these signals in the frequency domain has been systematically studied. In contrast, only a few studies have addressed issues concerning the neural mechanisms which mediate temporal pattern recognition in frogs. To gain further insights into these processes we have chosen to systematically examine the neural basis of temporal pattern recognition in the auditory system of the northern leopard frog, Rana pipiens pipiens.

Single unit recordings were made to determine the response patterns of neurons at three different loci within the frog's auditory pathway; the 8th nerve, dorsal medullary nucleus (DMN) and central thalamic nucleus (CTN). Our results demonstrate an increasing selectivity for the temporal parameters (pulse repetition rate, pulse duration, pulse rise-fall time) of complex acoustic signals as one ascends the auditory pathway. Units of the 8th nerve and most of those in the DMN behave as envelope detectors, i.e., the stimulus envelope is accurately reflected in their discharge patterns. However, a few (<10%) DMN neurons behave as low-pass temporal filters with respect to pulse repetition rate or rise-fall time. All 8th nerve and DMN units show V-shaped frequency tuning curves and are narrowly tuned. In contrast, CTN neurons show broad frequency tuning but are highly selective for the temporal components of acoustic stimuli (pulse repetition rate and pulse duration). They do not reproduce variations in stimulus envelope, but respond with changes in firing rate. Low-, high-, and band-pass as well as band-suppression properties are evidenced. These neurons are probably important components of a pattern recognition system operating in the time domain.

Using both natural and synthetic stimuli it is clear that neural responses at lower levels are determined primarily by stimulus frequency rather than temporal features. At higher levels, such as the CTN, the converse is true.

89.4 DETERMINATION OF AUDITORY AND FREQUENCY DISCRIMINATION THRESHOLDS IN A FROG, XENOPUS LAEVIS, BY CONDITIONING. E.Günther*, B.Traub*, A.Elepfandt (SPON: H.Leppelsack). Dept Biol, Univ Konstanz, D-7750 Konstanz, FRG.

The study of the amphibian auditory system suffers from inability to test the animals' auditory capabilities with psychophysical methods: attempts of auditory conditioning in amphibians have failed so far. Recently one of us conditioned the aquatic clawed frog, Xenopus laevis, to discriminate between water waves (A.Elepfandt, Naturwissenschaften 72,492,1985). Using that paradigm, we succeeded to induce tone-discriminations in X.laevis, and present here the first psychophysically determined auditory threshold of a frog. In addition, we give initial data on the frog's frequency discrimination. Since Xenopus is purely aquatic, these data refer to underwater sound.

At or closely below water surface, Xenopus spontaneously responds to an impinging water wave by turning toward the wave origin. We trained the frogs to discriminate between stimulus waves that were or were not paired with a tone or, in the case of frequency discrimination, that were paired with tones of different frequency. The paradigm was go/no-go conditioning: responses to the one stimulus were rewarded by feeding the frog a small cricket, responses to the other were punished by a stroke with forceps on the animal's head. Nearly all animals learned the discrimination, about half of them within five sessions of 80 stimuli. Whenever discrimination was significant, the stimulus difference was reduced and testing continued until the threshold was attained.

The hearing range of the animals was 200-3600 Hz. In this range the threshold was relatively flat except that at 1000-1400 Hz it was lower by 15 dB. Interindividual differences were less than 5 dB.

Two types of frequency discrimination were compared at 1 kHz: in continuous-tone discrimination each wave was paired with a monofrequent tone of 3 s duration (interstimulus intervals were 1-5 min), in pulse-tone discrimination the tones consisted of 6 pulses with alternating or constant frequency. Surprisingly, there was no difference between these discriminations, neither in the speed of learning nor in the discrimination limen. On the other hand, their limen of 0.4 was considerably higher than known from other vertebrate classes.

89.5 STIMULUS PARAMETER ENCODING IS EQUAL IN LATERAL LINE AND IN LOW-FREQUENCY AUDITORY AFFERENT FIBERS OF VERTEBRATES. L.Wiedemer*, A.Elepfandt (SPON: E.Liske). Dept Biol, Univ Konstanz, D-7750 Konstanz, FRG.

Our recordings of afferent lateral line responses in the clawed frog, Xenopus laevis, to water waves revealed intriguing similarities of stimulus encoding to that in vertebrate low-frequency auditory afferents.

Two types of stimulus encoding were found: discharge modulation and firing rate. Low intensities are encoded by the degree of phase locking. It increases linearly with the logarithm of stimulus amplitude, the slope does not depend on wave frequency. Response phase is independent of stimulus intensity. From some intensity firing is completely suppressed for an increasing section of the wave cycle. Phase locking saturates at about 30 dB above threshold with vector strengths of 0.75-0.90. At high intensities response peaks get slightly skewed with the leading edge rising faster than the trailing edge falls.

20-30 dB above the threshold for phase locking, the mean firing rate begins to rise. Like in phase locking, the increase is a linear function of stimulus logarithm and the slope is independent of stimulus frequency. Further, sensitivity is positively correlated with the resting discharge rate, and the slope of the rate-intensity function is less when the threshold is higher.

This matches the response behavior of low-frequency auditory units below their characteristic frequency as described for mammals, birds and other vertebrates. There exists strong evidence that the mechano-electrical transduction is equal for all vertebrate sensory hair cells. The present data suggests that also the subsequent transformation to afferent discharge rates is done according to common mechanisms.

Alternatively to the mean firing rate, we determined the firing rate during the excitatory phase of the response cycle. This allowed to replace the two only partially overlapping codes, phase locking and mean firing rate, by one uniform code that over the whole intensity range follows Weber's law. We suggest that the same would be possible for auditory afferent encoding.

Supported by DFG (EL 75/2).

89.6 WAVE LOCALIZATION WITH LATERAL LINE: ORGANIZATION OF LEMNISCAL INPUT TO THE MIDBRAIN IN THE CLAWED FROG, XENOPUS LAEVIS. A.Elepfandt. Dept Biol, Univ Konstanz, D-7750 Konstanz, FRG.

The purely aquatic frog Xenopus can, by means of its lateral lines, detect the direction of impinging water waves. It responds to them by turning toward the wave origin. Previous investigation had shown peripheral redundancy of wave localization: many subsets of the lateral line organs, e.g. the organs of one side, are sufficient to allow the animal accurate localization of waves from any direction (A.Elepfandt, J Comp Physiol 148,535,1982). In the midbrain, however, a topology and lateralization was found: each midbrain side is responsible for localization of contralateral waves (A.Elepfandt, Brain Behav Evol, in press). The present investigation is concerned with the organization of the input ascending to the midbrain through the lateral lemniscus. The method is behavioral testing for detection of wave directions after unilateral lateral lemniscus dissection at isthmic level.

After that dissection, the frogs can still localize waves from any direction. Thus, lateral line input from either lateral lemniscus is conveyed not only to the ipsilateral but also the contralateral midbrain. A bilateral lateral-line connection above the level of the medulla was not known as yet. Wave localization was not impaired when the lemniscus dissection was combined with an ipsilateral hemisection between di- and mesencephalon. Thus, such a bilateral connection must exist at midbrain level, and this connection is sufficient to enable detection of all wave directions on the basis of input from only one lateral lemniscus.

When the lemniscus dissection was combined with unilateral destruction of the lateral line organs, all wave directions still could be detected, irrespective of whether deafferentation was made ipsi- or contralaterally of the dissected lemniscus. Thus, the duplicity described above for the lemniscal level is duplicated once more: not only is the input through either lemniscus sufficient for the detection of any wave direction, but even the input from the organs of either side through either lemniscus.

Supported by DFG (El 75/2).

89.7 INDEPENDENT MAPS OF INTERAURAL TIME AND INTENSITY DIFFERENCES IN THE OPTIC TECTUM OF THE BARN OWL. J.F. Olsen* and E.I. Knudsen. (SPON: U.J. McMahan). Department of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

Neurons in the optic tectum of the barn owl respond to sounds from restricted areas of space, and form a map of azimuth and elevation. We investigated the binaural basis of the auditory space map. We compared the tuning of single units to the location of free-field noise with their tuning to interaural time (Δt) and interaural intensity differences (ΔI) of digitally controlled noise presented through earphones. Our sample consists of 63 units from 5 owls. Best azimuths ranged from 60° contralateral to 15° ipsilateral, and best elevations ranged from 70° up to 40° down. Most units responded only when Δt was within 50 microsec of the best Δt and when ΔI was within 20 dB of the best ΔI. Within these ranges, the best Δt was not altered by changes in ΔI, and vice versa. Varying stimulus level over a 20-40 dB range did not affect the best Δt, although, for most units, increases in level caused the best ΔI to increase in favor of the dominant ear. None of the units responded to monaural stimulation. Best azimuth varied with the anteroposterior location of the recording site and was strongly correlated with the best Δt ($r = 0.89$ to 0.96, depending on stimulus level; Fig. 1), but only weakly correlated with ΔI ($r = 0.14$ to 0.36). Conversely, the best elevation varied with the dorsoventral location of the recording site and was a function of ΔI (Fig. 2), but weakly correlated with the best Δt ($r = 0.55$ to 0.66). The data indicate that the auditory map of space is created primarily by the integration of two independently mapped acoustic parameters.

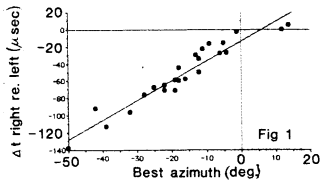

Fig 1

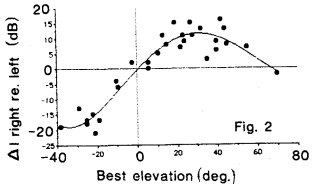

Fig. 2

Supported by grants from the March of Dimes, the Sloan Foundation, the NIH, and the McKnight Foundation.

89.8 FLOW OF INFORMATION IN THE NETWORK THAT COMPUTES AZIMUTH IN THE BARN OWL. H. Wagner, T. Takahashi and M. Konishi. Div. Biology, 216-76, California Institute of Technology, Pasadena, CA 91125.

The central nucleus of the barn owl's inferior colliculus (ICc) is a major source of afferents to the neural map of contralateral space found in the external nucleus of the inferior colliculus (ICx). ICc itself consists of a centrally located "core," defined by the terminal field of nucleus laminaris (NL), surrounded by a "shell," defined, in turn, by the terminal field of nucleus angularis (NA). Interaural time difference (ITD), the binaural cue for the location of a sound source along the horizontal axis, azimuth, is systematically represented in ICc. ITDs corresponding to loci in the ipsilateral hemi-field are represented in the core, and ITDs corresponding to loci in the contralateral hemi-field are represented in the lateral portion of the shell. The representation of ipsilateral space in the core is simply explained. NL of the left, for example, which represents predominantly the right, or contralateral hemi-field, projects contralaterally thereby conferring upon the core of the right ICc selectivity for right or ipsilateral space. NA does not contain cells sensitive to ITD. How then, does the lateral shell acquire selectivity for ITDs corresponding to loci in the contralateral hemi-field?

Small quantities of horseradish peroxidase were iontophoresed into the lateral shell under guidance of extracellular recordings (four cases), and the distribution of retrogradely labeled somata was assessed by the tetramethyl benzidine method. Ipsilateral to the injection site, retrogradely labeled somata were scattered throughout ICc, with the greatest density occurring in the immediate vicinity of the injection site. The functional significance of this labeling pattern is difficult to interpret. Interestingly, however, contralateral to the injection, retrogradely labeled somata were concentrated in the core of ICc. This suggests that the core of the right ICc, for example, which contains a representation of right or ipsilateral space, projects to the lateral shell of the left ICc, thus presenting to it a representation of right or contralateral space. Finally, from the lateral shell, the representation of contralateral space is transmitted to the ipsilateral ICx.

89.9 BINAURAL PROPERTIES OF AUDITORY NEURONS IN THE GRANULE CELL LAYER OF THE POSTERIOR CEREBELLAR VERMIS IN THE CAT. C. Huang. Division of Structural and Systems Biology, School of Basic Life Sciences. University of Missouri-Kansas City, Kansas City, MO 64110.

There is an auditory area in the posterior cerebellar vermis. Within this area, neurons in the granule cell layer are sensitive to differences in sound intensity at the two ears. When the sound intensity at one ear was fixed, and that at the other ear was gradually increased, the response of a given neuron may increase, decrease, or show no change. Moreover, all three types of response pattern may be demonstrated in the same neuron depending upon the level of sound intensity at the other ear. The dynamic range of a given neuron ranged from narrow (10-20 dB) to wide (70-80 dB). The sound intensity at one ear strongly influenced its dynamic range for the other ear. By systematically varying the sound intensities at the two ears, it can be shown that a given auditory neuron in the cerebellum was most sensitive to a certain combination of binaural intensities but not to others. The limited excitation by binaural sound seems to code the direction of sound source in space. Three major types of neurons can be identified based on their binaural properties. Neurons located in the lateral part of a folium were more likely to be excited by sound from the contralateral ear. At the same time, these neurons were often inhibited by sound from the ipsilateral ear. Neurons located in the medial part of a folium were best excited by sound combinations in which the intensities at the two ears were equal or nearly equal. The third type of neuron generally showed a more complex binaural behavior and may actually contain several sub-types. Thus, neurons in the cerebellar auditory area were sensitive to the interaural intensity difference; as a collection of neurons, they were organized topographically according to their binaural properties. (Supported in part by NIH, ONR, MRAA, and the Lettie B. McIlvain Frederic Fund.)

89.10 RESPONSES OF CELLS IN THE INFERIOR COLLICULUS OF THE CAT TO INTERAURAL TIME DIFFERENCES OF CLICKS: COMPARISON WITH NOISE AND TONES AND AN ANALYSIS OF EXCITATORY AND INHIBITORY COMPONENTS. L.H. CARNEY* and T.C.T. YIN, Dept. of Neurophysiology, University of Wisconsin-Madison, Madison, WI 53706.

We have recorded responses of low-frequency cells in the central nucleus of the inferior colliculus (ICC) of the cat to 100 usec clicks presented binaurally with interaural time differences (ITDs). Previous work has characterized the cyclic properties of discharge rate vs. ITD in response to tones and wideband noise. Since low-frequency auditory nerve fibers have periodic responses to clicks as well as to tones and noise, we hypothesized that the responses to ITDs of these three sets of stimuli should be similar. For some cells in the ICC this prediction was confirmed, as the main features of the ITD curves for clicks agree with those measured in response to noise and tones. However, some cells that showed sensitivity to ITDs of tones and noise did not to clicks. Furthermore, unlike responses to noise and tones, the dynamic range of ITD sensitivity to clicks is often quite narrow, usually about 10-20 dB. This appears to be due to the fact that the responses to single clicks, either monaural or binaural, are no more than 2-3 spikes.

Click responses provide an interesting opportunity to study binaural interaction since one can attribute individual spikes to binaural facilitation, ipsilateral excitation or contralateral excitation based on monaural response properties. Thus, when studying responses to binaural clicks, it is possible to monitor the excitatory and inhibitory components of both inputs as ITD is varied. By changing the relative levels of the binaural inputs, we have been able to examine the laterality, strength, and time course of the various excitatory and inhibitory influences from each side. There is evidence for an early, short-lasting (usually < 1 msec) inhibition, followed by excitation, which is in turn followed by a late, long-lasting (from 2 to 50 msec or more) inhibition. These components may be present in both ipsilateral and contralateral inputs, and they vary in strength and duration from cell to cell. One effect of the early inhibition is to limit the range of ITDs, near 0 ITD, which produce facilitation or excitation, thereby keeping the central peak within the cat's physiological range of ITDs. The late, long duration inhibition following excitation is consistent with intracellular records of click responses (Nelson and Erulkar, 1963).

89.11 A COMPARISON OF INTERAURAL TIME SENSITIVITY OF NEURONS IN THE MEDIAL GENICULATE AND INFERIOR COLLICULUS OF THE UNANESTHETIZED RABBIT. T. R. Stanford*, R. Batra, and S. Kuwada. Dept. of Anat., Univ. of Connecticut Health Ctr., Farmington, CT 06032.

Low-frequency sounds are localized along the azimuth using the difference in the time of arrival of the sound at the two ears. Neurons in the binaural pathways respond preferentially to a particular range of interaural time differences (ITD's) reflecting their sensitivity to a range of azimuthal sound locations. The range of ITD's to which a cell is sensitive appears to decrease from the superior olive to the inferior colliculus (IC). We report here that low-frequency neurons in the medial geniculate body (MGB) of the unanesthetized rabbit are tuned to an even more restricted range of ITD's than neurons in the IC. Consequently, azimuthal sensitivity may be sharpened as binaural information progresses up the auditory pathway.

The rabbit was placed in a body stocking and its head immobilized by clamping to a bar fixed to its skull. Lidocaine was applied to the dura and a Pt-Ir electrode was lowered through a small hole in the skull. Acoustic stimuli were delivered dichotically through custom-fitted earmolds. Recordings were made from either single neurons or from small groups of neurons.

For each unit, interaural phase sensitivity was assessed at several frequencies. Interaural phase was continuously varied by delivering tones that differed by 1 Hz to the two ears. For all frequencies to which the neuron was sensitive to interaural phase, the response was plotted as a function of the equivalent ITD. At each frequency, one cycle on either side of zero was plotted to represent both ipsi and contralateral delays. These interaural delay curves were averaged to yield a composite curve. Using a parabolic fit to the upper 30% of the composite curve, the peak delay and the width of the composite curve were estimated. The peak delay corresponds to the point in space to which the neuron is maximally sensitive whereas the peak width reflects the sharpness of its azimuthal tuning.

Neurons in the MGB of the unanesthetized rabbit demonstrate a sensitivity to ITD that is qualitatively similar to that of cells in the IC. Neurons of both nuclei respond preferentially over a restricted range of ITD's. However, it appears that the widths of the composite peaks are on the average 30% narrower for neurons in the MGB. This implies that neurons in the MGB are tuned to a more restricted range of azimuths than those in the IC and supports the hypothesis that azimuthal tuning becomes progressively sharper at higher levels of the auditory system.

This work was supported by NIH grant NS18027 to S. Kuwada.

89.12 MONAURAL AND BINAURAL RESPONSES OF NEURONS IN THE RABBIT INFERIOR COLLICULUS: EFFECTS OF BARBITURATE ANESTHESIA. S. Kuwada, R. Batra, and T. R. Stanford*. Dept. of Anatomy, Univ.of Connecticut Health Center, Farmington, CT 06032.

Most neurophysiological studies of the auditory system have used barbiturate anesthesia. However, barbiturates are known to have a potent depressive action in the CNS, presumably through the potentiation of inhibitory GABAergic mechanisms. Furthermore, GABAergic cells have been observed in the inferior colliculus. Does barbiturate anesthesia affect the responses of neurons in the inferior colliculus? We have answered this question directly by testing the same neuron before and after the i.v. administration of sodium pentobarbital.

Rabbits were surgically prepared for recording under general anesthesia. A catheter was inserted into the external jugular vein to permit later infusion of barbiturate. A stainless steel rod was cemented to the skull and a small hole was drilled for entry of the electrode. The hole was capped with silastic and the animal was allowed to recover for several days. During recording, the rabbit was placed in a stocking and its head immobilized by clamping the steel rod. The silastic was removed, dura swabbed with lidocaine, and a Pt-Ir electrode was lowered using approximate stereotaxic coordinates. Sounds were delivered dichotically via custom-fitted earmolds.

At the start of the recording session, the rabbit was unanesthetized. Upon isolating a neuron, we measured the responses to monaural tone bursts at or near the neuron's best frequency. If the cell was tuned to low frequencies (<2000 Hz), we also assessed its sensitivity to interaural time differences using a binaural beat stimulus. We then injected sodium pentobarbital (10-25 mg/kg) through the catheter and repeated these characterizations. If time permitted, we injected supplemental doses to mimic procedures used in acute experiments employing anesthesia.

In most cases, anesthesia altered spontaneous activity, response rate, response latency, and discharge pattern. We have observed response latencies lengthening by tens of milliseconds, and monaural response patterns changing from sustained to onset, sustained to pauser, and build-up to pauser, among others. Sensitivity to interaural time differences was also changed. Barbiturate anesthesia often reduced the frequency range over which ITD sensitivity was observed, and measures such as the characteristic delay, characteristic phase, and composite delay were also altered.

This work was supported by NIH grant NS18027 to S. Kuwada.

89.13 THE FREQUENCY OF "OVER-REPRESENTATION" IN THE MUSTACHED BAT INFERIOR COLLICULUS. R.F. Huffman, D.C. Fitzpatrick*, A.W. Keating, Jr.* and O.W. Henson, Jr.* Curriculum in Neurobiology and Dept. of Anatomy, Univ. of North Carolina, Chapel Hill, NC, 27514.

The inner ear of the mustached bat (Pteronotus p. parnellii) resonates near a frequency of 61 kHz in response to echolocation signals. Large populations of neurons throughout the auditory system are sharply tuned near this frequency, resulting in an "over-representation" of 61 kHz within the CNS. A question of interest is the exact relationship of this over-represented frequency to the resonance frequency and to the resting frequency (the frequency which the bat emits while stationary).

For each bat studied the resting frequency was determined prior to surgery. The resonance frequency (RF) of the cochlea and best frequencies of single- and multi-units in the inferior colliculus (IC) were obtained from fully awake animals at least three days following surgery, and repeated for each bat over several days. The RF was obtained by FFT spectral analysis of cochlear microphonic potentials. Using glass microelectrodes, orthogonal and oblique penetrations were made in the IC. The best frequencies of units in the dorsal posterior division (DPD) of the central nucleus of the IC (Zook, et al., 1985, J. Comp. Neurol. 231:530) were determined with an accuracy of ± 25 Hz using an automated computer program.

To date, two bats have been studied. In one bat the RF was 63.47 kHz and the resting frequency was 63.11 kHz. Four penetrations into the DPD yielded 53 sharply tuned units with best frequencies within ± 300 Hz of the RF: 70% of these had best frequencies 100-300 Hz below the RF; 26% were tuned within 100 Hz of the RF; and 4% were tuned 100-300 Hz above the RF. The second bat had a RF of 61.55 kHz and a resting frequency of 61.49 kHz. Five penetrations yielded 61 units of which 28% were tuned 100-300 Hz below the RF; 51% were within 100 Hz of the RF; and 21% were tuned 100-300 Hz above the RF. Thus, one bat whose resting frequency was well below its RF had most units tuned below the RF; while the other bat whose resting frequency and RF were similar had the majority of units tuned near the RF. In both cases many units were tuned to a frequency other than the RF.

These preliminary results indicate that DPD neurons have best frequencies which are distributed within a range that encompasses both the RF and the resting frequency. Although emitted pulses and echoes cause the bat's ear to resonate, only a fraction of the neurons in the DPD appear to be maximally responsive to the resonance frequency. More animals are being studied to determine the range of variation among the resonance frequency, resting frequency and the frequency of over-representation within the IC. (Supported by NIH grant NS 12445)

89.14 MAJOR OUTPUTS OF THE FM_2 REGION OF THE MUSTACHED BAT INFERIOR COLLICULUS GO TO MEDIAL AND DORSAL DIVISIONS OF THE MEDIAL GENICULATE BODY. M.L. Zettel, W.E. O'Neill, and R.D. Frisina. Dept. of Physiology, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

The FM components of the multi-harmonic CF/FM mustached bat sonar cry carry target range information which is processed by delay-tuned neurons in the auditory cortex and medial geniculate body (MGB). A subpopulation of these units encodes time delays between the FM_1 and FM_2 components of the sonar pulse and the echo, respectively. HRP injections restricted to the physiologically-defined region representing FM_2 frequencies of the central nucleus of the inferior colliculus (ICC) revealed connections which are generally similar to the pattern seen in other mammals, except for the MGB.

Anterograde projections were examined in TMB- and DAB-stained sections from 5 bats. Aside from the MGB there were two major ipsilateral projection zones: the deep layers of the superior colliculus (SC) and the dorsal and ventral lateral pontine nuclei (LPN). The projections to SC course through the brachium of the IC (BIC) to merge with the brachium of the SC, within which small, simple, en passant endings are made by delicate fibers onto many cells. Fibers also ramify dorsally in the SC itself. Endings in LPN are dense and more complex than those in SC, and convergence is evident on individual cells. Minor projections can be seen entering the dorsal periaqueductal gray, ending in small clusters, usually ipsilateral to the injection.

Most intriguing were the projections to the MGB. Large numbers of labeled fibers travel ipsilaterally in the BIC and enter the MGB medially in a tract elongated dorsomedial-ventrolaterally. Large, complex endings occur on clusters of cells within the BIC as it courses through the medial division. Large numbers of fibers entering the dorsal division branch off laterally from the BIC to form complex endings onto many cells in rostral MGB. In DAB sections, individual fibers divide into several branches which terminate onto widely separated clusters of cells within the dorsal division.

In stark contrast to the pattern seen in other mammals where the ventral division receives the heaviest projection from the ICC, in the mustached bat few fibers were seen to enter this area, and no endings were visible. This suggests that the ventral division has a significant gap in its tonotopic axis for the FM_2 frequency band, the pathway for which is diverted to the dorsal and medial divisions where FM_1-FM_2 delay-tuned neurons are found (Olsen & Suga, SNS Abstr. 225.13, 1983). A lack of FM_2 representation in the ventral division may explain the lack of FM_2 representation in the tonotopic region of primary auditory cortex of this species. Supported by NSF grant BNS 8311627 to WEO and NIH-NRSA NS07343 to RDF.

89.15 ANATOMY OF THE MUSTACHED BAT'S MEDIAL GENICULATE BODY: CYTOARCHITECTONICS, NEURONAL ARCHITECTURE, AND GAD-IMMUNOREACTIVITY. Jeffery A. Winer and Jeffrey J. Wenstrup. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

As part of a study of the functional relations between the inferior colliculus and the medial geniculate body in the bat (*Pteronotus parnellii*), we classified thalamic auditory neurons in Golgi and Nissl preparations, and compared the architectonic scheme with the patterns of glutamic acid decarboxylase (GAD) or gamma-aminobutyric acid (GABA) immunoreactivity. We wished to provide an anatomical framework for parallel physiological experiments on midbrain afferents to medial geniculate subdivisions (J.J. Wenstrup and J.A. Winer, *Proc. Soc. Neurosci.*, 1987, **13**: in press), and to consider the organization of the mustached bat's medial geniculate body within the context of comparative neuroanatomy.

As in most species, three primary parts of the medial geniculate complex were recognized. The *ventral division* filled much of the ventrolateral two-thirds of the medial geniculate body. It contained medium-sized neurons (~10 μm in diameter) whose dendrites formed richly branched tufts which ran in long, parallel strips. Single dendritic tufts often ran parallel to the preterminal segments of midbrain afferents. In the *dorsal division*, which filled the superficial and caudal parts of the auditory thalamus, neurons with radiating dendrites and cells with tufted branches occurred. The former were numerous and had spherical dendritic fields and moderate numbers of branches, while the latter were bushier and common in the superficial dorsal nucleus. The *medial division* formed the medial limb of the auditory thalamus and had a heterogeneous neural population, including small (<10 μm), medium sized (10-12 μm), and large (>12 μm) neurons with radiating or weakly tufted dendrites. In each division, small neurons with thin, sparse dendrites were impregnated.

The distribution of GAD-immunoreactive puncta supported this architectonic parcellation. Thus, ventral division puncta were numerous and fine; their disposition confirmed the architectonic borders discerned in Nissl preparations. Puncta in the dorsal division were extremely small and few in number. Medial division puncta were much larger and coarser than in other medial geniculate divisions, and as plentiful as ventral division puncta. Few medial geniculate neurons were immunoreactive for GAD or GABA, even in sections where the superior colliculus or extrathalamic nuclei contained many such cells.

We conclude that three primary medial geniculate divisions exist in the mustached bat, each with a characteristic neuronal architecture and pattern of GABAergic innervation. These divisions are readily comparable to those in other mammals, with some important differences, especially in the relative reduction of the neuropil and the large size of the ventral division. The divisions are also distinguished by their differential input from funtionally defined inferior colliculus subdivisions, suggesting that even finer physiological and architectonic subregions might exist within the auditory thalamus.

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89.16 PROJECTIONS TO THE MEDIAL GENICULATE BODY FROM PHYSIOLOGICALLY DEFINED FREQUENCY REPRESENTATIONS OF THE MUSTACHED BAT'S INFERIOR COLLICULUS.
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The projections of different frequency representations from the inferior colliculus to the medial geniculate body were examined in the mustached bat, *Pteronotus parnellii*. A goal of these experiments was to contribute to our study of the functional organization of the mustached bat's auditory thalamus. We placed small, iontophoretic deposits of HRP in regions of the inferior colliculus that were physiologically characterized by their best frequencies and sharpness of tuning. Axons and their terminals were visualized in alternate sections with either heavy metal-intensified DAB or TMB as chromogens.

Our preliminary observations showed that the projections of restricted frequency bands from the central nucleus of the inferior colliculus (ICC) to the auditory thalamus were divergent (*cf.* J.A. Winer and J.J. Wenstrup, *Proc. Soc. Neurosci.*, 1987, **13**: in press, for thalamic subdivisions). Low frequency ICC deposits, centered in regions with best frequencies between 30-35 kHz, produced a dorso-ventral crescent of labeled terminals in the lateral part of the *ventral division*. However, discontinuous groups of terminals were present in the medial part of the *ventral division*. HRP placed in the enlarged 60 kHz representation of the ICC produced heavy terminal labeling of the middle part of the *ventral division*. HRP deposits in the high frequency representation of ICC, centered at best frequencies between 91-96 kHz, labeled the medial and dorsal parts of the *ventral division*. These deposits resulted in tonotopic, retrograde labeling within the cochlear nuclei, the medial and lateral superior olives, and the nuclei of the lateral lemniscus. Deposits of HRP placed in the ICC often labeled other thalamic divisions as well. Thus, deposits centered near 30 kHz, 60 kHz, and 90 kHz each labeled axonal terminals in the *medial* and *dorsal divisions*. It is unlikely that these divergent projections resulted from damage to fibers passing through the midbrain deposit sites.

Axons from the brachium of the inferior colliculus, ranging from <1 μm to about 3 μm in diameter, entered the medial geniculate body dorsomedially. Viewed in the DAB material, their terminal ramifications were highly varied, ranging from extremely fine, widspread boutons, to axonal varicosities with irregular configurations, to dense clusters of coarse terminals.

These results, and those from our cytoarchitectonic studies, suggest that the mustached bat's medial geniculate body contains elements in common with other mammals (*e.g.*, the primary cytoarchitectonic subdivisions), and certain unique features (*e.g.*, the divergent terminal fields of the 30 kHz projection in the *ventral division*). This pattern of ICC projections to the medial geniculate body could underly the responses of some thalamic cells to combinations of frequencies in the mustached bat's sonar call (J.F. Olsen and N. Suga, *Proc. Soc. Neurosci.*, 1983, **9**:768).

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89.17 COMPLEX RESPONSE PROPERTIES OF FM-FM COMBINATION-SENSITIVE NEURONS IN THE VENTRAL FRINGE (VF) AREA OF THE MUSTACHED BAT'S AUDITORY CORTEX.
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In the mustached bat, *Pternotus parnellii parnellii* , different types of combination-sensitive neurons are clustered in separate areas of the auditory cortex. In the FM-FM area, FM-FM combination-sensitive neurons are tuned to particular echo delays, i.e. target ranges. Anatomical studies with tritiated amino acids indicate that the FM- FM area projects to the DF area, which, in turn, projects to the VF area. The FM-FM area consists of three types of FM-FM neurons (FM_1- FM_2, FM_1-FM_3, and FM_1-FM_4) and has an axis representing echo delays of 0.4-to-18 ms (target ranges of 7- to-310 cm), while the DF area has the axis representing echo delays of 0.8-to-9 ms. [Suga,N. & Horikawa, J. Neurophisiol., 55,4:776- 805, 1986]. Since the VF area is two steps higher than the FM-FM area, neurons in the VF area are expected to be more specialized in processing biosonar signals than those in the FM-FM and DF areas.

In 8 unanaesthetized mustached bats, single unit activity was recorded with a tungsten-wire electrode from the VF area which is located along the sulcus and is very small (0.29-to-1.13 mm^2). As in the FM-FM area, there are 3 clusters of combination-sensitive neurons. Unlike the FM-FM area, however, these clusters are quite variable among bats in their relative locations, shapes, and sizes, and there is no systematic representation of echo delays, although best delays varied somewhat systematically in a few electrode penetrations. All VF neurons are tuned to short echo delays, 0.9-to-4.5 ms (2.87 ms on the average). Since these data suggest that VF neurons process target-range information only at short distances from the target, we delivered the pulse- echo pairs consisting of a short CF and FM components, mimicking biosonar signals in the terminal phase of target-directed flight. We then found that the facilitative responses evoked by combinations of two FM components in the pulse-echo pair were reduced by the CF component. This reduction of facilitative response was not evoked by a 30-ms-long CF component but only by a short CF component. Neurons in the FM-FM area did not show such a reduction of responses by CF sounds. Therefore VF neurons are more complex in response properties than those of the FM-FM area. The biological significance of this reduction remains to be explored. (This work was supported by PHS research grant NS 17333.)

89.18 PARALLEL-HIERARCHIAL PROCESSING OF RANGE INFORMATION IN THE MUSTACHED BAT: A SUBTHALAMIC NUCLEUS PRODUCES DELAY LINES.
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The mustached bat, Pteronotus parnellii,emits orientation sounds (pusles) each consisting of eight components (CF_{1-4} and FM_{1-4}). In the medial geniculate body (MGB), neurons responding to the FM_1 of the pulse and those responding to the FM_n (n=2,3 or 4) of an echo are integrated to produce FM_1-FM_n conbination-sensitive neurons. These neurons are tuned to particular echo delays, i.e., target ranges. To produce such FM-FM neurons, delay lines are necessary. FM-FM neurons in the MGB often respond weakly to FM_1 and FM_n even when presented alone. The response latency for the FM_1 is usually longer than that for the FM_n. When the FM_n is presented after the FM_1 with a delay which is the same as the difference in latency, the FM-FM neurons show a strong facilitation. The response latency for the FM_1 is linearly related to the best delay for facilitation, while that for the FMn is the same regardless of the best delay, suggesting the creation of delay lines by neurons responding to the FM_1 (Olsen, 1986). To examine whether the delay lines are created within the MGB or a subthalamic nucleus, the distributions of the response latencies of brachial fibers of the inferior colliculus were studied.

In 6 unanesthetized mustached bats, action potentials were recorded from 1511 brachial fibers. The response of each neuron to a tone burst presented at its best frequency and amplitude was plotted as PST and PSTC histograms, and the latency and peak latency of the response were measured. The latencies of neurons tuned to the FM_1 ranged from 4.3 msec to 17.3 msec, and the distribution of the FM_1 latencies was very similar to that of best delays of FM-FM neurons in the MGB. On the contrary, the latencies of neurons tuned to the FM_n fell within a narrow range from 3.8 msec to 6.6 msec. Most of them were approximately 5 msec. Our data indicate that a subthalamic nucleus creates the delay lines, and then the thalamus produces FM-FM neurons to extract the range information. Thus, the processing of range information is parallel-hierarchial. (Supported by PHS research grant, R01-NS17333)

90.1 BINAURAL ORGANIZATION OF THE AUDITORY CORTEX IN THE ALBINO RAT. J.B. Kelly and S.L. Sally*. Lab. of Sensory Neuroscience, Psychology Dept., Carleton University, Ottawa, Canada K1S 5B6.

Microelectrode mapping techniques were used to determine the binaural response properties of cells in the auditory cortex of the albino rat. The animals were anesthetized with Equithesin (3.0 ml/kg i.p.), the surface of the cortex was exposed, and recordings were made with tungsten microelectrodes. A dissecting microscope was used to determine the position of electrode penetrations with respect to surface landmarks on the cortex. An enlarged photograph of the exposure allowed precise location of electrode penetrations by cross-reference to the vascular pattern on the cortex in individual animals. Pure tone pulses were delivered to each ear independently through sealed loudspeakers fitted to specula inserted into the external meatus. Sound pressure levels were determined by probe tube measurements within a few millimeters of the tympanic membrane. Characteristic frequency (the frequency for which excitatory thresholds was lowest) was determined for each neuron or neuron cluster encountered. Responses were obtained separately for contralateral alone, ipsilateral alone and binaural stimulation. In some cases detailed information was obtained regarding the responses to a wide range of interaural intensity differences. Results confirmed our previous findings with regard to frequency organization of the auditory cortex, namely, that the primary auditory cortex is tonotopically organized with high frequencies represented rostrally and low frequencies caudally. Isofrequency contours were aligned dorsoventrally. Also, secondary auditory areas were found dorsal and ventral to the primary auditory cortex. Binaural response types were classified as either summation (35.3%), suppression (42.2%), mixed (18.5%), or other (4%). Each of these response categories was represented at all frequencies within the rat's hearing range. Similar response types were grouped together within the auditory cortex forming binaural regions which cut across the isofrequency contours. The majority of neurons in the summation and suppression classes exhibited binaural interaction at interaural intensity differences around 0 db. Neurons in the mixed category typically showed binaural summation around 0 dB and binaural suppression when the ipsilateral intensity was greater than contralateral by 15-25 dB.

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90.2 POSTNATAL DEVELOPMENT OF CORTICAL CYTOARCHITECTURE AND LAMINA V PYRAMIDAL CELLS IN AREA 41 OF RAT. J. R. Coleman, J-M. Ding*, A. Wei*, and H. Dorn*. Departments of Psychology and Physiology, University of South Carolina, Columbia, SC 29208.

Area 41 of neocortex is the target of the core colliculo-geniculate system carrying tonotopically organized information. The present work demonstrates patterns of laminar and neural development in cortical area 41 during postnatal life in rat. The results show that substantial growth of auditory cortex occurs prior to onset of auditory function. Groups of five Sprague-Dawley rats were perfused with 10% formalin-saline on the first day postpartum (day 0) and on postnatal days 3, 5, 7, 11, 14, 16, 35 and 80. Coronal sections cut at 50 µm were stained with thionin for observations of cortical cytoarchitecture. Other groups were perfused at the same ages with 4% paraformaldehyde and the material postfixed in osmium tetroxide and placed in 0.75% silver nitrate solution then embedded in paraffin. In Golgi stained sections of 150 µm containing auditory cortex qualitative and quantitative observations were made on branching patterns of apical and basal dendrites of lamina V pyramidal cells and spine counts made on the apical dendrite and secondary branch in layer IV.

There is a dramatic growth of the six laminae of area 41 between birth and postnatal day 7 ($p < .001$). The sharpest growth occurs between days 5 and 7, which is followed by modest growth to postnatal day 11. A substantial increase in cortical thickness occurs by postnatal day 14 which includes the period of functional onset of hearing. Examination of individual laminae show that the growth spurt observed by postnatal day 7 is due to expansion in laminae II, III-IV, V and VI. Increments between postnatal days 11 and 14 largely correspond to enlargement of laminae III-IV.

In Golgi material, examination of pyramidal cells of lamina V show that emergence of the apical dendrite and axon precedes that of basal dendrites which accounts for the lack of pyramidal appearance of these cells at birth. During the period following birth, when the deep white matter exibits a transitional cascading appearance, the apical dendrite possesses thick varicosities probably associated with growth cones. Within the first three days postpartum branching of the apical dendrites occur, and by 5 days varicosities on the apical dendrite take on a more elliptical arrangement and some spines are observed. In 7-11 day-old animals varicosities are more associated with secondary branches off the apical dendrite. By 35 days spine counts/unit length on the apical dendrite and its branches are similar and adult-like in number. Branching of basal dendrites within 150 µm radius increases from 5 to 35 days from a mean of 3.8 to 4.3 for primary fibers, and from 0.3 to 5.6 secondary branches, and from no tertiary branches to 4.0. (Supported by NIH grant NS-20785).

90.3 DEVELOPMENT OF THE RAT MEDIAL GENICULATE BODY: QUANTIFICATION OF NEURONS IN THE VENTRAL DIVISION. W. J. Clerici, B. Maxwell*, D. R. Byrd* and J. R. Coleman. Departments of Psychology and Physiology, University of South Carolina, Columbia, SC 29208.

The rat medial geniculate body (MGB) contains ventral (MGv), dorsal (MGd) and medial (MGm) divisions. The present study examines cytoarchitectural features of the MGB in thionin stained material and quantifies somatic areas and densities in the MGv of rats at postnatal days (PND) 0, 5, 7, 11, 16 and 80+ (adult).

The adult MGv contains small dark staining round to almond shaped somata, the MGd has loosely packed pale cells; MGm is cytologically diverse and includes large, dark, vertically oriented triangular to oblate cells. In the MGv, the ovoid nucleus (OV) contains a double spiral of neurons in register with brachial axons sequestered from the ventral nucleus (LV) by a cell sparse zone; transition zone neurons align with radially ordered axons. MGd dorsal nucleus cells are sparsely packed and pale. The deep dorsal nucleus is cell sparse, lying within the midgeniculate bundle (MB); fusiform cells align with the MB. Suprageniculate neurons are vertically oriented, medium and large sized and medium darkly stained.

At PND 0 neurons within each MGB subdivision are homogeneous, very small and dark staining; nuclei and nucleoli are minimally discernable. At birth the MGv is relatively well formed, although the LV/OV distinction is not clear; the dorsal nucleus is very small. Between PND 0 and 16 nuclear differentiation involves cells aligning with afferent axons: DD fusiform cells strictly align with the MB, bipolar cell orientation in OV spirals and mediolateral orientation dominates the transition zone; OV double spirals are most distinct at PND 7-11. Dendrites of MGd stellate cells show no preferential orientation. Beyond PND 16, cell density is low, only somata stain and nuclear boundaries become less discriminable as adult patterns emerge. Diversity of somal shapes increase and alignment with fibers is less strict in the adult -- fusiform cells are a smaller proportion of the DD population, the OV double spiral is less obvious and somatic orientation in LV is less clear.

Somatic densities in LV, OV and DD are approximately three times greater at PND 0 as in the adult, and decrease exponentially. Densities decrease most rapidly between PND 0 and 5, with slightly less decline from PND 5-7 and PND 7-16; densities are almost at adult levels by PND 16. Somatic size increases exponentially during this same period; the most rapid increase in cell size is between PND 5 and 16, with only a slight further increase to adulthood. Somata measured from medial and lateral portions of the LV in register with brachial axons do not show differential growth rates. (Supported by grant NS-20785).

90.4 THE EFFECTS OF ACETYLCHOLINE ON SINGLE NEURON RESPONSES TO TONES IN CAT AUDITORY CORTEX. Raju Metherate, Josée F. Bourg* and Norman M. Weinberger. Center for the Neurobiology of Learning & Memory, Department of Psychobiology, University of California, Irvine, CA. 92717.

Cholinergic agents affect auditory perception and cognitive processes, and may do so by altering auditory sensory processing. To pursue this question, the present study examines the effects of iontophoretically administered acetylcholine (ACh) on single neuron responses to tones in the auditory cortex of barbiturate anesthetized cats. A further goal was to determine the extent to which pairing ACh with a single frequency tone would subsequently affect the cell's frequency receptive field (FRF).

Cats prepared for chronic recording sessions (performed at 1 week intervals) were initially anesthetized with sodium pentobarbital (35 mg/kg) and maintained areflexic by continuous infusion of barbiturate (1 mg/hr) and lactated Ringer's solution (12 ml/hr). Multibarrel glass or tungsten and glass microelectrodes were inserted through a burr hole into the auditory cortex. Drug barrels contained ACh chloride (1 M, pH 4), sodium glutamate (0.5 M, pH 8) and sodium chloride (1 M) for current controls. When a single neuron was initially isolated, its level of spontaneous activity and FRF were determined. The responses to a single, repeated tone were then noted before and during iontophoresis of ACh. Following this, the cell's FRF was re-determined.

ACh (5-70 nA) was applied to 51 neurons in 14 recording sessions. The spontaneous and/or tone-evoked activity of 39 cells (76%) was altered in the presence of ACh. The spontaneous rate increased in 34 cases, but never decreased during the ACh application. Responses to tones were increased by ACh in 16 cases and decreased in 11 cases. ACh often differentially affected a cell's activity, increasing, for example, the spontaneous rate while decreasing the evoked response. Six additional cells that did not respond to tones in the absence of drugs did so during ACh administration. When FRFs were determined following pairing of ACh with a single frequency tone, some cells displayed a decreased response to tones close to the paired frequency while responses to frequencies further away were less affected.

These data suggest that ACh can modify the activity of a large number of auditory cortical neurons. The differential effects on spontaneous and tone-evoked activity are consistent with previous observations from this laboratory (McKenna et al. 1986) using pressure ejection of ACh in unanesthetized cats. Finally, the observation of altered neuronal receptive fields subsequent to the ACh treatment bears significant implications for studies on auditory sensory processing.

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90.5 LIGHT MICROSCOPIC EVIDENCE FOR THALAMOCORTICAL INPUT TO GABA-ERGIC NEURONS IN AUDITORY NEOCORTEX: COMBINATION OF PHA-L AND TARGET NEURON IMMUNOCYTOCHEMISTRY. N.T. McMullen and A.M. Prescott*. Dept.of Physiology, Univ. of Maryland School of Medicine, Baltimore., MD 21201.

We have combined anterograde labeling using PHA-L (Gerfen & Sawchenko, Brain Res., 1984) with GABA immunocytochemistry of presumptive target neurons (Wenthold et al, Brain Res., 1986) using procedures described by Wouterlood et al (J. Histo. Cyto., 1987). PHA-L was iontophoretically injected (20 um tip, 4 uA for 30 min.) into the MGB of NZW rabbits using stereotaxic procedures. Ten days post surgery, each rabbit was deeply anesthetized and intracardially perfused with 4% paraformaldehyde with 0.1% glutaraldehyde and 0.2% picric acid in 0.1 M PBS at 4° C (pH 7.4). Frozen sections (50 um) through the auditory cortex and injection site were collected in Tris buffered saline (TBS). We have found that incubation in pooled primary antibody solutions yielded optimal color differentiation of PHA-L-labeled afferents and GABA-ergic neurons in frozen sections. Free floating sections were incubated for 48 hours at 4° C in a solution of goat anti-PHA (1:2000, Vector Labs) and rabbit anti-GABA (1:3750) containing 1% normal donkey serum (NDS), 1% normal swine serum (NSS) and 0.3% Triton X-100 in 0.05 M TBS. Sections were then incubated for 2 hours in pooled secondary antisera consisting of donkey anti-goat IgG (1:40 Nordic) and swine anti-rabbit IgG (1:40 Nordic) with 0.3% Triton in 0.05M TBS followed by goat PAP (1:400 Nordic) with 1% NDS for 2 hours. Visualization of the PHA-L labeled afferents was carried out by incubation in a solution of .015% DAB, 0.6% nickel ammonium sulfate and .015% H_2O_2 in Tris buffer. After rinsing, sections were incubated in rabbit PAP (1:600) with 1% NSS (2 hours). Visualization of the GABA-labeled neurons was carried out using a solution of .04% DAB and .015% H_2O_2 in Tris buffer. With this protocol, PHA-L labeled fibers were labeled jet black and GABA positive neurons were labeled brown. PHA-L labeled terminal fibers within laminae III/IV form en passant and terminal varicosities on the somata and proximal dendrites of GABA-ergic neurons. Thalamocortical afferents also ascend to lamina I where they course tangentially for long distances and form en passant varicosities on the somata of GABA-ergic neurons in this layer. Because these methods are compatible with electron microscopy, the combination of anterograde tracing methods and target neuron immunocytochemistry will be a powerful method for future studies of synaptic circuitry in this sensory cortex. (Supported by NIH Grant NS17861)

90.6 SINGLE UNIT, EVOKED POTENTIAL AND MAPPING STUDIES OF GUINEA PIG AUDITORY CORTEX. J.W. Fleshman and S.A. Shamma*. Systems Research Center, Univ. of Maryland, College Park, MD 20742.

The guinea pig has long been used as the subject of studies of the peripheral auditory apparatus, but very little is known about the organization of its central auditory system, the cortex in particular. This study begins to provide a picture of the functional organization of the guinea pig auditory cortex.

Young adult guinea pigs (300-600 gm) were anesthetized with a combination of pentobarbital (12 mg/kg) and a mixture of fentanyl and droperidol (Innovar-Vet, 0.9 ml/kg). The pinnae were bilaterally resected and the temporal aspect of the cortex was exposed by craniotomy and covered with a 2% solution of agar in saline. A single-channel glass-coated platinum-iridium microelectrode or a multichannel silicon-based microelectrode was inserted normal to the cortical surface during presentation of contralateral sound stimuli.

Large evoked-potentials are characteristic of guinea pig auditory cortex and were used in conjunction with unit responses to perform mapping studies. In the area referred to as primary cortex, the evoked potentials were sharply tuned to sinusoidal stimuli and occurred at peak latencies of 18-28 ms. In agreement with Hellweg et al. (Exp. Brain Res. 29:467, 1977), we found that the gradient of tonotopic organization was reversed relative to cat primary auditory cortex, with low frequencies represented rostrally and high frequencies caudally, and isofrequency contours approximately parallel to the medio-lateral axis. The primary cortex was surrounded by areas responsive to auditory stimuli, but with different response characteristics. Preliminary results indicate the presence of at least two large fields, one temporal and one caudomedial to primary cortex, characterized by broad tuning and, at least in some locations, by rapidly adapting and highly variable responses evoked by sinusoidal stimuli. It was often possible to obtain more robust responses using broad-spectrum stimuli, such as low frequency square waves.

The most common unit responses were quite phasic, consisting of 1-5 spikes at the onset or offset of the stimulus or onset responses lasting about 100 ms during a 300-500 ms tone. Responses sustained for the duration of the stimulus were rare.

More detailed mapping and single unit studies of the primary and nonprimary cortices are proceeding.

90.7 REGION AND SPECIES SPECIFIC DISTRIBUTION OF SMI-32-IMMUNOREACTIVE (IR) NEURONS IN MONKEY AND HUMAN TEMPORAL LOBE (TL) NEOCORTEX. M.J. Campbell, K. Cox*, H.J. Noack*, J.H. Morrison. Scripps Clinic and Res. Fdn., La Jolla, CA 92037

SMI-32, a monoclonal antibody (Sternberger-Meyer) developed against rodent nonphosphorylated neurofilament protein, was used in immunohistochemical studies of cynomolgus monkey and human TL neocortex. A distinct subset of pyramidal neurons (PN) were SMI-32-IR in both primate species. Thus the epitope recognized by SMI-32 appears to be a molecular marker for a specific class of neurons. There were consistent differences between members of this subpopulation of PN in the degree to which they were completely and/or intensely immunoreactive. There were intensely immunoreactive PN that had a Golgi-like appearance where the soma and dendritic processes up to secondary and tertiary branches were visualized. In contrast, only the outline of the soma and limited portion of the apical and basilar dendrites were visualized in other SMI-32-IR PN. These observations suggest that even within a morphologically similar cell class like PN, distinct chemical phenotypes can be recognized by subtle differences in the molecular structure of their basic cytoskeletal proteins.

The size, density and laminar distribution of SMI-32-IR neurons differed substantially across TL neocortical areas in both species. A comparison of the two species revealed substantial differences in some TL areas, while other areas appeared somewhat similar. For example, the primary auditory cortex (PAC) of the human contained a substantial number of large, intensely reactive PN; the majority were located in deep layer III with the remainder in layer V. In addition, numerous examples of the less completely stained PN were found in layers II-III and V-VI. In contrast, there were no large intensely reactive PN observed in the monkey PAC, where deep layer III, V and VI contained a fair population of medium to small, less completely stained PN. Although differences in cell size were often less obvious in other areas, the species related differences in distribution were often even more dramatic than seen in PAC. For example, the posterior inferior temporal gyrus of the monkey had a striking bilaminar distribution of SMI-32-IR PN in layer III, a feature that was not observed in any human cortical areas. Areas that tended to be more similar in the distribution of SMI-32-IR PN included parahippocampal and perirhinal cortices, where the most intensely reactive PN were in layer V while the superficial layers contained only very lightly reactive PN. These observations suggest that detailed analyses of the distribution of different chemical phenotypes provide a powerful approach for further elucidation of regional and species related differences in neuronal organization of the primate neocortex. Supported by AG015131, AA07456.

90.8 PROJECTION PATTERNS AMONG THREE AUDITORY CORTICAL FIELDS A, AI, AND P IN THE CAT. W.A. Irons* and T.J. Imig (SPON: R. Bunag) Dept. of Physiology, Univ. of Kansas Medical Center, Kansas City, KS 66103.

The cortical projections among three tonotopic fields (anterior field, A; primary auditory field, AI; and posterior field, P) of cat auditory cortex were studied using retrograde axonal transport techniques. Horseradish peroxidase conjugated with wheat germ agglutinin was injected under pressure into one auditory field per experiment. Best frequency maps of the injected fields generated from microelectrode recordings were used to guide the placement of injections. All injections were confined to the middle (9-13 KHz) frequency representation within each field. The cortical locations of fields containing labeled neurons were identified by reference to previously obtained best frequency maps. The laminar architecture of cortical fields containing labeled cells was identified by microscopic examination of tissue sections reacted for peroxidase activity and counterstained with thionin. Outlines of brain sections and locations of labeled cells were transferred from slides to paper using a drawing tube attached to the microscope. Results from one injection into A, four injections into AI, and one injection into P indicate that each of the three fields receives projections from auditory areas in both hemispheres. Injection into field A resulted in labeling in ipsilateral fields AI and P and contralateral field A. AI injections labeled cells in ipsilateral fields A, AI, P and VP and contralateral field P and VP. Preliminary results indicate that projections from cells whose axons terminate in auditory fields within the same hemisphere (intrahemispheric) are confined primarily to cortical layers V and VI (70.4%), with fewer cells located in layers II (0.9%), III (12.3%) and IV (7.4%). Cells giving rise to projections which terminate in the opposite hemisphere (interhemispheric) are distributed primarily within cortical layer III (80.1%), with fewer cells found in layers IV (9.3%), V, and VI (10.6%). Few labeled cells were found in layer IV. Cells from fields AI, P and VP which gave rise to intrahemispheric projections to field P displayed a larger percentage of cells confined to layer III compared to cells projecting to other intrahemispheric fields. (Supported by NINCDS Grant NS17720)

90.9 CONNECTIONS OF PRIMARY AUDITORY CORTEX IN PRIMATES. L. E. Luethke, L. A. Krubitzer, and J. H. Kaas. Department of Hearing and Speech Sciences and Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

The location, response characteristics and connections of the primary auditory cortex (A-I) were investigated in the tamarin (Saguinus fuscicollis), a New world monkey with a relatively smooth brain. Microelectrode multiunit recordings were used to determine the best frequencies for recording sites in A-I. In each case, A-I was defined by a characteristic pattern of tonotopic organization and lesions were placed to mark physiological borders for later correlation with cortical architecture. Following mapping, single injections of each of three tracers (wheat germ agglutinin conjugated with horseradish peroxidase, plus two of the following: fast blue, rhodamine, or diamidino yellow) were placed in different frequency representations within A-I. Three days later, animals were perfused and cortex was separated from the thalamus and brainstem, flattened and cut parallel to the surface. The thalamus and brainstem were cut in the frontal plane. Alternate sections were reacted with tetramethylbenzidine, mounted for fluorescent microscopy, or stained for fibers or cells. The recordings revealed that A-I has high frequencies represented caudally, low frequencies rostrally, and isofrequency contours oriented mediolaterally. The injections demonstrated tonotopic patterns of connections with a number of cortical and subcortical structures. Reciprocal connections were found between A-I and at least three adjoining architectonically distinct subdivisions of cortex of the same hemisphere, including subdivisions caudomedial, rostrolateral and lateral to A-I. Injections in the high frequency portion of A-I labeled caudal regions of both the caudomedial and lateral fields, injections in the middle frequency portion of A-I demonstrated connections with middle portions of the caudomedial and lateral fields, and the caudal and middle portions of the rostrolateral field, while injections in the low frequency portion of A-I labeled caudal portions of the rostrolateral field, and middle and rostral portions of the lateral field. Callosal connections of A-I were with A-I and adjoining cortical regions. Injections in the low, middle, and high frequency regions of A-I labeled lateral, middle, and medial regions of the principle division of the medial geniculate nucleus, respectively. Connections with the inferior colliculus and other subcortical structures were also evident. An injection confined to the lateral field of cortex exhibited dense reciprocal connections with A-I and less dense connections with surrounding cortex. Callosal connections of this lateral field were primarily with the lateral field. These results are consistent with the conclusion based on previous findings in other monkeys that auditory cortex of monkeys contains four or more tonotopically organized fields, and extend these findings by providing detailed information about the topographic organization of connections of primary auditory cortex in primates. (Supported by NIH Grant NS-16446).

90.10 A CONNECTIONAL ANALYSIS OF THE INTERHEMISPHERIC AND IPSILATERAL ASSOCIATION AFFERENT NEURONS OF THE SUPERIOR TEMPORAL REGION IN THE RHESUS MONKEY. P.B. Cipolloni and D.N. Pandya, ENRM VA Hospital, Bedford, MA 01730 and Depts. of Anatomy and Neurology, Boston Univ. Sch. of Medicine, Boston, MA 02118.

In order to determine if interhemispheric and ipsilateral association afferents of the superior temporal region (STR) originate from common neurons, flourescent retrograde tracer (FRT) dyes were injected in selected cortical regions of the STR of each hemisphere in four animals. Although there was considerable overlap of labeled neurons of both afferent systems, only occasional double-labeled neurons were found.

The interhemispheric afferents originated not only from the homotopic but also from heterotopic areas. The heterotopic areas giving rise to the interhemispheric projections corresponded to some of the ipsilateral projection zones. Whereas the laminar patterns of the ipsilateral neurons of origin varied considerably, the interhemispheric projections neurons were only in layer III.

For interpreting the distribution patterns of the projection neurons, the architectonic parcellation of the STR described by Galaburda and Pandya (J. Comp. Neurol., 221: 169-184, 1983) was utilized. They divided the STR into three distinct rostrocaudal lines, designated as "root", "belt" and "core" lines. Each of these three lines were subdivided into four rostrocaudal stages. Because of more restricted injections of FRT dyes, our study has provided additional information about the connectional organization of the STR. Thus, the primary auditory area (KA) receives projections not only from the "belt" and "root" areas of the same stage but also from all three components of the adjacent rostral and caudal stages. This means that a "core" region is connected with all adjacent cortical subdivisions. Unlike the "core" areas, the "belt" regions receive projections from "core" and "root" regions of the same stage, as well as from the "root" and "belt", but not "core", areas of adjacent stages.

On the basis of architectonic analysis, Sanides (The Primate Brain, Vol. 1, pp. 137-208, 1970) postulated that a highly differentiated "core", ie. primary cortical area, developed from a surrounding less-differentiated association region and conceptualized this as a growth-ring pattern of cortical development. Our connectional observations support this growth-ring concept since the "core" area, ie. KA, receives projections from all the immediately surrounding regions and since the "belt" area of a given stage connects more with the other association areas surrounding the "core" than with the "core" itself.

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90.11 AN ULTRASTRUCTURAL ANALYSIS OF PRIMARY AUDITORY CORTICAL AFFERENTS ONTO IDENTIFIED NEURONS OF THE LATERAL AUDITORY ASSOCIATION CORTEX OF THE RHESUS MONKEY. V.M. Knowlton(1), D.A. Pandya(1), B.A. Ekstein(1) and P.B. Cipolloni(2). Depts. of Anatomy(1,2) and Neurology(2), Boston Univ. Sch. of Medicine, Boston, MA 02118 and the ENRM VA Hospital, Bedford, MA 01730(1,2).

Architectonic parcellation and the ipsilateral connectivity patterns of the auditory cortices of the rhesus monkey have been described at the light microscopic level (Galaburda, A.M. and Pandya, D.N., J. Comp. Neurol., 221: 169-184, 1983). Using this study for guidance, cortical lesions of the primary auditory area (KA) were made by aspiration in a series of monkeys. In order to mark the various neurons of the lateral auditory association cortex (paAlt) for subsequent ultrastructural examination, several methods were employed including the retrograde transport of horseradish peroxidase (HRP), Golgi impregnation and NADPH-diaphorase staining. In the application of the HRP method, the enzyme was injected into area Ts3 (the cortical area immediately adjacent to area paAlt rostrally) filling pyramidal neurons in layers II and III of area paAlt. Since this labeling technique marks only pyramidal neurons, the Golgi-EM method (Fairen, A., et al., J. Neuorcytol. 6: 311-337, 1977) was employed to mark all neuronal types and the diaphorase stain was used to mark a subset of the nonspinous interneurons.

Survey of the degenerating axon terminals in area paAlt showed them to be distributed in cortical layers I-V, with the overwhelming majority found in layers III and IV. The synapses formed were always of the asymmetric type. Preliminary observations show that the postsynaptic elements found forming synapses with these terminals were spines (90%), dendrites (9%) and somas (1%). Using the cell marking techniques, in combination with lesion-induced degeneration, axon afferents from area KA were demonstrated forming asymmetric synapses on spinous and nonspinous neurons of area paAlt.

Supported by NIH Grant NS26841, the ENRM VA Hospital, Bedford, MA 01730 and the Institute for Neurologic Research, Inc.

90.12 AUDITORY INVASION OF "VISUAL" CENTERS IN THE MOLE RAT (SPALAX EHRENBERGI): EVIDENCE FROM 2-DEOXYGLUCOSE. P. Heil, G. Bronchti*, Z. Wollberg, H. Scheich. Depts. of Zoology, TH Darmstadt, 6100 Darmstadt, F.R.G., and Tel-Aviv Univ., 69978 Tel-Aviv, ISRAEL.

Mole rats (Spalacidae) live subterraneously in south east Europe, Israel, Egypt, and Libya. Their largely reduced eyes are covered by muscles, skin, and fur. The central visual system also shows numerous reductions: the optic nerve is very thin and has no myelinated fibers, of the lateral geniculate body (LGB) only the ventral part (LGBv) is developed, the superior colliculus has only 5 layers, the nerves N.oculomotorius, N.trochlearis, N.abducens, and their nuclei are absent (Leder,M.-L.,Zool.Jb.Anat. 94:74,1974). In spite of these observations it is not clear to what degree these animals can see. However, their acoustic and olfactory communication seems to be highly developed (Nevo,E., Ann.Rev.Ecol.Syst. 10:269,1979).

We have tested the hypothesis whether the remaining structures of the central visual system might have adopted auditory functions by analyzing (14C)-2-fluoro-2-deoxy-D-glucose (2DG) patterns in specimens of Spalax ehrenbergi, which had been subjected to unilateral cochlea destructions and thereafter been stimulated with noise bursts after 2DG-injection. Methods for preparation and evaluation of 2DG autoradiographs correspond to those in Heil and Scheich (J.Comp.Neurol. 252:279,1986).

The success of the cochlea destructions was controlled by asymmetric labeling in the auditory system. Reduced 2DG accumulation was seen in the dorsal (DCN) and ventral (VCN) cochlear nuclei on the side ipsilateral to the lesioned ear. The medial nucleus of the trapezoid body (MNTB), the ventral (LLv) and dorsal (LLd) nuclei of the lateral lemniscus, the inferior colliculus (IC), the medial geniculate body (MGB), and the auditory cortex all showed weaker 2DG labeling contralaterally. Interestingly, the LGBv also showed asymmetric labeling with reduced 2DG accumulation contralaterally. Moreover, an area of cortex, extending for about 2mm along the dorsoventral and 1mm along the rostrocaudal axis, and located dorsally and caudally to the auditory cortical fields, was found to be asymmetrically labeled, with weaker labeling ipsilateral to the lesion. In intact controls all of these structures were labeled symmetrically. According to the Spalax brain atlas (Leder,M.L.,Zool.Jb.Anat. 93:353,1974) this cortical area would correspond to fields A17 and A18a. Electrophysiological experiments revealed the presence of auditory units there.

Our data indicate that LGBv and the cortex area described above, which have visual functions in other rodents, receive auditory input. We are presently investigating the connectivity and functional anatomy of these structures. (Supp. by DFG-SFB45.)

90.13 COMPARATIVE STUDIES OF THE ACOUSTICAL COMMUNICATION BEHAVIOR AND ORIENTATION OF WILD RATS. M.Th. Kaltwasser* (SPON: S. Chang), Zoophysiology, Morgenstelle 28, D-7400 Tübingen, F.R. Germany

The purpose of this study was to determine the complete vocalization repertoire of two closely related species, RATTUS NORVEGICUS and RATTUS RATTUS, and to evaluate its functional significance. Wild trapped rats of both species were kept in the laboratory under seminatural coniditions. Behavior was monitored on a video tape, vocalizations were recorded on a high speed tape recorder. By means of a special synchronization set-up behavior and sounds were exactly correlated. Behavioral categories were scored from the video recordings, and sound analyses were done with a real time spectrum analyzer. Analyses of behavior and sound of well established and undisturbed groups of rats indicate that the vocalization repertoire consists of nine different types of sounds. TOOTH CHATTER, SNIFF-WHISTLE and BROADBAND PULSE were in the hearing range of man, and typically occurred during orientation behavior. While sniff-whistle and broadband pulse were characteristic for R. RATTUS only, the tooth chatter was also produced by R. NORVEGICUS. It occurred as an orientation signal and also during attack behavior of a dominant male. When attacked or bitten an animal of either species emitted a SREAM of 3 to 4 harmonics with the 1. harmonic at about 2 kHz. The 22-kHz-CALL and SHORT PULSES in the range of 50 to 80 kHz were typical for agonistic behavior as well as for sexual behavior. Additionally, LONG PULSES, ranging between 30 and 60 kHz, and WAVE-LIKE MODULATED PULSES of 40 to 70 kHz were recorded during mating. New born pups isolated from their mother, emitted a DISTRESS CALL in the range of 50 kHz. These sounds mature to the adult pattern within 3 weeks.

Out of all analyzed signals some were tested for their functional significance. In a four alternative forced-choice procedure with blinded rats the orientation sounds were tested for their use as echolocation signals when subjects are deprived of sensory cues other than hearing. As no correlation between sound production and target detection was found, the results do not support the hypothesis of echolocation in rats. In resident-intruder tests with sham operated and devocalized intruders the role of the 22-kHz-call was investigated. The data show that different hierarchically organized social and environmental factors influence defensive behavior and especially the use of the 22-kHz-call of a submissive rat. The intensity of defensive behavior is suggested to be a measure of the aggression level of the resident. Therefore, resident-intruder tests may serve as an ethological model for pharmacological investigation of aggression.

90.14 THE RAT'S HEART RATE AND AROUSAL RESPONSE AS A FUNCTION OF STIMULUS INTENSITY AND RISE TIME. K. Rudolph*. (SPON : B. Mambrito). Dept. of Zoophysiology, Univ. Tuebingen, Morgenstelle 28, D-7400 Tuebingen, West Germany.

Auditory stimuli can evoke motor as well as autonomous and centralnervous responses in an organism. Depending on the intensity of the stimulus, an orienting response (OR) or a defense response (OR) can be elicited. OR's are characterized by habituation upon repeated presentation of the same stimulus whereas DR's do not habituate. Another difference between these two types of response is the direction of the reponse: in humans the cardiac OR consists in heart rate (HR) deceleration, whereas the cardiac DR consists in acceleration (GRAHAM, 1979 and TURPIN, 1986) A third response type, the cardiac startle response (SR) which consists in HR acceleration can be elicited by high intensity stimuli with a sufficiently short rise time (TURPIN, 1986).

The present study thought to characterize the rat's cardiac OR, DR and SR and the accompanying cortical response under a well defined state of vigilance (slow wave sleep, SWS). Electrocardiogram (ECG) and electrocorticogram (ECoG) of unrestrained animals were transmitted via miniature telemetry. Additionally, respiration and motility were registered. Stimuli were 2-sec bursts of white noise with intensities between 40 and 100 dB SPL and either fast, uncontrolled rise time or a rise time of 100 ms. One session consisted of 10 stimuli with an interstimulus.interval between 45 and 75 sec.

The results of this study show that the rat's cardiac and arousal response is very similar to that of man. Low intensity stimuli evoked a HR deceleration and a cortical arousal response. Since both responses showed habituation within session, they can be considered as part of the rat's OR. No long-term habituation of the OR was observed: The response had recovered completely, when the same stimuli were presented one week later. High intensity stimuli, on the other hand, showed a non-habituating HR acceleration and an incomplete habituation of the cortical arousal response. This seems to reflect the rat's DR. Further data will be presented to describe the influence of stimulus rise time upon the rat's OR and DR. This work was supported by the Deutsche Forschungsgemeinschaft grant Schn. 138/13-15 and by the SFB 307 of the University of Tuebingen.

AUDITORY SYSTEM IV

91.1 LATE ONSET OF ACOUSTIC DEPRIVATION RETARDS DEGENERATION OF COCHLEAR NUCLEUS IN GERBILS. M.D. McGinn and B.T. Faddis. Department of Otolaryngology, University of California, School of Medicine, Davis, CA 95616.

Gerbils exhibit an unusual spongioform encephalopathy, characterized by extensive microcystic lesions that are most pronounced in the neuropil of the posterior ventral cochlear nucleus. These lesions become evident at 5 to 6 weeks of age, they are progressive and, eventually, result in neuronal degeneration.

We have recently reported that the progress of these lesions is slowed by neonatal acoustic deprivation (Assoc. Res. Otolaryngol. Abstr., Vol. 10, p. 208, 1987). A conductive hearing loss, induced prior to the onset of hearing (postnatal day 12), resulted in dramatic reductions in lesion area density and lesion number density in the cochlear nuclei ipsilateral to deprived ears. However, we now find that late onset of conductive block is as effective as early onset in retarding the progress of this disease.

We induced monaural deprivation in 4 gerbils, on postnatal day 35, by occlusive ligation of the right external auditory canal. Although gerbils of this age are still immature, their auditory sensitivity has reached adult levels. As in our earlier study, the gerbils were sacrificed on postnatal day 96, transcardially perfused with mixed aldehydes, and tissue blocks containing the brainstem were prepared for histology.

The late onset of monaural deprivation resulted in unilateral reduction of lesions. In the cochlear nuclei ipsilateral to the deprived ear lesion number density was only 14% of that in the non-deprived ear (number density; left CN, $\bar{x}$=11.86, right CN, $\bar{x}$=1.684; df=3, 't'=11.20, p=0.0012; two-tailed 't'-test for dependent samples). This compares to a 17% reduction in lesion number density with early onset of auditory deprivation. Thus, the course of this disease can be altered by a reduction in functional activity after lesions begin to develop. Whether this effect is restricted to immature animals is yet unknown.

91.2 AGING IN THE RAT LATERAL SUPERIOR OLIVARY NUCLEUS: QUANTITATIVE EM STUDIES OF AXOSOMATIC SYNAPTIC TERMINALS. M.A. Casey. Department of Cell Biology and Anatomy, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294.

Recent studies have shown that principal cells and synaptic terminals in the medial nucleus of the trapezoid body (MTB) are significantly reduced in number in aged albino rats (Casey and Feldman, Neurobiol. Aging, 3:187-195, 1982; Casey and Feldman, Neuroscience, In press, 1987). The primary projection of MTB principal cells is to the ipsilateral lateral superior olivary nucleus (LSO), and the fibers terminate mainly on the cell bodies of LSO principal cells. Thus, an age-related loss of MTB principal cells would be expected to result in a significant loss of axosomatic synaptic boutons terminating on LSO principal cells, and the present study was done to test this hypothesis.

Twelve animals (four per age group) were studied at the following ages: 3-5 MO, 18 MO, and 24 MO. The animals were perfused with aldehydes, and brainstem slabs containing the LSO were prepared for transmission electron microscopy. Fifteen to twenty photographic montages of LSO principal cells from each animal were analyzed. Using a computerized planimeter, the perimeter of LSO principal cell profiles was measured, and the amount of somal surface in close contact with axosomatic synaptic terminals was determined. In young adult rats (3-5 MO of age) 75.7% (SEM = 1.5) of the surface of LSO principal cells is apposed to synaptic terminals. In rats aged 18 MO and 24 MO, the proportions are 59.5% (SEM = 3.1) and 64.7% (SEM = 2.1), respectively. These significant (ANOVA, $F = 12.6$, $p<0.001$) decreases in the amount of somal surface apposed to synaptic terminals may signify a partial deafferentation of LSO principal cells with aging. Whether this deafferentation is associated with other morphological changes, such as cell atrophy or cell loss in the aging rat LSO, is currently being studied.

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91.3 ULTRASTRUCTURE OF THE ANTEROVENTRAL COCHLEAR NUCLEUS (AVCN) IN YOUNG AND OLD C57BL/6J MICE. W.E. Briner and J.F. Willott. Dept. Psychol., Northern Illinois Univ., DeKalb, IL 60115

The C57BL/6J mouse is a model of presbycusis, developing severe sensorineural pathology during the second year of life. We examined the AVCN of young (2-mo.-old) and old (24-mo.-old) C57BL/6J mice using transmission electron microscopy. The old mice had about a 50% loss of ganglion cells and eighth nerve fibers.

The young mouse's AVCN appears to have features similar to that of other mammals, with bushy and multipolar (stellate) cells being identified. The former are characterized by perinuclear endoplasmic reticulum cisternae and numerous terminal oppositions, including end bulbs of Held. The latter are opposed by fewer terminals, and these are non-end bulb types.

Compared to young mice, AVCN neurons of old mice typically had: (1) nuclei likely to be heterochromatic; (2) more and deeper invaginations of nuclei; (3) reduced perinuclear cisternae; (4) more and larger lipofuscin deposits; (5) a lower percentage of the cell occupied by mitochondria; (6) fewer terminal contacts and a smaller percentage of neuronal perimeter opposed by terminals; (7) more myelinated axons contacting the soma en passant; (8) fewer somatic spines. In spherical cells, buildup of lipofuscin and nuclear invaginations were most pronounced in the dorsal AVCN (the high frequency region, which is first to lose input due to progressing sensorineural degeneration). However, throughout the AVCN age-related differences were generally more pronounced in multipolar cells than in spherical cells.

AVCN neurons of old, presbycusic, C57 mice demonstrate a number of changes suggesting impairment of cellular function. However, different cell types, bushy and multipolar (and, presumably, the functions they mediate), and different AVCN regions (re: tonotopic organization) may be differentially vulnerable to aging and/or presbycusis.

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91.4 RESPONSES OF INFERIOR COLLICULUS NEURONS IN C57BL/6J AND CBA/J MICE: CORRELATES OF AGING AND PRESBYCUSIS. J.F. Willott, K. Parham, and K.P. Hunter*. Dept. Psychol., N. Illinois Univ., DeKalb, IL 60115

Extracellular recordings were obtained from inferior colliculus (IC) neurons of C57 (1- to 15-mo.-old) and CBA (1- to 29-mo.-old) mice. C57 mice undergo progressive sensorineural hearing impairment (severe by 15-mo.); CBAs develop only moderate losses by 2-yrs.

Post-stimulus time histograms in young mice of both strains showed numerous examples of typical tone-evoked IC response patterns--phasic on, sustained excitation and/or inhibition, pauser, etc. These response types proved remarkably robust in the face of aging without severe presbycusis, being observed in very old (29-mo.) CBA mice. However, there were many examples of old neurons that were "sluggish" in their response to sounds or did not respond at all. Nevertheless, in those neurons that continued to respond well to tones, the relative proportions of neurons showing each response type changed little with age. Other response properties of these neurons (e.g., spontaneous activity, intensity functions) also showed little age-related change.

In severely hearing-impaired aging C57 mice many examples of "normal" PSTH response types were also found. However, the incidence of neurons responding with phasic on responses increased with aging. Many older neurons were auditorily "sluggish" or unresponsive to sound. It was often impossible to classify intensity functions in older C57s due to elevated thresholds and reduced dynamic range; nonmonotonic intensity functions were rare. Many older neurons responded to tones at unusually high rates within 10 dB of their (elevated) thresholds. The incidence of spontaneously active neurons increased in aging C57s.

The data indicate that extreme aging without severe presbycusis (CBA mice) is accompanied by an increasing number of IC neurons that respond poorly to tones; however, many "normal" response features persist. The same is true in the case of moderate aging with severe presbycusis (C57 mice); however, changes in the proportion of response types, intensity functions, and spontaneous activity are more pronounced. Thus, presbycusis appears to disrupt the coding of suprathreshold sounds more severely than aging per se.

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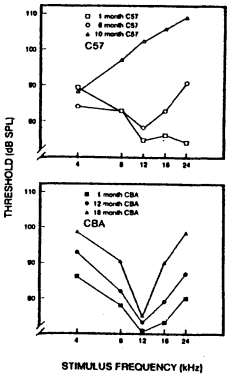
91.5 THE ACOUSTIC STARTLE RESPONSE IN YOUNG AND AGING C57BL/6J AND CBA/J MICE. K. Parham, and J.F. Willott. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

C57BL/6J mice demonstrate progressive age-related hearing loss during the first year of life (threshold elevations at 4, 8, 12, 16, and 24 kHz: 4, 5, 4, 18, and 55 dB, respectively; from Willott, J.F., J. Neurophys., 56: 391-408, 1986). CBA/J mice lose little sensitivity through 18 mo of age. We measured the acoustic startle response (ASR) to determine behavioral correlates of aging with and without presbycusis. ASR thresholds (the minimum SPL required to elicit ASRs more than 50% of the time) of C57s increase dramatically at high frequencies, but remain unchanged at 4 kHz . CBA startle thresholds increase minimally at middle frequencies and moderately at high and low frequencies (see figure). The CBA data indicate that aging per se has little effect on ASR threshold; the C57 data show that hearing loss is a cogent factor. However, elevations of ASR thresholds are much greater than elevations of absolute thresholds. Both peripheral and central mechanisms can be proposed to account for the discrepancy. High intensity ASR stimuli must displace a considerable length of basilar membrane. The basal cochlear regions (e.g.; > 20 kHz) of aging C57 mice are severely impaired, reducing the number of sensory/ganglion cells providing input to the CNS. Higher frequencies would involve relatively greater proportion of impaired organ of Corti off the "center frequency". Alternatively, central factors (e.g., greater loss of potency of excitatory than inhibitory cells) might reduce the number of centrally excited auditory neurons that "drive" the ASR.



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91.6 AUDITORY BRAINSTEM RESPONSE DEVELOPMENT IN PRECOCIAL AND ALTRICIAL RODENTS. L.J. Hood*, E.K. Barlow*, and D.B. Webster. Kresge Hearing Research Lab, Department of Otorhinolaryngolgy, LSU Medical Center, New Orleans, LA 70112.

The development of auditory brainstem responses (ABR's) was measured in four rodent species chosen because of their difference in onset of hearing relative to time of birth. Guinea pigs and spiny mice develop detectable ABR's a few days before birth, cotton rats at birth and CBA/J mice at 14 days after birth.

ABR's were obtained for three subjects from each species at 1, 3, 6, 12, 24 and 48 days after onset of airborne hearing (which coincided with days after birth except for CBA/J mice where Day 1 of detectable ABR's was obtained at 14 days after birth). ABR's were also recorded for 3 adults of each species. Click stimuli were presented through an insert earphone beginning at 98 dB peak SPL and decreasing in 10 dB steps to 10 dB below ABR threshold. Data analyzed were: 1) intensity of the response threshold, 2) latency of the first vertex-positive wave (wave I representing the VIIIth Nerve CAP), and 3) the latency interval between the first and fourth vertex-positive waves (Waves I-IV).

Response thresholds indicated two distinct groups. Guinea pigs and spiny mice showed sensitivity at Day 1 which did not differ statistically from adult thresholds. Cotton rat and CBA/J mice thresholds reached adult values by 12 days after the onset of airborne hearing. Wave I latencies varied by species with latencies statistically consistent with adult values at Day 1 for guinea pigs, Day 3 for spiny mice, Day 6 for CBA/J mice, and Day 12 for cotton rats. Interwave latencies for Waves I-IV were consistent with adult values at Day 1 in guinea pigs and did not differ significantly from adult values by Day 12 for spiny mice and cotton rats and by Day 24 for CBA/J mice. Although CBA/J mice have a more rapid development than cotton rats, it must be remembered that airborne hearing onset as measured by the ABR is close to Day 1 after birth for cotton rats while CBA/J mice do not demonstrate ABR responses until 14 days after birth.

Since all the measures (sensitivity, Wave I latency, and Wave I-IV latency interval) are at adult values in 1 day old guinea pigs, it is evident that airborne hearing is not necessary for maturation of these parameters of hearing. However the very rapid maturation of these three measures in the altricial species (cotton rats and CBA/J mice) suggests that airborne hearing may accelerate both peripheral and central auditory maturation.

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91.7 EFFECTS OF COCAINE ON THE BRAINSTEM AUDITORY EVOKED POTENTIAL (BAEP). P. Gritzke* and M.W. Church (SPON: M.J. Titmus). Research Institute on Alcoholism, Buffalo, NY 14203.

Because cocaine is both an abused substance and a central nervous system (CNS) stimulant, its effects on sensory function are of interest. For example, cocaine reportedly enhances auditory sensitivity and the acoustic reflex, can cause auditory hallucinations, and decreases cortical sensory evoked potential amplitudes. The present study examined the effects of an acute psychoactive dose of cocaine on the rat BAEP over a broad range of stimulus intensities as an objective quantitative measure of this drug's effects on subcortical auditory electrophysiology.

So far, data have been collected from 6 adult Long-Evans rats. The stimuli were 0.1 msec clicks (12.5/sec) with intensities ranging from 33-110 dB p.e. SPL. BAEPs were recorded before and after cocaine HCl treatment (10 mg/kg, i.p., 2% solution). Rectal temperatures were maintained within a narrow range to control for the possible influence of core temperature.

At the higher stimulus intensities, the rat BAEP had 6 components (labelled P1 through P6). Only components P2 and P4 were visible at lower stimulus intensities. All BAEP latencies were prolonged and all amplitudes were decreased as a function of decreasing stimulus intensities ($P<.002$). There were no significant main effects for the drug on BAEP latencies or amplitudes. There were, however, significant intensity-by-drug interactions for P2 and P4 latencies ($P<.05$). In brief, cocaine treatment prolonged P2 and P4 latencies at low stimulus intensities (60 dB and below) and shortened these latencies at high intensities (90 and 110 dB) in comparison to pre-drug levels. A similar trend was observed for P5 and P6 latencies, but not P1 (auditory nerve). The average BAEP threshold was also increased by cocaine treatment (37.3 vs. 41.0 dB, $P=.065$).

The various cocaine-induced changes in the BAEP were not strong. They do suggest, however, that the increased auditory sensitivity associated with cocaine use probably occurs only in response to loud sounds with the reverse effect occurring in response to soft sounds. Such a pattern is suggestive of a recruitment-type change (i.e., loudness recruitment) in auditory neural function. Our results with cocaine treatment are similar in some respects to the effects of amphetamine on auditory threshold (Delay et al., 1978) and theophylline on the BAEP (Church & Shucard, 1987).

91.8 EFFECTS OF MORPHINE, NALOXONE AND TEMPERATURE ON THE AUDITORY BRAINSTEM RESPONSE (ABR) IN THE GUINEA PIG. T.L.Sahley*, T.C.Chimento* and J.N.Gardi. Coleman Memorial Lab. HSE 871, University of California, San Francisco California, 94143.

Acetylcholine (ACh) is the major transmitter of the efferent olivocochlear system (OCS). Enkephalin and dynorphin-like activity also exist in the lateral OCS cells of origin, and in cochlear structures ascribed to the same system. There is also evidence of opioid-kappa and mu receptors in the cochlea, the significance of which is unknown. Amplitude reductions in N1 and ABR potentials, induced by electrical or chemical stimulation of the OCS have been well documented. To date, however, there are no published reports of opioid effects on cochlear outputs.

Changes in ABR interpeak latencies (IPLs) and amplitudes following morphine (10mg/kg) and naloxone (1mg/kg) administration were determined in a series of adult ketamine (40mg/kg) and rompun (20mg/kg) anesthetized guinea pigs. Stimuli were 100usec. rarefaction clicks, monaurally delivered at 20/sec. from 0 to 60dBSL in 10dB increments. Responses to 500 click presentations were averaged at each intensity level through a baseline period, and at 5 minute post-morphine and post-naloxone intervals. Ear temperature (ET) was continuously recorded by a thermister sealed in the contralateral ear canal. This method affords an accurate estimate of brainstem temperature (Williston & Jewett, 1982, Audiology 21:457), and ET changes are highly correlated with wave I-V or I-IV IPL shifts [$F(1,5)=29.35, p<.005; r=.92$; or $F(1,5)=17.62, p<.01; r=.88$] respectively. ET was kept to within ±0.1°F during all ABR recording.

With ET controlled, morphine and naloxone produced no measurable IPL changes. Preliminary analyses did indicate that morphine produced significantly larger (15%) wave I amplitudes at 60dBSL [$t(5)=3.98, p=.01$ but not at 30dB [$t(5)=1.81$, ns]. Naloxone injectio failed to reverse this significant effect of morphin on ABR peak amplitudes by 30 minutes [$t(5)=0.05$, ns]

This research supported by Hearing Research Inc.

91.9 EFFECTS OF ATROPINE SULFATE ON THE BRAINSTEM AND CORTICAL AUDITORY EVOKED POTENTIALS IN THE RAT. M.W. Church and R. Gritzke*. Research Institute on Alcoholism, 1021 Main Street, Buffalo, NY 14203.

Because brainstem auditory evoked potentials (BAEPs) are frequently recorded in anesthetized animals and humans, it is important to become familiar with the effects on the BAEP of drugs used during anesthesia, including pre-anesthetics. The dose-dependent and stimulus intensity-dependent effects on the BAEP of one pre-anesthetic, atropine sulfate, were studied in the unanesthetized rat. The animal subjects were 11 young adult female Long-Evans rats. BAEPs in response to 0.1 msec clicks (12.5/sec) were recorded from skull screw electrodes during a baseline period as well as after saline and atropine treatments while each animal was restrained in a fixed position relative to a piezoelectric speaker inside a sound isolation chamber. Click intensitities were 70, 90 and 110 dB p.e. SPL. Rectal temperatures were maintained within ± 0.2 °C of baseline to control for possible temperature-related effects.

Contrary to a prior report, doses in the standard pre-anesthetic range (i.e., 0.250 to 1.000 mg/kg) did not convincingly influence the BAEP. Only the highest dose (40 mg/kg) produced a significant and noteworthy change. This effect was characterized by significant amplitude increases in the P1, P2, and P3 components, but not in the P4, P5 and P6 components. This selective effect occurred at the highest stimulus intensity of 110 dB p.e. SPL, but not at lower intensities. There were no convincing atropine-induced changes in BAEP latencies. Atropine-induced changes in the cortical auditory evoked potential (CAEP) were characterized by amplitude decrements. Thus, atropine seemed to have an excitatory effect on the BAEP and an inhibitory or depressive effect on the CAEP. Atropine-induced changes in the routine electroencephalogram (EEG) were also noted. The atropine-induced changes were not attributable to changes in core temperature, circadian variation, stress associated with handling and restraint, or the tensing of middle ear muscles.

The results of the present study suggest that a standard pre-anesthetic dose of atropine sulfate does not influence the BAEP. Instead, a relatively massive dose is required. Research by others, however, suggests that low doses of pre-anesthetics might interact with other drugs to alter the BAEP (Bhargava et al., Neurosci., 1978, 3: 821-826).

91.10 EXAMINATION OF IRRELEVANT AUDITORY EVOKED POTENTIALS DURING AN AUDITORY ATTENTION TASK. L.C. Oatman. U.S. Army Human Engineering Laboratory, Aberdeen Proving Ground, MD 21005-5001.

This investigation was designed to examine whether selective auditory attention in cats involves a filtering or gating of irrelevant auditory stimuli at the peripheral stages in the afferent auditory pathway. Previously, we demonstrated that when cats were attentive to a visual discrimination task, the amplitudes of irrelevant auditory evoked potentials at the receptor and cortical levels were suppressed. Since suppression of the N1 response of the auditory nerve was observed under carefully controlled conditions and in the absence of middle-ear muscles, we suggested that, during attention to visual stimulation, a central inhibitory mechanism suppresses irrelevant auditory stimuli through the action of the olivo-cochlear bundle (OCB). Since these studies demonstrated a cross-modality gating effect during visual attention, perhaps a similar gating function occurs within the auditory modality during attention to auditory stimuli.

To examine this possibility, four adult female cats learned a two-tone (1000 Hz and 800 Hz) frequency discrimination using food reinforcement. The two tones (relevant stimuli) were presented successively at 85 dB SPL (re 0.0002 microbar). The auditory clicks (irrelevant stimuli) were presented at 1/sec at each 10-dB intensity step from 35 to 125 dB SPL (re 0.0002 microbar) during the presentation of the two-tone discrimination task.

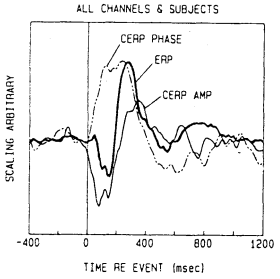
Click-evoked potentials were recorded from the round window (cochlear microphonic and auditory nerve), cochlear nucleus, and auditory cortex of unanesthetized cats during periods of auditory attention and non-attention. The clicks (irrelevant stimuli) of increasing intensity were presented continuously as background before, during, and after the presentation of a two-tone frequency discrimination task (relevant stimuli) which attempted to alter the attentive state of the cats. Amplitude measures of the auditory evoked potentials recorded concurrently at the receptor and the cortical levels were used to assess the effects of auditory attention on irrelevant auditory click stimuli. At all electrode sites (auditory nerve, cochlear nucleus, and auditory cortex) the mean peak-to-peak amplitudes of click-evoked potentials were not significantly different during attention to the two-tone frequency stimuli when compared with the pretest and posttest control sessions. The results suggest that during auditory attention, irrelevant auditory stimuli are not affected by inhibition of the olivo-cochlear bundle at the peripheral stages in the afferent auditory pathways. In contrast to a cross-modality gating effect during visual attention, a similar gating function does not occur within the auditory pathways during auditory attention.

91.11 EVENT-RELATED PERTURBATIONS IN 40 HZ AUDITORY STEADY-STATE POTENTIALS. S. Makeig* and R. Galambos. Childrens Hospital Research Center, 8001 Frost St., San Diego, CA 92123.

When a train of monaural clicks is delivered to the human ear at repetition rates near 40/s, a steady-state evoked response (SSR) is recorded from the scalp. An experimental event (such as changing the intensity of one of the clicks) may perturb the amplitude and phase of the SSR, and these perturbations can be quantified by averaging the responses time-locked to the events, narrow-band filtering at the stimulus rate, and measuring the amplitude and phase of the filtered response using a brief (circa 50 msec) moving window. A complex event-related potential (CERP) is the result. The CERP is a two-dimensional, narrow-band, frequency-domain analog of the usual one-dimensional time-domain event-related potential (ERP).

If a single click is omitted from the stimulus train the CERP amplitude drops for 200 msec then rises (peaking at 400 msec), whereas the phase shifts 40 or more degrees (at 100 msec), crosses the baseline near 400 msec, reaches a minimum at 600 msec and takes fully 900 msec post-stimulus to regain baseline phase. When the perturbing stimulus is a click to the ear contralateral to that receiving the probe train a similar CERP appears; however, when the perturbing stimulus is visual (checkerboard reversals), no CERP is observed.

The window on event-related brain dynamics provided by the 40 Hz CERP differs from that given by the conventional auditory ERP sequence; the CERP both parallels and complements the ERP. The CERP may index gating of events in cortical and subcortical structures, and we speculate that its initial 200 msec drop in amplitude may be related to the several 200 msec time constants characteristic of central auditory integration.



91.12 EFFECTS OF TEMPORAL AND PARIETAL LESIONS ON TEMPORAL COMPONENTS OF THE HUMAN AUDITORY EVOKED POTENTIAL. R. T. Knight, D. Scabini*, D. L. Woods and C. C.Clayworth* Neurology Dept., Univ. of California Davis, V.A.M.C., 150 Muir Rd., Martinez, Ca 94553

Based on scalp topography and dipole modelling data, the human auditory evoked potential (AEP) generated to a transient sound is felt to consist of superimposed neural activity from multiple intracranial sources. The T-component of the human AEP is a negative potential which is maximal over temporal recording sites and is proposed to arise from a radial dipole source situated in lateral regions of the superior temporal gyrus (STG). In order to assess the contributions of temporal and parietal cortex to the T-component, we recorded AEPs to tone pips (50 msec duration, 1Khz, 60 dbSL, 1/sec ISI) in controls and in patients with focal unilateral lesions centered in STG (n=10) or in lateral parietal cortex (PAR; n=6). Control subjects generated a T-component peaking at 128 msec with comparable amplitudes over T3 (-1.09 uv) and T4 (-1.33 uv) electrode sites. In controls the T-component was markedly enhanced in amplitude (by 88%) over the temporal lobe contralateral to the ear of stimulation ($p < 0.001$). Data from STG and PAR patients were analyzed as a function of whether the temporal electrode was ipsilateral (Ti) or contralateral (Tc) to the lesioned hemisphere. Latencies of the T-component were similar to controls for both STG and PAR groups (PAR, Ti=120 msec, Tc=128 msec; STG, Ti=124 msec, Tc=128 msec). The T-component was slightly reduced over lesioned and non-lesioned hemisphere in the PAR group but the amplitudes were not significantly different from controls (Ti=-0.66 uv, Tc=-0.82 uv). In contrast, a near abolishment of the T-component was observed over lesioned STG with normal amplitudes recorded over non-lesioned hemisphere (Ti=-0.13 uv, Tc=-1.27 uv, $p < 0.02$). These data support an origin of the human T-component in STG. The results will be discussed in relation to other lesion data concerning the origins of the human AEP.

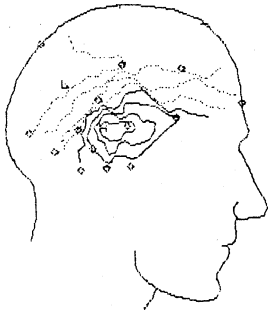
Research supported by the VA Research Service and NIH Grant NS21135 to R.T. Knight

91.13 THE 45 MSEC TEMPORAL POSITIVE PEAK (TP45) OF THE HUMAN AUDITORY EVOKED POTENTIAL. A.T. Cacace* and J.R. Wolpaw (SPON: K.D. Barron). Depts of Surg, Neurol, & Anat, Albany Med Coll, Albany, NY 12208; and Wadsworth Labs, NYS Dept Hlth, Albany, NY 12201.

Detailed topographical analysis of middle latency (20-80 ms) auditory evoked potentials has received only limited attention. Knowledge of these distributions may allow development of significant new measures of CNS function. We studied these distributions in 30 normal adults (20-49 years, 15 male, 15 female). Monaural and binaural clicks were delivered at 0.3 Hz and recorded from 32 channels referred to a balanced noncephalic reference.

In addition to the expected central positive peaks, Pa and Pb, at about 30 and 50 ms, very localized positive activity occurred over posterior temporal scalp locations at about 45 ms. This TP45 peak, focused over auditory cortex, was simultaneous with frontocentral negative activity. TP45 was larger over the right hemisphere and over the contralateral hemisphere.

TP45 has probably been obscured in previous studies by use of active reference electrode locations, such as mastoid and earlobe, and possibly also by high stimulation rates. We are defining the intersubject variability of TP45 and evaluating electrode montages which may minimize variability for clinical applications.



Right-side isovoltage topography for TP45 to left ear stimulation. Solid lines indicate positive voltage, dotted indicate negative. "H" is the highest point, "L" is the lowest.

(Supported by NIH NS19891)

91.14 CONTRIBUTIONS OF MULTIPLE NEURAL ELEMENTS TO THE CLICK-EVOKED RESPONSE OF MONKEY AUDITORY CORTEX. C.E. Tenke, G.V. Simpson, C.E. Schroeder, J.C. Arezzo and H.G. Vaughan, Jr. Neuroscience and Neurology Depts., Albert Einstein Coll. Med., Bronx, NY.

Auditory koniocortex is the principal generator of surface-recorded auditory evoked potentials (AEP) in monkeys, and is thought to be a major contributor to scalp-recorded AEP in humans. Pyramidal cells have traditionally been identified as the major neural element contributing to surface responses. This study uses high resolution, intracortical recording techniques to examine the laminar distribution of neural activity associated with the AEP as well as the possible contribution of non-pyramidal elements. AEPs and concomitant multiunit activity (MUA) were recorded during electrode penetrations through the supratemporal plane (STP) in 2 alert monkeys (m. Fasicularis). Recordings were made using 16 channel, multicontact electrodes with intercontact spacings of 75 or 100uM. Current source density (CSD) was computed from the AEP depth profiles. To permit an accurate one-dimensional CSD, penetrations were made normal to STP. Stimuli consisted of binaural clicks (50usec rectangular pulses at 100db SPL) at repetition rates of 1.4/sec to 40/sec, varied across trial blocks.

The early portion of the cortical AEP consists of 4 components: an initial negativity (N9), a double lobed positive wave (P12/14), a small, second negativity (N18) and a later positivity (P24). Each component inverts in polarity within STP: P12/14, N18 and P24 within the middle laminae, while the inversion of N9 is completed only in the deepest cortical laminae. CSD analysis resolves three temporally and spatially separable sinks in the immediate vicinity of the thalamocortical terminations. The earliest sink is sharply localized to lamina IV and is coincident with N9 and with the initial burst of MUA recorded within A1 and the subjacent white matter. Both the initial sink and N9 are selectively spared at high repetition rates. The second sink, coincident with P12/14, is co-located with the N9 sink but is characteristically larger in amplitude and more widely distributed. A third sink peaking between 18 and 20 msec is centered within lamina III. The sources associated with the N9 sink are primarily confined to laminae IV and V, while those associated with P12/14 extend superifically to include laminae III and II.

These data are consistent with the localization of the major generators of the early AEP components to neural elements within lamina IV. Terminal depolarization of thalamocortical afferents and the initial activation of lamina IV stellate cells appear to be exclusively responsible for N9 and partially responsible for P12/14. The activation of pyramidal cells contributes to P12/14 and to the later components. Supported by MH06723, MH15788.

91.15 CONTRIBUTIONS OF AUDITORY CORTEX TO AUDITORY EVOKED POTENTIALS IN THE RAT. G. V. Simpson and R. T. Knight, University of California, Dept. of Neurology, V.A. Med. Ctr., Martinez CA 94553

Auditory evoked potentials (AEP) were recorded before and after bilateral ablations of auditory cortex to examine its contribution to the AEP in the rat. AEPs were recorded in the alert, unrestrained rat from screw electrodes implanted (under pentobarbital anesthesia) in the lateral skull directly over auditory cortex (ACx) 4.5 mm anterior to the interaural line; and in the dorsal skull over frontal cortex (FCx), at the vertex (Vx), and at the posterolateral edge of the dorsal skull (PL). AEPs were recorded to clicks (50 dB above BAEP threshold) in eight Fischer 344 male rats 12 months of age, twice in each of two pre-lesion sessions and at one and six days following bilateral auditory cortex lesions. Post-lesion data are reported for the 4 animals in which complete bilateral ablations of auditory cortex had been verified.

One series of components was maximal over the dorsal skull surface (at Vx) and consisted of a P13, N18, P25, N40, N50 and P70. The later components (N40, N50 and P70) were also recorded on the lateral skull (ACx). Another series of components was found to be maximal on the lateral skull over auditory cortex (ACx), the P7, P11, N14, and N32. Higher stimulation rates (10/sec.) diminish the P11 and increase its latency while the P7 is largely unaffected.

The P7, P11 and N14 components were eliminated on the first day following bilateral auditory cortex ablations and remained so at day six post-lesion. The N32 component was greatly reduced, but the percentage decrease could not be unequivically determined due to its overlap with the N40 component. In marked contrast, those components maximal at the dorsal skull were relatively unaffected by the lesions. The basic waveforms remained intact and only partial amplitude reductions were found, some not evident until the sixth day post-lesion.

The findings indicate that auditory cortex is the generator of the laterally recorded P7, P11 and N14 components and at least the major contributor to the laterally maximal N32. All AEP components recorded on the dorsal skull appear to receive relatively little, if any, direct contribution from auditory cortex generators.

91.16 FOURIER BESSEL DECOMPOSITION OF AUDITORY EVOKED POTENTIALS. F.M. Chen* and K.M. Mudry (SPON: L. Abel). Dept. Biomedical Engineering, The University of Akron, Akron, OH 44325.

A series of evoked potentials recorded in response to a sequence of stimuli are often difficult to quantitatively describe and to compare to each other if more than one or two predominant peaks occur in the signal. A method to decompose evoked potentials into orthogonal components which can approximate the original signal with a small number of basis components are being investigated. There are many orthogonal series which could be evaluated including the Fourier and Exponential series. The Fourier Bessel functions, because of their decaying oscillatory shape which resembles the auditory evoked responses, have the potential to be a more efficient descriptor of these signals than some other orthogonal series. Bessel functions of the 1st kind and above the 1st order have a shape related to the evoked reponses. The decomposition using the 1st kind, 1st order were used to analyze a series of whole VIIIth nerve responses to a series of tone bursts at different frequencies. Because of the latency of the evoked response, the optimal starting time used to decompose the signals was found to be important. The number of components needed to reconstruct the potentials was dependent on the shape with fewer components needed for signals with rapid onsets and more transient responses than signals which exhibited slower onsets and continued responses throughout the stimulus period.

This work was supported by The U. Akron.

FEEDING AND DRINKING II

92.1 **ABDOMINAL VAGAL PATHWAYS MEDIATING THE CEPHALIC PHASE INSULIN SECRETION IN THE RAT.** H.-R. Berthoud and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue U., W. Lafayette, IN, 47907.

In contrast to the widely held belief that the celiac branch(es) of the abdominal vagus innervates the pancreatic B-cells to stimulate insulin secretion, we recently found that the plasma insulin response to electrical stimulation of the cervical vagi depends almost exclusively on the integrity of the two gastric branches and the hepatic branch (Berthoud et al., Soc. Neurosci. Abstr. 12, p. 766,1986). In order to test the validity of these observations in a more physiological situation (electrical stimulation activates all vagal fibers indiscriminately) we investigated the cephalic phase of insulin secretion in separate groups of rats that had received selective bilateral celiac vagotomies, bilateral gastric (plus hepatic) vagotomies, or sham operations.

Vagotomies and the implantation of chronic jugular vein catheters were performed in male SD rats of 320 - 360g which had been adapted during 2 weeks to a liquid sweetened condensed milk diet. Fifteen days later, testing began with either normal rat chow (2 trials), 20% α-D-Glucose (1 trial) or the maintenance diet (1 trial) given to the 10 - 15h food deprived animals. At the end of the testing period, the animals were injected i.p. with 3mg True Blue and 3 days later were sacrificed. Inventory of the fluorescently labeled dmnX cells was taken in order to verify the selective vagotomies, according to the method of Powley et al. (Am. J. Physiol., in press, 1987).

Controls and animals with verified bilateral celiac vagotomies, both significantly increased their plasma insulin levels at 2min when eating chow (+17.5 ± 3.1 μU/ml and +15.9 ±5.2 μU/ml above basal, respectively). In contrast, rats with verified bilateral gastric plus hepatic branch vagotomies showed only a small and nonsignificant increase (+1.4 ± 1.3 μU/ml, $p<0.01$). Plasma glucose changes at 2 min were small and non-significant for any group. Cephalic phase insulin responses to milk or glucose ingestion were confounded by the significantly elevated glycemia at 2 min, due to rapid absorption, but the same pattern emerged if insulin responses were corrected for glycemia. In other regulatory tests, gastric plus hepatic branch vagotomized rats exhibited (1) depressed intake of the maintenance diet for the first 7 days following vagotomy, (2) chronically reduced water intake, (3) lower body weight, (4) delayed gastric emptying of chow, and (5) accelerated emptying of fluids. Celiac vagotomized rats failed to show any of these disturbances and only displayed abnormally high plasma glucose and insulin levels between 10 and 20 min after the start of a chow meal, which was most likely due to changes in gastric emptying and/or absorption.

It is concluded that the integrity of the gastric and the hepatic abdominal vagal branches is necessary and sufficient for mediation of the cephalic phase insulin response. Together with our earlier electrical stimulation experiments, this is strong evidence that these branches innervate the endocrine pancreas. The targets and functions of the celiac branches remain to be indentified. (NIH Grant DK27627)

92.2 EFFECT OF NUCLEUS OF SOLITARY TRACT LESIONS ON FOOD INTAKE RESPONSES AND DIETARY CHOICES OF RATS FED DIETS CONTAINING PROTEIN IN EXCESS. P.M.B. Leung, D.W. Gietzen and Q.R. Rogers. Dept. of Physiological Sciences, School of Vet. Med., Univ. of California, Davis, CA 95616.

Bilateral electrolytic lesions were placed in nucleus of solitary tract (SOL) of adult Sprague-Dawley rats at rostrocaudal extent of medial and lateral aspects rostral to the area postrema (AP) (SOL 1, 13.3 mm posterior to bregma) and at the level of the AP (SOL 2, 13.8 mm posterior to bregma). Earlier we reported that rats with AP lesions showed chronic reduction in food intake (FI) and body weight. They also exhibited similar initial FI depression as did intact controls (INT) but showed facilitated adaptation when fed a high-protein (75% casein) diet (HP) (Leung, P.M.B., et al., Fed. Proc. 45:1091, 1986). Also when offered the choice between a low protein (6% casein) diet (LP) and the HP, rats with AP lesions consumed more HP in the choice-regimen than INT (Leung, P.M.B., et al., Soc. Neurosci. 12:1556, 1986). Since SOL has demonstrated neural and vascular connections with AP and contains both visceral and gustatory connections, animals with SOL 1 and SOL 2 lesions as well as INT were equilibrated with the LP and fed the HP for 23 days before refeeding the LP. Following LP re-equilibration, rats with SOL 1 and SOL 2 lesions as well as INT were offered the choice between the LP and the HP. The two identical spillage-proof food containers in choice-regimen were rotated daily at random from side to side and among all animals tested. Food and water were available ad libitum. An average of 3 days of LP intake just prior to the switching to HP was used as basal baseline intake (BL). When fed HP, animals with SOL 1 and SOL 2 lesions as well as INT showed similar marked initial reductions in FI (45.0±2.3%, 52.5±2.4% and 49.6±2.8% of respective BL of 24.6 g, 24.4 g and 24.5 g for the two lesioned and INT groups). Slow adaptation to HP was observed for all lesioned and non-lesioned animals reaching 80.3±3.1%, 75.8±6.4% and 83.4±5.2% of their respective BL for rats with SOL 1 and SOL 2 lesions as well as INT, respectively, in 23 days. All animals, whether lesioned or non-lesioned, showed transient hyperphagia on the 3rd day but returned to BL thereafter when refed LP. When offered the choice between LP and HP, the initial FI of HP comprised only 22.9±8.7%, 24.5±7.8% and 27.4±4.9% of their respective combined total FI in choice-regimen for SOL 1 and SOL 2 lesioned groups as well as INT. The initial protein-energy selected expressed as % of total caloric intake (PE%) were 22.6%, 23.6% and 25.4%, respectively, for SOL 1 and SOL 2 lesioned animals as well as INT. The preference for LH steadily increased while the reverse was true for HP. Averages of 7 days PE% for SOL 1, SOL 2 and INT groups were respectively 17.7%, 17.8% and 18.6%. These results indicate that the SOL and the AP responded differently to HP feeding. In contrast to AP, SOL is not involved in the adaptive FI of HP or normal avoidance for HP under dietary choice situations. (Supported by USPHS NIH grant #DK13252.)

92.3 STRIA TERMINALIS TRANSECTIONS PRODUCE DRAMATIC CHANGES IN TASTE REACTIVITY WITHOUT CONCOMITANT HYPER- OR HYPO-PHAGIA IN THE RAT. R. M. Black* and H. P. Weingarten. Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

An over-reactivity to the sensory qualities of a food (*i.e.* finickiness) is one of the defining features of the ventromedial hypothalamic (VMH) syndrome. The neural damage responsible for this disturbance is unknown. It is possible that finickiness results from damage to cell bodies in the VMH *per se*. In contrast, it may result from interruption of amygdaloid (AMY) inputs to the VMH via the Stria Terminalis (ST). This view is supported by evidence that: 1) AMY inputs to the VMH are destroyed in the classic VMH lesion; 2) hyperphagia and finickiness may be anatomically separable in the VMH; and 3) AMY damage results in feeding disturbances suggestive of an over-responsiveness to taste stimuli. To test the contribution of damage of amygdalo-hypothalamo connections via the ST to VMH induced finickiness, the taste responsiveness of four groups of rats (VMH lesion, ST transection, combined VMH/ST damage and controls) were assessed in a sham feeding paradigm.

When feeding palatable sucrose solutions of various concentrations, ST rats were as finicky as VMH rats. However, the effects of VMH and ST damage were additive suggesting that the taste disturbances induced by VMH and ST damage are independent. Neither VMH, ST nor VMH/ST rats over-respond to unpalatable tastes such as quinine. When the continuity of the gastro-intestinal tract was maintained, *i.e.* the fistula was closed, the excessive taste responsivity of the ST rats, but not the VMH or VMH/ ST rats, was completely masked. Similarly, when fed *ad lib*, ST rats were not hyperphagic compared to controls, whereas VMH and VMH/ST rats were. It was concluded that ST damage produces dramatic alterations in the sensory processing of sweet but not bitter tastes, independent of the VMH, and this effect is masked when the continuity of the gastro-intestinal tract is maintained.

Supported by grant funds from NSERC.

92.4 TASTE RESPONSES IN THE NTS OF NA-DEPRIVED RATS. G. P. Mark*, K. M. Jacobs* and T. R. Scott. Dept. of Psych. and Inst. for Neurosci., Univ. of Delaware, Newark, DE 19716.

Maintenance of sodium balance is crucial to mammals and is expressed in the innate salt appetite. With depletion, sodium preference is exaggerated, hypertonic solutions accepted, and salt balance restored. This compensatory behavior is thought to result from a centrally-induced change in taste responsiveness, a proposal we tested by recording taste activity from 94 single neurons in the nucleus tractus solitarius of sodium replete (N=44) and of deprived (N=50) rats. Twelve Wistar rats were given a nominally sodium-free diet for 10-13 days, and the resulting Na depletion confirmed by flame photometry of their urine. Nine rats provided control data. Taste stimuli included five concentrations of NaCl (.003-.3M) plus 8 other salts, acids, sugars and alkaloids. Taste responsiveness was generally reduced in Na-depleted rats. Spontaneous activity was 33% lower while responses to sodium salts lagged by a mean of 30%, to acids by 25% and to bitter salts and quinine by 17%. Remarkably, mean activity to sugars was 60% higher in the deprived group. Activity in sweet-sensitive and Na-sensitive neurons was most affected. In deprived animals responses to sodium salts were lower by 80% among Na-sensitive cells while among sugar-sensitive neurons activity to these stimuli was nearly 10 times greater than in controls. Multidimensional stimulus spaces based on average activity in each of the four identifiable neuron subgroups (sugar, sodium, acid, quinine) demonstrated a shift in the affiliation of Na-salts away from bitter stimuli and toward sugars. These results confirm earlier findings from the chorda tympani that Na-deprivation suppresses activity evoked by Na salts and augments responses to sugars. Beyond this, however, they reveal a shift in the major responsibility for encoding sodium from Na-sensitive neurons to those whose primary sensitivity is to sugars. The implication is either that sodium assumes a sweet taste in a deprived rat or that the underlying dimension we are measuring is one based on hedonics along which sodium is shifted toward the positive pole by deprivation and so toward the sugars that reside there.

92.5 TASTE REACTIVITY IN RATS WITH VENTROMEDIAL-HYPOTHALAMIC AND SEPTAL LESIONS
J. M. Dopp* and J. C. Mitchell, Psychology Dept. Kansas State University, Manhattan, KS., 66506.

The taste reactivity test (Grill & Norgren, 1978) was used to evaluate the magnitude of taste hyperresponsivity displayed by rats with lesions to the VMH and septum. Septal, VMH, and Control groups (n=6) received 1-ml intraoral infusions of solutions of sucrose (.01, .03, .3, 1.0 M) and quinine hydrochloride (.00003, .00003, .0003, .003 M). The resulting stereotyped behavioral responses or "fixed action patterns" (FAPs) were videotaped and analyzed frame-by-frame.

Responses were divided into three categories: ingestive, aversive, and neutral (neutral responses were those found to be negatively correlated with solution concentration).

Septal and VMH animals displayed significantly more ingestive responses to sucrose than did controls, and all groups of animals showed similar decreases in aversive responses as sucrose concentration increased. These results support the notion of positive finickiness (Weingarten, 1982) in septal and VMH animals.

Lesion animals did not show evidence of negative finickiness; there were no sigificant differences in numbers of aversive responses to quinine hydrochloride.

Additionally, ingestive responses were plotted against sucrose concentration and it was found that while septal animals showed more ingestive responses than controls at all concentrations, the slopes of the lines for the two groups were approximately the same. In contrast, the slope for the VMH group was significantly steeper than that of the septal group. This indicates that the nature of taste hyperresponsivity in septal animals is different from that in VMH animals: The septal lesion appeared to produce a constant increase in responsivity across the sucrose concentrations tested; the VMH lesion produced a multiplicative increase in responsivity as sucrose concentration increased.

92.6 ANGIOTENSIN II EVOKES DRINKING-LIKE JAW AND TONGUE MOVEMENTS IN THE ANESTHETIZED GUINEA PIG. L. J. Goldberg, K. De Bruyne* and G. E. Gerstner*. Depts. of Oral Biology, Kinesiology, Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Angiotensin II (AII) has been shown to elicit drinking in a variety of water replete awake animals when administered either intravenously or directly into the cerebral ventricles. Its effects are presumably via activation of dopaminergic systems that initiate and maintain drinking motor movements. We have observed that the dopamine agonist apomorphine when administered intravenously in the anesthetized guinea pig induces drinking-like jaw and tongue movements (Lambert et al., J. Neurophysiol. 55,301, 1986). The purpose of the present study was to determine if AII could induce such drinking-like motor movements in the anesthetized guinea pig.

In ketamine anesthetized guinea pigs fine wire bipolar recording electrodes were inserted into the jaw opener digastric muscles for recording EMG activity. The movements of the mandible were monitored by a photoelectric position sensor placed in front of a small tungsten light source fixed to the inferior surface of the mandible. An infusion pump was used to deliver (i.v. through the external jugular vein) 1 μg of AII dissolved in 0.4 ml of normal saline at a rate of 0.02 ml/min for 20 min.

The level of anesthesia was adjusted so that all animals demonstrated spontaneously occurring rhythmic jaw movements prior to AII infusion. This previously described behavior (Lambert et al., J. Neurophysiol. 55,301, 1986) consists of prominent lateral excursions of the mandible occurring in association with both jaw opening and closing movements. From between 6 and 8 minutes after the beginning of AII infusion jaw and tongue movements and digastric EMG activity characteristic of drinking behavior were observed. This activity consisted of rhythmic jaw movements with the opening component of the cycle confined almost totally to the midline. In contrast to the spontaneous cycles, visually observed prominent tongue protrusions accompanied each jaw opening movement. The mean and standard deviation of the cycle times of the drinking-like behavior was 386±88 ms (n=96); the EMG burst durations of the digastric muscle were 128±23 ms, and there was a highly significant correlation between digastric EMG burst duration and cycle time ($r=0.47$; $p<0.001$). Cycles with drinking characteristics gradually replaced the spontaneous type. From 9 to 13 minutes after the onset of AII infusion the presence of drinking-like cycles increased to 70-95% of the total observed rhythmic jaw movements. Drinking-like cycles ceased to occur and all rhythmic jaw movements were of the spontaneous type 18 minutes after the beginning of AII infusion.

The jaw movement trajectories, tongue movement patterns, digastric EMG burst durations and cycle time-digastric burst duration correlations are similar for AII and apomorphine induced drinking-like behavior in the anesthetized guinea pig, as well as for actual drinking in the awake guinea pig. These results indicate that the regulatory peptide AII can drive motor systems responsible for drinking behavior in the unconscious animal.

This research was supported by NIH grant DE 4166.

92.7 PATTERNS OF FEEDING AND BITING ELICITED BY DEPRIVATION OR BY HYPOTHALAMIC STIMULATION *M. L. Volkov*, T. R. Maone, S. Mann, J. M. Kaplan* and S. M. Feldman.* Department of Psychology, New York University, New York, NY 10003.

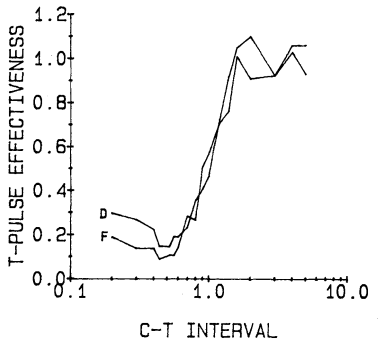
We used a discrete trials paradigm to study the microstructure of food intake in rats. A single daily feeding session consisted of 30 or 32 trials, during which a dish of wet mash (Purina rat chow and water, 3:2, w/w) was available for 30-sec feeding trials (bouts) separated by 2-min intertrial intervals. Feeding was achieved during experimental sessions either by using 30-sec trains of electrical stimulation of the lateral hypothalamus (ESLH: 0.1msec pulses @ 60pps) or by food deprivation. Intake during ESLH sessions was targeted by varying current intensity. Under deprivation conditions, animals were adapted to a feeding schedule in which food was available during trial-structured meals that always followed a 23-hr fast. Food intake was measured after each trial, and sessions were recorded on videotape for subsequent analysis of latency, duration and bite frequency. Deprived rats were studied either in an open field environment, or were suspended in a harness (Kaplan and Feldman, *Soc. Neurosci. Abstr.*, 9:190, 1983) during the feeding sessions to restrict their behavioral options. Harness restraint was also used for ESLH-induced feeding. Both restrained and open field subjects adapted to the paradigm and consumed a sufficient amount to maintain body weight. ESLH subjects and a comparable group of deprived subjects studied using harness restraint were matched for total session food intake.

Results showed that the shape of the intake curves were the same for food deprived and ESLH subjects. Analysis by linear regression of the eating curves produced by subjects under all conditions showed that intake rate decreased monotonically throughout the course of a feeding session (mean slope = -0.048 for deprived animals in harness, -0.050 for deprived animals in open field, and -0.040 for ESLH animals) with a high correlation between trial order and intake level (mean r = -0.83 for deprived animals in harness, -0.77 for deprived animals in open field, and -0.79 for ESLH animals). Microstructural analyses, carried out on ESLH and deprived animals in the harness, revealed that intake rate was a direct function of bite size, which declined through the course of the session for both groups, with a *constant* bite frequency. This constant bite frequency under ESLH conditions was earlier reported by Kaplan and Feldman, cited above. However, under ESLH conditions the number of bites was approximately double that seen under deprivation-induced conditions, even when intake rate was the same for both groups. We have shown that for both ESLH-elicited and deprivation-induced feeding, intake can be described by the same linear function. By sampling intake rate over time, this paradigm reveals a strong correlation between decreasing intake rate and meal progress.

92.8 A COMPARISON OF REFRACTORY PERIOD ESTIMATES FOR STIMULATION-INDUCED FEEDING AND STIMULATION-INDUCED DRINKING. M.B. Noel and R.A. Wise. Dept. of Psych., Concordia University, Montreal, Canada H3G 1M8.

Electrical stimulation of the same site in the lateral hypothalamic medial forebrain bundle (MFB) can induce a variety of behaviors including eating and drinking. It is not clear whether these two behaviors result from activation of two different fiber systems of the MFB, or whether the same MFB fiber population is involved in the mediation of the two behaviors. In the present experiment, the refractory periods (RPs) for the two behaviors were estimated and compared.

Rats were implanted with monopolar MFB stimulating electrodes. Subjects were four animals that both ate and drank in response to stimulation. Refractory periods for the substrates of stimulation-induced feeding (SIF) and for stimulation-induced drinking (SID) were estimated using a paired-pulse technique, in which the animal's latency to respond during a train of pulse pairs is compared to its latency to respond to a train of single pulses. The distribution of RPs in the population of behaviorally relevant fibers was inferred by varying the delay (C-T interval) between the two consecutive pulses (C and T pulse) of each pair in the paired-pulse condition. The initial rising portion of the RP curves of the two behaviors begins at C-T intervals of about 0.5-0.7 msec. In either behavior the RP curve approaches an asymptote at C-T intervals of 2.0-3.0 msec. It appears that the two behaviors are mediated at least in part by the same MFB fibers, or by different MFB fibers having similiar RP distributions.



92.9 STUDIES ON THE FEEDING AND DRINKING ELICITED IN RATS FOLLOWING INJECTIONS OF MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS. M.A. Klitenick and D. Wirtshafter, Dept. Psychology, Univ of IL at Chicago Chicago, IL 60680.

In previous studies we have demonstrated that injection of the GABA-A agonist muscimol into the median raphe nucleus (MR) leads to a pronounced increase in food and water intake. Mapping studies have shown that the MR is a more sensitive site for the elicitation of feeding and drinking than are either the dorsal raphe nucleus or the ventral tegmental area (Klitenick & Wirtshafter, 1986) and, in other studies, we have observed a similar pattern of anatomical sensitivity with respect to the ability of muscimol to increase locomotor activity. In the current report we attempted to investigate in more detail the ingestive behaviors produced by intra-MR muscimol injections.

Several authors have reported that the effects on feeding elicited by intra-hypothalamic injections of norepinephrine differ depending on whether the injections are made during the light or dark phases of the day/night cycle. In order to determine whether similar effects occur with muscimol injections, we prepared rats with chronic indwelling cannula aimed at the MR. Feeding and drinking in response to intra-MR injections of muscimol (0,6.25,12.5, 25ng/0.25ul) were then examined either during the first two hours of the dark phase or during the middle of the light phase. The results indicate that baseline intakes were much higher during the night than the day. Muscimol, however, produced equivalent, dose dependent, increases over baseline feeding and drinking regardless of the time of administration.

Since the MR is well known to contain a population of serotonergic cells, we next examined the effects of serotonin depletion on muscimol-induced feeding. Rats were prepared with chronic cannula aimed at the MR and feeding and drinking were measured for 2 hours after injection of vehicle or 25ng of muscimol. Subjects were then divided into two groups matched for previous intakes and given intra-MR injections of either 5,7-DHT (7.5ug/1.5ul) or its vehicle following DMI pretreatment. After 12 days of recovery, food and water intake and food spillage were again measured for 2 hours after intra-MR muscimol (0 & 25ng/0.25ul).

Preliminary results indicate that depletion of 5-HT does not attenuate muscimol-induced feeding behavior. The present results do not support a role for serotonergic systems originating in the MR in mediating the ingestive behaviors produced by intra-MR muscimol. In other studies we have obtained similar results with respect to muscimol-induced hyperactivity.

(Supported by NIH Grant ROl NS21350)

92.10 ONTOGENY OF POLYCOSE AND SUCROSE APPETITE IN INFANT RATS. M. Vigorito and A. Sclafani. Dept. of Psychology, Brooklyn College of CUNY, Brooklyn, NY 11210.

Rats taste starch and starch-derived polysaccharides and find the taste very attractive. Their starch taste is qualitatively different from their taste for sucrose and it appears that rats have two different carbohydrate taste systems. It is known that rats have an innate preference for sweet taste but whether their preference for starch taste is innate or conditioned by the postingestive effects of starch is not known. We addressed this issue by comparing the behavioral responsiveness of infant rats to Polycose (a starch hydrolysate) and sucrose using the intraoral infusion technique.

Infant rats were tested once with either water, Polycose or sucrose (.03 M or .3 M) at 3, 6, 9, 12, or 15 days of age. One hour prior to testing the pups were removed from the mother, implanted with an oral cannula and placed in a test incubator. Their behavioral responses (mouthing, general activity) to five infusions of the taste stimulus was measured during a 10 min test session; intake was determined by pre- and post-test body weight measures.

The pups consumed more .3 M Polycose and sucrose than water at ages 9 days and older, and more .03 M saccharides than water at 12 days and older. Their intakes of the two saccharides did not differ. The mouthing data revealed that the pups first discriminated the .3 M sucrose from water at 6 days and the .3 M Polycose at 9 days of age. However, the pups discriminated the .03 M Polycose at an earlier age (9 days) than they did the .03 M sucrose (12 days). These results are consistent with the findings that adult rats prefer Polycose at lower concentrations, but sucrose at high concentrations in two-bottle preference tests.

The findings demonstrate that polysaccharide appetite, like sucrose appetite, is present in rats at a very young age prior to any experience with starch. In fact, the rats' appetitive response to polysaccharides develops before they have the enzymes to digest starch (salivary and pancreatic amylase). The differential development of the mouthing response to Polycose and sucrose provides further evidence for the existence of two different taste (sub)systems for carbohydrates. (Supported by NIH Grant DK-31135)

92.11 CONDITIONED FLAVOR PREFERENCES INDUCED BY INTRAGASTRIC POLYCOSE INFUSIONS: AN ELECTRONIC ESOPHAGUS PREPARATION
A. Sclafani and J.W. Nissenbaum*. Dept. of Psychology, Brooklyn College of CUNY, Brooklyn, NY 11210.

Rats are very attracted to the taste of starch-derived polysaccharides (e.g., Polycose). Some data suggest that the postingestive consequences of polysaccharides are also rewarding to rats. The present study assessed the relative importance of pre- and postingestive factors in polysaccharide appetite using an "electronic esophagus" preparation.

Adult female rats were fitted with two chronic gastric catheters. The catheters were connected to infusion pumps that were automatically operated whenever the rat drank from drinking tubes that were available 24 hr/day. On alternate days, as the rats drank one flavored nonnutritive solution (CS+ = cherry or grape Kool-Aid) 16% Polycose was infused intragastrically (IG) and when they drank a different flavored solution (CS- = grape or cherry Kool-Aid) water was infused intragastrically. (Approx. 1.3 ml was infused for each 1 ml orally consumed.) Chow was available ad libitum. After 4 training days the rats were given two-bottle preference tests (24 hr/day). The rats displayed a robust preference (95%) for the CS+ solution over the CS- solution. They also preferred the CS+ solution to water (87%) but did not prefer the CS- solution to water (56%). The rats continued to prefer (99%) the CS+ solution to the CS- solution even when both flavors were associated with IG water infusions (extinction tests).

In separate one-bottle tests the rats' oral intakes of 16% Polycose solution (paired with IG water), CS+ solution (paired with IG 16% Polycose), and .2% saccharin solution (paired with IG 16% Polycose) were compared. The rats consumed approximately twice as much of the Polycose and saccharin solutions as of the CS+ solution.

These results demonstrate that the postingestive effects of polysaccharides are reinforcing to rats and condition strong and persistent flavor preferences. Nevertheless, stimulation of the rats' polysaccharide or sweet taste receptors (with Polycose and saccharin, respectively) appears necessary in order to obtain maximal intakes. Thus, both taste and postingestive factors play a critical role in carbohydrate appetite.

(Supported by NIH Grant DK-31135)

92.12 OROPHARYNGEAL CORRELATES OF MEAL PROGRESS IN THE RAT.
J.M. Kaplan* and H.J. Grill. Department of Psychology and Institute of Neurological Sciences, University of Pennsylvania.

Because the ingestion rate for a given feeding bout is equal to the product of the swallow frequency and the average volume per swallow, any modulation of the rate of ingestion must be mediated by an adjustment of either or both of these swallowing parameters. Recently, using controlled intraoral infusions of sucrose solutions, Kaplan & Grill (1986) showed that both swallowing parameters covaried positively to accommodate adjustments, imposed by the experimenter, in ingestion rate (= infusion rate). In the present study, we sought to determine whether comparable swallow patterning mechanisms operate during normal meals of semi-solid food where ingestion rate varies systematically under the animal's voluntary control.

Prior to feeding tests, fine-wire electrodes were implanted into the inferior pharyngeal constrictor muscle of adult male Sprague-Dawley rats for chronic EMG recording. During test sessions, a video frame-splitter enabled simultaneous recording of the rat's oromotor behavior (biting, chewing) along with an oscilloscope screen displaying the EMG activity associated with swallowing. Rats were 20 to 24 hrs food-deprived and placed in an experimental chamber to which they had previously been adapted. Access to food (wet mash - 3:2 water:lab chow v/w) was limited to 60 sec test trials (inter-trial interval = 120 sec). Thus, meals taken were of the form of a series of consecutive 1 min feeding bouts.

For each meal studied: (1) there was a gradual decline in ingestion rate over trials, presumably reflecting the satiating consequences of the accumulating postingestive load and, (2) both swallow frequency and average volume per swallow declined over trials in direct proportion to reductions in the rate of ingestion. Correlation coefficients relating ingestion rate to either swallowing parameter ranged from 0.75 to 0.91 and were, for every subject (N=4), significant at or beyond the .05 level.

Comparing the present results with those obtained via the intraoral infusion method, we conclude: (1) swallowing is adjusted similarly for liquid and for solid foods and, (2) swallowing is adjusted similarly whether ingestion rate is manipulated by the experimenter or whether ingestion rate varies over the course of normal meals.

Supported by NIH-AM21397.

92.13 RELATION BETWEEN DIURNAL RHYTHM OF FEEDING AND NEURON ACTIVITY IN LATERAL AND VENTROMEDIAL HYPOTHALAMUS IN FREELY BEHAVING RATS.
R. Shibata* and T. Ono. (SPON: S. Mori). Dept. of Physiol., Fac. of Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan

The lateral (LHA) and ventromedial (VMH) hypothalamus are involved in control of circadian rhythm of feeding. We report here, recording of single unit LHA and VMH activity for 1-8 days through chronically implanted electrode in freely behaving rats, and relations between neuronal activity, circadian rhythm, and individual feeding acts and episodes. Activity of 55 of 64 LHA neurons varied with circadian rhythm, 49 increased activity during EEG arousal and decreased during slow wave sleep, and one reacted inversely. Activity of 5 was independent of sleep-wake conditions; 3 gradually increased activity in the dark to a maximum in the morning, then subsided rapidly in 1-2 h; and 2 increased intermittently, at night, prior to and during eating and drinking episodes. Activity of 23 of 50 neurons that were related to sleep-wake cycles decreased during individual feeding acts (consumption of each pellet), and that of one increased. Nine neurons had no relation to circadian rhythm; 4 responded to individual feeding acts; 5 did not. Of the 5 neurons that were unrelated to circadian rhythm and feeding, activity of 3 increased at light on and decreased at light off for 4-13 min. Activity of 74 of 78 VMH neurons recorded varied with circadian rhythm, and 4 had no relation to circadian rhythm or feeding. Activity of 31 of 74 increased during EEG arousal and decreased during slow wave sleep; that of 26 changed inversely. Of 17 neurons that were independent of sleep-wake patterns, 16 were more active in the dark than in the light; 8 of these increased activity during feeding and for 4-10 min after, 2 were depressed during feeding but recovered immediately, and 6 were not influenced by feeding. None responded to individual feeding acts. Activity of 1 of 17 was increased by photostimulation. Activity of 26 of 57 neurons related to the sleep-wake cycle decreased during feeding episodes, but none responded to individual feeding acts. Bilateral suprachiasmatic lesions abolished circadian rhythm that was sleep-wake dependent or independent, but did not affect relation to EEG change or feeding responses. The results suggest that the LHA processes external and internal signals while the VMH processes mainly internal signals in the control of short term feeding (feeding episodes), and that sleep-wake cycles are related to LHA (76.6% increased upon arousal) and VMH (39.7% increased, 33.3% decreased upon arousal, 20.5% increased in dark period) activity in the long term (circadian) control of feeding.

92.14 ADRENALECTOMY ALTERS CENTRAL MONOAMINE METABOLISM IN GENETICALLY OBESE MICE (ob/ob).
J. S. Sims, S. J. Meloni*, and J. F. Lorden. Dept. Psychol., UAB, Birmingham, AL 35294.

A role for adrenal cortical hormones in the obesity syndrome of the ob/ob mouse is suggested by the fact that adrenalectomy reduces weight gain, hyperglycemia, hyperinsulinemia and fat deposition in the mutant. These effects are reversed by glucocorticoids. The effects of adrenalectomy cannot be reproduced by food restriction, although adrenalectomy does reduce food intake. We have previously shown that in addition to the metabolic and hormonal abnormalities displayed by the ob/ob mouse, hypothalamic monoamine metabolism is altered. The present study examined the effects of adrenalectomy on this aspect of the ob/ob syndrome.

Female (C57BL/6J-ob/ob) and C57BL/6J control mice were subjected to bilateral adrenalectomy or sham surgery under ketamine/xylazine anesthesia at 6-7 wks of age. Prior to surgery, all animals were given 325 ug of corticosterone acetate, s.c. Following surgery mice were supplied with standard mouse chow, tap water and .9% sodium chloride ad libitum. The mice were weighed every three days for one month. During the last week of observation, food intake was measured twice. On Day 31 postsurgery, all mice were decapitated. Brains were removed and dissected. Trunk blood was collected for enzymatic assay of glucose and radioimmunoassay of corticosterone.

Adrenalectomy produced similar reductions in serum corticosterone in both lean and obese mice. As expected, adrenalectomy slowed weight gain in the obese mutants. Obese-adrenalectomized mice (ob-ADX) gained an average of 4.9 + 2.1g (SE) in comparison with obese sham controls (ob-SHM) that gained 15.4 + 2.1g. Food intake was reduced by an average of 16% in the ob-ADX group in comparison with sham-operated mice. Lean mice were not significantly affected by the treatment. Mean (+ SE) blood glucose levels in the ob-ADX group were reduced to 143.6 + 3.1 mg% in comparison with the ob-SHM mean of 216.1 + 43.7 mg%. In the lean groups, values did not differ significantly. In hypothalamic samples assayed by HPLC, norepinephrine levels were significantly elevated in the ob-SHM group in comparison with lean controls, but were reduced significantly in the ob-ADX group. Adrenalectomy also reversed a significant decrease in levels of the serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA) in the obese mice. 5HIAA levels in ob-ADX mice did not differ from lean control values. These effects on hypothalamic monoamines were not duplicated by weight reduction brought about by restricting food intake, nor were they seen in Lean-ADX mice. Thus, the CNS effects are due to the altered hormonal status of the ob-ADX mice and furthermore, the obese mice may be abnormally sensitive to the effects of adrenal hormones. (Supported by grant NS14744).

92.15 MICRODIALYSIS AND BEHAVIOR: FEEDING INDUCED BY DEPRIVATION OR HYPOTHALAMIC STIMULATION INCREASES DOPAMINE TURNOVER IN THE NUCLEUS ACCUMBENS. L. Hernandez and B. G. Hoebel, Dept. Psychology, Princeton Univ., Princeton, NJ 08544

Extracellular DA was measured in the accumbens of freely moving rats using a small, removable microdialysis probe[1]. Ten rats were implanted with 26 gauge guide shafts in the nucleus accumbens. Seven rats had lateral hypothalamic electrodes in addition. Microdialysis in the accumbens was performed in three experiments: (1) before, during and after a meal in rats at 80% body weight (n=5); (2) before, during and after bar pressing for food after 20 hours of food deprivation (n=5); and (3) before, during and after electrically induced feeding (n=7). Dopamine, DOPAC and homovanillic acid were measured in the dialysate by reverse phase high pressure liquid chromatography with electrochemical detection. In all three experiments feeding significantly increased extracellular dopamine, DOPAC and homovanillic acid (ANOVA $p<0.05$). This enhancement of dopamine turnover outlasted feeding behavior. This shows that the mesolimbic dopamine system is involved in some aspect of feeding behavior, and the increase in dopaminergic activity is a neurochemical event common to feeding induced by either food deprivation or electrical stimulation of the lateral hypothalamus.

This research was supported by USPHS grant DA-03597 and Campbell Soup Co.

[1]Hernandez, L., Stanley, B. G., Hoebel, B. G., Life Sciences, 1986, 39, 2629-2637.

92.16 FENFLURAMINE INCREASES EXTRACELLULAR SEROTONIN MEASURED BY MICRODIALYSIS IN THE LATERAL HYPOTHALAMUS OF FREELY MOVING RATS. D. H. Schwartz, J. B. Kloecker*, L. Hernandez & B. G. Hoebel, Dept. Psychology, Princeton Univ., Princeton, NJ 08544

Extracellular serotonin (5-HT) was measured in the lateral hypothalamus (LH) of freely moving rats using a small, removable microdialysis probe[1]. Animals received dexfenfluramine injections ip or directly into the lateral hypothalamus. Dialysis samples taken during 20 min intervals were analyzed by high pressure liquid chromatography with electrochemical detection before and after injection. Injection of ip dexfenfluramine increased extracellular 5-HT (3 mg/kg, 2 fold increase; 10 mg/kg, 6 fold* increase). Direct administration of dexfenfluramine (vehicle, 0.1, 1.0, 10 ug) to the LH caused dose dependent increases in extracellular 5-HT (0.1 ug, 8 fold; 1.0 ug, 52 fold*; 10 ug, 64 fold* increase). Similar results were obtained by infusing dexfenfluramine via the microdialysis probe itself. Dexfenfluramine (10 ug) depressed food intake when administered bilaterally to the same LH site. This dose, which increased extracellular 5-HT, also suppressed food intake with a similar time course. These findings support the view that the anorectic drug, dexfenfluramine, releases 5-HT in the hypothalamus, and that 5-HT is involved in the termination of feeding behavior.

*ANOVA followed by Dunnett's Test: $p<0.05$.

This research was supported by USPHS grant DA-03597 and Servier Co.

[1]Hernandez, L. F., Stanley. B. G., Hoebel, B. G. Life Sciences, 1986, 39, 2629-2637.

92.17 Comparison of Regional Cerebral Metabolic Rates for Bulimics vs. Normal Controls J.C. Wu, B. Blinder, M. Derrfler, T. Asavisanu, E. Hazlett, N. Sicotte, M. Buchsbaum, Psychiatry Dept., Univ. Calif. Irvine, Irvine, CA 92717

Introduction: This study assesses differences in the regional cerebral glucose metabolic rates in bulimics vs. normal controls.

Methodology: Six bulimic subjects (women, age 24.5 ± 3 yrs) and five normal controls (women, age 28.9 ± 4 yrs) were studied. Bulimic subjects met DSM-3 criteria for bulimia. Subjects were asked to perform the Continuous Performance Test (CPT) during 18F-2dg uptake. Subjects were scanned on CTI Neuroecat IV (FWHM = 7.6mm). Nine scans parallel to the CM line were obtained. Glucose use was calculated according to the Sokoloff model.

Results: A cortical survey will be performed to analyze anteroposterior, dorsal-ventral and hemispheric dimensions. Metabolic rates for caudate, putamen and thalamus will also be analyzed.

Discussions: Treatment response and family history have led some investigators to suggest the possibility that bulimia may be an affective variant. Recent PET scan studies of affective illness have independently found decreased relative caudate activity in depressed subjects. If bulimia is an affective variant, then bulimia may share a common neural metabolic substrate with depression.

(This study was supported in part by Lilly Pharm.)

92.18 HYPOTHALAMIC HYPERINSULINEMIA AND OBESITY: A COMPARISON OF RADIO-FREQUENCY AND ELECTROLYTIC LESIONS. B. M. King, P. M. Daigrepont*, M. F. Dallman* and L. A. Frohman*. Department of Psychology, University of New Orleans, New Orleans, LA 70148.

Electrolytic lesions of the ventromedial hypothalamus (VMH) result in overeating, obesity, and a marked increase in plasma insulin levels. Rats with VMH lesions become obese even when pair-fed with sham lesioned control animals, leading many to conclude that the obesity is primarily the result of the hyperinsulinemia. However, in addition to tissue ablation, electrolytic lesions leave deposits of metallic ions that can potentially irritate adjacent tissue. King and Frohman recently reported (Am. J. Physiol. 248: E669, 1985) that small, nonirritative VMH lesions resulted in no increase in basal insulin levels, but a moderate increase in weight gain (60% of that observed with electrolytic lesions). The present experiment compared the effects of large electrolytic and radio-frequency VMH lesions in female rats. The absence of metallic ion deposits was verified with a Perls' stain.

Both groups with VMH lesions became obese, but the weight gain was greater in animals with electrolytic lesions (189.8 g vs. 120.0 g). The rats with electrolytic lesions were markedly hyperinsulinemic both during food restriction and when given food ad libitum, but as in the previous experiment, the animals with nonirritative lesions displayed elevated basal insulin levels only when allowed to overeat. Both groups with lesions displayed elevations in morning plasma corticosterone levels.

In a second experiment, weanling female rats were given sham, electrolytic, or radio-frequency lesions when 28-days old and were then allowed food ad libitum for 30 days. Weanling rats with electrolytic lesions displayed a significantly greater weight gain and Lee Obesity Index than either of the other two groups. Their mean basal insulin level was double that observed in rats with radio-frequency lesions (99.3 μU/ml vs. 48.8 μU/ml).

Recent studies have found that like radio-frequency VMH lesions, electrolytic paraventricular lesions, parasagittal knife cuts between the VMH and lateral hypothalamus, and procaine injections into the VMH do not result in basal hyperinsulinemia unless the animals are allowed to overeat. Electrolytic lesions of the VMH is the only condition in which animals are found to be hyperinsulinemic during food restriction. It is concluded that VMH lesion-induced hyperinsulinemia (i.e., in absence of overeating is the result of irritative ion deposits rather than tissue ablation.

92.19 MONOCYTE PROLIFERATION FOLLOWING PVN KNIFE-CUTS IS NOT ADRENALLY MEDIATED. Patrice B. Evers, Richard M. Gold, and Sue Anne Assimon, Psychology Department, Univ. of Massachusetts, Amherst, MA 01003.

Parasagittal knife-cuts alongside the paraventricular nucleus (PVN) of the hypothalamus produce hyperphagia and obesity. These cuts also compromise adrenal regulation, presumably by cutting CRF positive axons going from PVN to median eminence, as well as projections to the intermediolateral column of the spinal cord. These cuts have recently been shown to also selective elevate the monocyte population of the white blood cells. This study asked whether the monocytosis is adrenally mediated.

Female albino rats were sequentially adrenally demedullated, knife-cut, and treated with the synthetic glucocorticoid Dexamethasone (DEX). Irregardless of the other treatments, the hypothalamic knife-cuts more than doubled the monocyte count while having a negligible impact on other leucocyte populations. Neither adrenal demed-ullation nor glucocorticoid (DEX) administration had any impact on the basal or knife-cut elevated monocyte count. (DEX has, however, been shown to block PVN knife-cut obesity.)

Monocytosis following obesifying knife cuts has been replicated, but the phenomenon is, surprisingly, not adrenally mediated.

Finally, ACTH (8 U bid) was admininstered for 8 days to determine whether the knife cuts might be producing monocytosis via a direct action of CRF elicited ACTH secretion on blood forming elements. ACTH completely blocked the monocytosis produced by PVN knife-cuts (but had no effect on body weight).

We conclude that PVN knife-cuts elicit monocytosis via a reduction in ACTH secretion, and that ACTH exerts this control extra-adrenally. Furthermore, monocytosis does not appear to mediate the hyperpagia and obesity associated with these knife-cuts.

ALCOHOL AND BARBITURATES I

93.1 ETHANOL PREFERENCE OF ADULT RATS CAN BE PREDICTED IN INFANCY BY DURATION OF ETHANOL-INDUCED ANESTHESIA. D.L. Kachele* and I.B. Johanson. Department of Psychology and the Institute for the Study of Drug and Alcohol Dependence. Florida Atlantic University, Boca Raton, Florida 33431.

McClearn and colleagues were among the first to report differences among inbred strains of mice in both ethanol preference and duration of loss-of-righting (or "sleep time") in response to an acute dose of ethanol. Subsequently, selectively bred lines of rats and mice have been developed based upon either ethanol preference or "sleep time". With some exceptions, it appears that these two traits are correlated, so that the alcohol-preferring lines display shorter "sleep times" than nonpreferring lines, and alcohol-resistant (short sleep) lines ingest more ethanol than alcohol-sensitive (long sleep) lines. An association between ethanol-induced sleep time and preference has been demonstrated, as well, in adult individuals of a heterogeneous population of rats.

In the present study, we determined that the ethanol-induced sleep time of infant Sprague Dawley rats was a reliable predictor of their adult ethanol preference. Infant rats (4, 10, and 16 days old) were given a hypnotic dose of ethanol (4.5 g/kg ip) and the duration of ethanol-induced loss-of-righting was recorded. Beginning at 60 days of age, "short sleepers" (less than 10 min sleep time as infants) and "long sleepers" (greater than 30 min sleep time) were tested for ethanol consumption, using a 2-bottle test of 15% ethanol vs. water over a 2-week period.

Duration of ethanol-induced anesthesia, even when measured at 4 days of age, was highly predictive of adult ethanol preference. Animals that displayed short sleep times as infants consumed large volumes of 15% ethanol when tested as adults (43-44% of total fluid intake by the end of 2 weeks of 2-bottle testing). Conversely, those animals that displayed long sleep times in infancy consumed very little ethanol as adults (< 5% of total fluid intake). These findings demonstrate that adult differences in ethanol preference can be predicted from differences in sensitivity to ethanol that exist in infants as young as 4 days of age. The establishment of a reliable predictor, in infancy, of adult ethanol preference makes it possible, for the first time, to study the ontogeny of experimental alcoholism in a heterogeneous animal population.

93.2 OPIOIDS MODULATE ETHANOL'S REINFORCEMENT. L. D. Reid, C. L. Hubbell*, S. H. Marglin*, M. L. Abelson* and K. D. Wild*. Department of Psychology, Rensselaer Polytechnic Institute, Troy, NY 12180.

Multiple procedures were used to assess the idea that surfeits of opiodergic activity enhanced rats' positive reactivities toward ethanol (E). In one set of procedures, water-deprived rats were given a daily opportunity of 1.5 hr to take water or sweet alcoholic beverage. Rats receiving placebos, across days, gradually increase their daily intakes to asymptotic values of 2.25 g/kg of E. Injections of morphine, 1.0 mg/kg, before the opportunity to drink (after asymptotic intakes are achieved) increase intakes to about 3.0 g/kg. Injections of morphine, day after day (up to 100 days), maintain enhanced intakes. When morphine injections are given across the first 20 days of opportunities to take water or E, morphine leads to greater increases in intake of E from the 3rd day forward. Rats fixed with osmotic pumps delivering constant infusions of morphine (0.6 mg/kg/hr) also have increased intakes of E during the development of and after asymptotic levels of intake are achieved. Small doses of morphine (1.0 mg/kg) in rats having pumps delivering morphine lead rats to take about 5.0 g/kg of E. Naloxone, the antagonist at opioid receptors, reduces intakes of E and blocks the development of high levels of intake. Naloxone also antagonizes morphine's enhancement of E-intake. Injections of morphine, in other procedures, were shown to be a setting condition for injections of E to establish a conditioned place preference i.e., morphine plus E established a larger place preference than either alone. Collectively, these data support the idea that high functional activity of opiodergic systems is a setting condition enhancing E's ability to reinforce behavior associated with E.

93.3 THE EFFECTS OF FLUOXETINE ON ETHANOL INTAKE IN THE ALCOHOL-NON-PREFERRING (NP) LINE OF RATS. G.J. Gatto*, J.M. Murphy, W.J. McBride, L. Lumeng* and T.-K. Li* (Spon: S.L. Morzorati). Depts. Psych. & Med., Inst. Psych. Res. and Regenstrief Institute, Indiana Univ. Sch. Med. and VA Med. Ctr., Indianapolis, IN.

Fluoxetine, a serotonin (5-HT) uptake inhibitor, attenuates ethanol consumption in the P line of alcohol-preferring rats (Murphy et al., Alcohol 2: 349, 1985). The present study examined whether fluoxetine has similar effects on ethanol intake in NP rats, which are selectively bred for their aversion to consume alcohol in a free-choice situation of 10% (v/v) ethanol, water and food. To overcome this aversion, a modification of the polydipsia design of Kulkosky (Pharm. Biochem. Behav. 10: 277, 1978) was employed. NP female rats (N=5) received an ad lib choice of food, water and a solution containing 3% polycose, 0.5% NaCl and 0.125% saccharin to which ethanol was gradually added until a concentration of 10% was reached. Two control groups of NP female rats (N=4 each) were given a polycose solution without ethanol: control group one (C-1) received a 3% polycose, 0.5% NaCl and 0.125% saccharin solution while control group two was given a 17.6% polycose, 0.5% NaCl and 0.125% saccharin solution (C-2) which had the same caloric content as the polycose-10% ethanol solution. Both control groups received water and food ad lib. After steady baseline intakes were established, rats were injected i.p. according to a counterbalanced design with sterile water or fluoxetine. Animals were allowed to return to baseline for at least five days before a subsequent injection. For the alcohol-drinking group, the mean ethanol intake was 8.7 ± 0.4 g/kg body wt/d (41 ± 2 ml/d); injection of the vehicle alone did not alter alcohol intake. The i.p. injection of 5 and 10 mg/kg of fluoxetine significantly reduced the intake of the alcohol-polycose solution to 77% and 36% of the control values, respectively. Food intake for the alcohol group was 12 ± 3 g/d. Fluoxetine decreased food intake to 72% and 32% of control levels at the 5 and 10 mg/kg doses, respectively. The control groups readily consumed the polycose solutions, reaching a maximum of 120 ml for group C-1 and 102 ml for group C-2. The dose of 10 mg/kg fluoxetine had no effect on the intake of group C-1 and only slightly reduced the intake of group C-2 to 86% of baseline. Food intake for C-1 and C-2 were 17 ± 1 and 9.5 ± 0.5 g/d, respectively. The dose of 10 mg/kg fluoxetine slightly reduced the food intake of groups C-1 and C-2 to 70 and 85% of baseline levels, respectively. The data support the contention that serotonin may be involved in alcohol drinking and also could suggest that chronic alcohol consumption may alter serotonergic control of food intake.

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93.4 TASTE REACTIVITY TO ALCOHOL IN RATS. S.W. Kiefer and J.M. Dopp. Dept. of Psychology, Kansas State University, Manhattan, KS 66506.

Taste reactivity has been defined as the gustofacial and body movements elicited by the infusion of a taste solution directly into the mouth (Grill & Norgren, Brain Res., 1978, 143). In the present experiment, this technique was exploited to determine the rat's response to various concentrations of alcohol. The goal of such work is to complement studies already done which have looked at preference/aversion responses in rats using traditional consumption measures.

Eight naive male rats were anesthetized and implanted with intraoral fistulae. Following two weeks of postoperative recovery, the rats were tested for reactivity to four concentrations of ethyl alcohol (3%, 6%, 9%, and 12%, v/v) in addition to the following solutions: .1 M sucrose, .1 M sodium chloride, .01 M hydrochloric acid, and .0001 M quinine hydrochloride. On a given day, 1 ml of solution was infused into the oral cavity over a 1 min period and the subsequent responses were videotaped for later analysis. Rats received all eight solutions, 1 per day, according to a balanced Latin square design. Distilled water was tested the day before solution presentation was begun. Scoring of the tapes was done frame-by-frame where the gustofacial responses and body movements were tabulated using the technique described by Grill and Norgren (1978). The responses were categorized as either ingestive (e.g., tongue protrusions), aversive (e.g., gaping), or neutral (e.g., face washing).

The results indicated that the reactivity to alcohol in rats was concentration dependent. As concentration of alcohol increased, the number of ingestive responses steadily decreased. And, as anticipated, the number of aversive responses increased as concentration increased. Whereas the predominant response to 3% alcohol was mouth movements (the profile of which resembled that of the response to water), 12% alcohol elicited a number of gapes and body movements indicative of rejection. Responses to sucrose were primarily ingestive; the remaining tastants elicited a complex pattern of responses.

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93.5 ETHANOL TOLERANCE DEVELOPED FOR DRL OPERANT BEHAVIOR IN RATS: RATE-INCREASING AND -DECREASING EFFECTS. D.C. Bird, and F.A. Holloway. (SPON: J. Criado) Univ. OK Hlth Sci. Ctr., Okla. City, OK 73190

We have reported that chronic exposure to ethanol resulted in the development of persistent tolerance for ethanol's rate-decreasing effects on rat operant behavior under VI or FR schedules of food reinforcement (Bird, D.C. & F.A. Holloway, Psychopharmacol., 87:414, 1985). We suggested that such data were compatible with a functional interpretation of tolerance in which animals acquired, through intoxicated practice, some compensatory response to counteract ethanol's rate-decreasing effects. The present study sought to examine tolerance acquisition in an operant task where one of ethanol's direct effects was to increase response rate (i.e., differential reinforcement of low rate of responding, or DRL).

Six male Sprague-Dawley rats were trained in standard operant chambers to lever press for food reinforcement under a DRL-20 sec schedule (i.e., reward only for presses with an inter-response time (IRT) interval of 20 sec or greater). After performance stabolized, an initial dose-effect curve (DEC-1) was obtained (0.375-3.0 g/kg ethanol). After completion of DEC-1, all animals received daily ethanol injections beginning with 1.125 g/kg, escalating as they became tolerant at each dose and continuing until no further tolerance was evident (3.0-3.75 g/kg). Maximum tolerance was achieved in a mean of 68.8 days. After the completion of this chronic injection phase, we obtained DEC-2 immediately and DEC3, 26 weeks later. Between DEC-2 and DEC-3, training continued with several intermittent 1.5 g/kg ethanol challenge tests.

The DEC-1 data indicated a significant rate-increasing effect on non-reinforced responses for doses up to 1.5 g/kg, with a peak rate at a mean dose of 1.18 g/kg. Reinforced responses declined mononically with increasing doses, with an ED50 (effective dose in reducing reinforcements 50% of baseline) of 1.48 g/kg. Tolerance effects were evident at both DEC-2 and DEC-3. At DEC-2, peak rate increased occurred at a mean dose of 1.81 g/kg and the ED50 for reduction in reinforcements was 2.14 g/kg. At DEC-3, some tolerance loss was evident with peak rate increases and the ED50 for reward loss both occurring at a mean dose of 1.63 g/kg.

These data clearly indicate that tolerance develops for both the rate-increasing and rate-decreasing effects of ethanol and that such tolerance, while declining somewhat over time, persists for up to six months. Fine-grain analysis of the IRT distributions at each DEC further indicates that the putative compensatory strategy developed by these animals during intoxicated practice appeared to be one of "rate-reduction." This analysis suggested that compensatory behaviors acquired through the development of tolerance may, in the DRL task, interact with the direct effects of ethanol to modulate performance efficiency with increases in efficiency at lower doses and decreases at higher doses.

93.6 VALPROATE POTENTIATES THE ANXIOLYTIC ACTION OF ETHANOL IN A NONSHOCK CONFLICT TASK. H.C. Becker and B. Bickerstaff*. VA Medical Center and Medical Univ. of SC, Charleston, SC 29403.

The inhibitory neurotransmitter, GABA, has been strongly implicated in mediating a variety of behavioral effects of ethanol (EtOH), such as its locomotor stimulant, motor incoordinating, and anesthetic properties, as well as altering seizure threshold after EtOH withdrawal. Recently, GABA has been implicated in the anxiolytic action of EtOH, as well (e.g., Koob and Thatcher-Britton, Alcoholism: Clin. Exp. Res. 9:204, 1985; Liljequist and Engel, Pharmacol. Biochem. Behav. 21:521, 1984). The purpose of this study was to examine the effects of the indirect GABA agonist, valproate (VPA), on the anti-conflict activity of EtOH in a nonshock task. This task, referred to as negative contrast, involves the quantification of how animals respond to an abrupt, unexpected reduction in reward. The depressed behavior engendered by reward reduction is accompanied by elevated plasma corticosteroid levels, and selectively antagonized by anxiolytic drugs (e.g., EtOH, benzodiazepines). Previous work indicated 1 g/kg EtOH to be an effective anxiolytic dose (Becker and Flaherty, Psychopharmacol. 77:253, 1982); the marginally effective dose of .5 g/kg was employed in this study. Male Sprague-Dawley rats were given brief (5 min) daily access to a 32% sucrose solution for several days, and then shifted to a 4% solution for 4 post-shift days. Unshifted controls received access to only the smaller 4% sucrose reward. Forty min prior to the second post-shift day session, shifted and unshifted rats were injected (IP) with either 0, 50, 100, or 200 mg/kg VPA, followed 30 min later by an injection of either saline or .5 g/kg EtOH. Results indicated that rats shifted from the 32% to 4% sucrose solution consumed substantially less of the latter in comparison to unshifted 4% controls, i.e. a significant negative contrast effect ($F(1,55)= 14.78$, $p< .01$). Moreover, while 0.5 g/kg EtOH did not significantly differ from saline treatment when given alone, co-administration of 100 or 200 mg/kg VPA reliably potentiated the marginally effective anxiolytic action of .5 g/kg EtOH, i.e. increased performance of shifted rats without significantly influencing that of unshifted controls ($F(4,55)= 2.65$, $p< .05$). VPA did not exhibit anti-conflict activity when given alone. These data are supportive of a role for GABA in mediating the anxiolytic activity of EtOH.

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93.7 BIPHASICITY OF CENTRALLY AND PERIPHERALLY ADMINISTERED CAFFEINE ON ACUTE ETHANOL-INDUCED MOTOR INCOORDINATION IN MICE. M.S. DAR, Department of Pharmacology, School of Medicine, East Carolina University, Greenville, North Carolina 27858.

We have previously reported that brain adenosine plays a role in the motor disturbing effects of ethanol in mice (Dar et al., Life Sci. 33:1363, 1983; Dar and Wooles, Life Sci. 39:1429, 1986) and rats (Clark and Dar, Pharmacologist 28:287, 1986). Recently we also reported up-regulation of brain adenosine receptors following chronic caffeine with a corresponding increase in acute ethanol-induced motor incoordination. Caffeine has been reported to exhibit biphasic effects on locomotor activity (Katims et al., JPET 227:167, 1983) and sleep (Yanik et al., Brain Res. 403:177, 1987). The possible biphasic effects of caffeine on acute ethanol-induced motor incoordination by rotorod evaluation was investigated in CD-1 male mice. Caffeine, 2.5, 5, 25, 75, 150ug/5ul of artificial cerebrospinal fluid was administered icv to mice at least five days after they were implanted with permanent indwelling stainless steel guide cannulae followed 5 min later by a test dose, 2g/kg ip, of ethanol. The animals were evaluated for motor coordination at 15, 30, 45, and 60 min post ethanol. The test dose of ethanol was selected based on a separate dose response study and was subsedative but produced marked motor incoordination. A motor coordination study in which caffeine, 2.5, 5, 20, 62.5 and 150mg/kg ip, followed 10 min later by the same test dose of ethanol ip, was also conducted in noncannulated mice. Caffeine <25 ug icv, dose dependently attenuated while 75ug icv potentiated ethanol(ip)-induced motor incoordination. Similarly, caffeine <20mg/kg ip, dose dependently attenuated while 62.5mg/kg ip, potentiated ethanol(ip)-induced motor incoordination. The higher, 150ug and 150mg/kg caffeine, given icv and ip respectively, practically exhibited no effect on ethanol-induced motor incoordination. The data obtained showed that caffeine <25ug icv and <20mg/kg ip, antagonized while at higher doses potentiated ethanol-induced motor incoordination demonstrating the biphasicity of caffeine on motor incoordinating effect of ethanol. Blood ethanol data suggested no effect of caffeine pretreatment on the clearance of ethanol. At low doses, (<25ug icv; <20mg/kg ip), caffeine has been known to display high affinity for adenosine binding sites. Therefore, the present study further supports our earlier reports that adenosine mediates the motor impairing effect of ethanol. The biphasic effects of caffeine may also suggest that at lower doses, caffeine appears to have greater affinity with A_1/A_2 subtypes of adenosine receptors that mediate the excitatory effects of adenosine while higher caffeine doses may have greater affinity with the subtypes of A_1/A_2 that mediate depressant effects such as motor incoordination.

93.8 INTERACTIONS OF IMIDAZODIAZEPINES WITH ETHANOL IN A HOLEBOARD TEST. R.G. Lister. Laboratory of Clinical Studies, DICBR, National Institute on Alcohol Abuse and Alcoholism, Bldg 10 Room 3C218, Bethesda, MD 20892.

There have been several reports that Ro 15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5a][1,4]benzodiazepine-3-carboxylate), which is a partial inverse agonist at central benzodiazepine (BDZ) receptors, can reverse some of the behavioral effects of ethanol. The present studies investigate the behavioral effects of several imidazodiazepines with high affinities for BDZ receptors and their interactions with ethanol. Ro 17-1812 (cyclopropylmethyl (S)-8-chloro-12,12a-dihydro-9-oxo-9H,11H-azeto[2,1c]-imidazo[1,5a][1,4]benzodiazepine-1-carboxylate) is a partial agonist at BDZ receptors (Clin. Neuropharmacol. 7 (Suppl 1) S363, 1984). Ro 15-3505 (ethyl 7-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate) is a BDZ receptor ligand that has been used to reverse the effects of benzodiazepines in humans (Br. J. Clin. Pharmacol. 18:541-547, 1984). In mice it lowers seizure thresholds to bicuculline suggesting that it is a BDZ-receptor partial inverse agonist (Nutt and Lister, in preparation). In all experiments, a holeboard test was used to examine the effects of each drug in mice. This test allows an animal's exploration (head-dipping) to be assessed independently of its locomotor activity.

Ro 17-1812 (0.75-6.0 mg/kg, i.p.), significantly increased locomotor activity but did not alter exploratory head-dipping. Ro 15-4513 (0.75-3.0 mg/kg) significantly reduced exploratory head-dipping but did not alter locomotor activity. Ro 15-3505, (0.75-6.0 mg/kg) caused a slight decrease in head-dipping but did not change motor activity. The effects of Ro 15-4513 on exploration could be partially reversed by Ro 15-3505, suggesting that Ro 15-3505 is a weaker partial inverse agonist than Ro 15-4513.

Ethanol (2 g/kg) increased locomotor activity and decreased exploratory head-dipping. Ro 17-1812 (0.75 and 1.5 mg/kg) enhanced the reduction in exploration caused by ethanol. Ro 15-4513 (1.5 and 3.0 mg/kg) and Ro 15-3505 (0.75 and 1.5 mg/kg) both partially reversed the ethanol-induced reductions in exploration. Ro 15-4513 (3.0 mg/kg) and Ro 15-3505 (1.5 mg/kg) also attenuated ethanol's locomotor stimulant effect. In neither case, however, was the reversal of ethanol's effects complete.

The results of these experiments indicate that BDZ-receptor ligands with varying intrinsic activities are capable of modifying the behavioral effects of ethanol. The direction of the effect appears to be related to the intrinsic effects of each drug. Thus the partial agonist Ro 17-1812 enhanced the effect of ethanol on exploration, and the partial inverse agonists Ro 15-4513 and Ro 15-3505 partially reversed ethanol's effects.

93.9 **DOSE-, TIME- AND SEX-DEPENDENT EFFECTS OF ETHANOL ON LOCOMOTOR ACTIVITY IN RATS. G.J. Schaefer and R.P. Michael. Department of Psychiatry, Emory University School of Medicine, Georgia Mental Health Institute, 1256 Briarcliff Rd, Atlanta, Georgia 30306.**

Adult male and female Sprague-Dawley rats (approx. 100 days old, n=20) were placed in a locomotor activity monitor (Digiscan RXY, Omnitech, Columbus, OH) and habituated to the apparatus for 30 minutes. Animals were then administered either saline or ethanol (0.1, 0.3, 1.0 and 1.7 g/kg) intraperitoneally (IP) and locomotor activity changes were recorded for the next 3 hours. The following measures are reported: (1) horizontal activity (total number of infrared beam interruptions), (2) time at rest (seconds), (3) time moving at a slow speed, and (4) time moving at a fast speed. Over the dose-range of 0.1-1.7 g/kg, biphasic effects were found and these depended upon the sex of the animal and the time of testing. Females were more active and spent less time at rest than males following saline administration. During the first hour after injection, the low dose (0.1 g/kg) increased horizontal activity nearly 60% above saline values in males, while the high dose (1.7 g/kg) reduced activity by 40%. In females the 0.1 g/kg dose increased activity by only 15%, while the 1.7 g/kg dose reduced activity by 55%. Under control conditions, animals spent greater than 85% of their time at rest and the effects of ethanol on this parameter were less dramatic. For example, during the first hour males spent only 3% less time at rest when administered 0.1 g/kg compared to saline values, and spend 2% more time at rest when administered 1.7 g/kg. In contrast, females spent 1% less time at rest following 0.1 g/kg and 6% more time at rest following 1.7 g/kg than their controls. Changes in time spent moving at the slow and fast speeds were similar in direction, but different in magnitude. For males during the first hour, ethanol increased movement at the slow speed by 47% and increased movement at the fast speed by 115% when administered 0.1 g/kg. At the 1.7 g/kg dose, slow speed was reduced by 34% and fast speed by 71%. For females, movement at the slow speed was increased by 1.0% and at the fast speed by 11% at the 0.1 g/kg dose, while at the 1.7 g/kg dose, slow speed was reduced by 48% and fast speed by 80%. During the third hour, the only consistent effects were those produced by 1.7 g/kg which continued to reduce horizontal activity as well as time spent moving at slow and fast speeds. Our results demonstrated that: (1) when rats were habituated to the apparatus, ethanol administered IP produced both increases and decreases in motor activity, (2) females showed less of an increase at low doses and more of a decrease at high doses than males, (3) movement at a fast speed showed greater changes in both increases and decreases than movement at a slow speed, and (4) while the increased activity with a low dose was a consistent effect only during the first hour, the decreased activity with a high dose of ethanol persisted throughout the entire 3-hour test session. Thus, these changes in motor activity paralleled the euphoric effects of low doses and the sedative properties of high doses of ethanol.

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93.10 INTERACTIONS OF THE 5HT REUPTAKE INHIBITOR FLUOXETINE WITH SEDATIVE-HYPNOTICS IN TESTS OF LOCOMOTION, EXPLORATION AND ANXIETY IN MICE. M.J. Durcan,* R.G. Lister, M.J. Eckardt, and M. Linnoila*. (SPON: J. Rohrbaugh) Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Blg 10 Rm3C218, Bethesda, MD 20892.

In previous experiments we have noted that pretreatment with the 5-hydroxytryptamine (5HT) uptake inhibitor fluoxetine (20mg/kg i.p., administered 90min prior to testing) selectively attenuated the anxiolytic effects of ethanol (2.4g/kg i.p., administered 30min prior to testing) without affecting the locomotor stimulant effects (Durcan et al, Alcohol. Clin. Exp. Res. 1987, in press.).

The present series of experiments were performed in mice to investigate the behavioral interactions of fluoxetine with a number of sedative-hypnotics; including ethanol (2.4g/kg), chlordiazepoxide (2.5,5,7.5mg/kg), diazepam (0.5,1,2mg/kg), sodium pentobarbital (10,15,20mg/kg) and phenobarbital (15,30, 60mg/kg), which were administered (i.p.) 30min prior to testing. The behavioral tests used were the holeboard test, which measures locomotor activity and exploratory head-dipping independently; and the elevated plusmaze test of anxiety, which assesses anxiolytic effects (Pellow et al., J. Neuro. Sci. Meth. 14:149 1985).

Ethanol increased activity both in the holeboard and on the plusmaze, decreased both number and duration of exploratory head-dips, and increased the percentage time and percentage entries on the open arm of the plusmaze reflecting its anxiolytic effects. Pretreatment with 20mg/kg fluoxetine (which is without intrinsic behavioral effects in these tests) significantly attenuated ethanol's anxiolytic effects without affecting its locomotor stimulating properties. Chlordiazepoxide and diazepam had dose-related effects similar to those of ethanol but pretreatment with fluoxetine did not significantly interact with any of these behaviors. Both pentobarbital and phenobarbital also showed dose-related effects similar to those of ethanol in both the holeboard and plusmaze tests. Pretreatment with fluoxetine had no significant interaction with the phenobarbital effects whereas it potentiated the behavioral effects of pentobarbital on locomotor activity, exploratory head-dipping and entries onto the open-arms of the plusmaze.

These experiments suggest that fluoxetine may interact with ethanol and barbiturates in a qualitatively different manner. Furthermore the extent of the involvement of 5HT uptake inhibition requires further investigation using other 5HT uptake inhibitors.

93.11 RESPONSE SUPPRESSION TO SEROTONERGIC DRUGS IS ATTENUATED IN THE P LINE OF ALCOHOL-PREFERRING RATS. J.M. Murphy, S.L. Morzorati, J.N. Hingtgen, S.L. Matney*, L. Lumeng* and T.-K. Li*. Departments of Psychiatry and Medicine, and Program in Medical Neurobiology, Institute of Psychiatric Research, Regenstrief Institute and Veterans Administration Medical Center, Indiana University School of Medicine, Indianapolis, IN 46223.

Rats of the alcohol-preferring P line have a lower content of serotonin (5-HT) and 5-hydroxyindoleacetic acid in forebrain regions than the alcohol-nonpreferring NP line (Murphy et al., Pharmacol. Biochem. Behav. 26: 389, 1987). This finding suggests that P rats have a deficient functioning and/or density of forebrain 5-HT innervation. To test this possibility, the P and NP lines were compared in an operant task where response suppression is known to be a sensitive measure of increased activity within brain 5-HT systems (Hingtgen et al., Pharmacol. Biochem. Behav. 20: 425, 1984). Adult male P (n=6) and NP (n=6) rats were maintained at 80% of their free-feeding body weights and were trained to bar press for sweetened milk on a VI 1 min schedule of reinforcement. Three 90-120 min sessions were given each week until a stable baseline responding was established. Subsequently, two sessions each week served as control sessions during which the rats received intraperitoneal injections of saline. During the third session of each week, the animals were injected with 25 mg/kg D,L-5-hydroxytryptophan (5-HTP), a 5-HT precursor, 15 min into the session or were given an injection of 5 mg/kg of fluoxetine, a 5-HT uptake inhibitor, 60 min prior to the 5-HTP injection.

The baseline response rate of the P rats was approximately twice that of the NP animals ($p<0.05$; 41 $\pm$ 6 vs. 22 $\pm$ 5 responses/min, mean $\pm$ SEM). The behavioral responding of the NP rats was similar to that observed for stock Wistar rats in previous studies. The period of response suppression (min) following 5-HTP alone tended to be longer for the NP rats compared with the P line ($p<0.10$; 70 $\pm$ 19 vs. 30 $\pm$ 5 min). Fluoxetine plus 5-HTP produced a significantly ($p<0.05$) longer response suppression in rats of the NP line relative to the P line (87 $\pm$ 20 vs. 36 $\pm$ 5 min).

The findings indicate that the P rats are behaviorally more active and exhibit less response inhibition than the NP rats, and that the shorter response suppression seen after 5-HTP and fluoxetine in the P line is consistent with a lower functional capacity in 5-HT neuronal systems of these alcohol-preferring rats. (Supported in part by AA03243).

93.12 TESTOSTERONE-ALCOHOL INTERACTIONS AND AGGRESSIVE BEHAVIOR IN DOMINANT AND SUBORDINATE SQUIRREL MONKEYS. J.T. Winslow*, K.A. Miczek, and J. Ellingboe. Dept. of Psychology, Tufts Univ., Medford MA 02155 and Alcohol and Drug Abuse Research Ctr., McClean Hosp.-Harvard Med. Sch., Belmont, MA, 02178

Social status and reproductive cycle determine the effects of acute, low doses of alcohol on the social behavior of squirrel monkeys. Alcohol produces biphasic effects on the behavior of dominant but not subordinate monkeys, and only during the mating season. Alcohol (0.1, 0.3, 0.6 g/kg) increased the frequency of threats, grasps, and displacements, and a higher dose (1.0 g/kg) decreased these behaviors in dominant monkeys. Neither dominant nor subordinate monkeys are affected by these doses during the non-mating season. The change in alcohol sensitivity measured in dominant monkeys coincides with changes in plasma testosterone levels. In order to directly study the interaction between alcohol, testosterone, and aggressive behavior, testosterone propionate (TP, 25 mg/kg/day, s.c.) was administered to either dominant or subordinate male monkeys belonging to four separate groups. TP was administered daily at 6:30 h and the behavior of treated monkeys observed in group between 8:30-10:30 h. Treatment elevated significantly plasma levels of testosterone, in excess of levels measured during the mating season (e.g. 905$\pm$ 43 ng/ml in subordinates). Studies were conducted during three consecutive non-mating seasons, period when aggressive interactions between members of established groups are infrequent. Two to three weeks after beginning testosterone treatment, treated monkeys were administered doses of alcohol (0.1-1.0 g/kg). We have previously reported that the behavior of subordinate monkeys was unaffected by testosterone treatment(even after the dominant monkey was removed from each colony and housed separately for 6 weeks). TP altered the sensitivity of subordinate monkeys to the effects of alcohol. Low doses of alcohol increased the frequency of threats, grasps, and displacements exhibited by subordinate monkeys with exogenously elevated testosterone. Daily administration of TP to dominant monkeys during the non-mating season did not affect the behavior of treated animals in group, although treatment was associated with increased body weight similar to that measured during the mating season. The sensitivity of dominant monkeys to alcoholwas affected by testosterone treatment. Low doses of alcohol increased the frequency of threats, grasps, and displacements.

These findings strengthen previous correlative data indicating a relationship between plasma testosterone levels and the effects of alcohol on the aggressive behavior of squirrel monkeys. High levels of testosterone may activate alcohol sensitive brain substrates of aggressive behavior.

93.13 INTERNAL CUES PRODUCED BY ETHANOL OR ETHANOL WITHDRAWAL: GENERALIZATION TO ANXIOLYTIC AND ANXIOGENIC DRUGS. R.D. Harland*, J.R. Criado, R.C. Michaelis, and F.A. Holloway. (SPON: M. Hettinger) Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190

In a new animal model of anxiety, rats are trained to detect the interoceptive discriminative stimulus (IDS) properties of pentylenetetrazol (PTZ) in a standard drug-saline discrimination paradigm (Lal, H. & M.W. Emmett-Oglesby, Neuropharmacol., 22:1423, 1983). The IDS properties of PTZ are thought to be anxiogenic and are blocked by anxiolytic drugs. Further, withdrawal from chronic benzodiazepines, a condition which is anxiety-provoking in humans, also produces a generalization to the PTZ-induced IDS. Ethanol (ETOH), also alleged to have anxiolytic actions, has a variety of behavioral effects which vary with dose, duration of exposure, and post-injection interval. The present study sought to examine the extent to which rate-increasing, rate-decreasing, and acute or chronic withdrawal actions of ETOH could produce IDS properties similar to that produced by chlordiazepoxide (CDP), an anxiolytic, or PTZ, an anxiogenic drug.

Rats (N=12) were trained to discriminate 3 mg/kg CDP from 20 mg/kg PTZ in a drug-drug discrimination paradigm (food reward on a VR5-10 schedule with 2-min extinction tests). Dose-dependent generalization to CDP and PTZ levers was found for CDP and PTZ respectively, with saline producing 46% CDP-lever responding. ETOH produced CDP-lever responding at doses of .5 and .75 g/kg when injected 4 min pre-test and at 1.25 g/kg when injected 15 min pre-test, and no PTZ-lever responding at any dose. At dose ranges of .25-1.25, ETOH did not block PTZ-lever responding produced by 10 mg/kg PTZ, but did produce dose-dependent blockade of the PTZ increases in overall response rates. Saline administered 9 or 12 hrs after a 3 g/kg ETOH dose of 12 hours after a 4 g/kg ETOH dose produced 80% or greater PTZ-lever responding. A .5 g/kg ETOH dose injected 15 min before tests, at 9 hr after a 3 g/kg ETOH injection, or at 12 hr after a 4 g/kg ETOH injection failed to attenuate PTZ-lever responding. The chronic ETOH regimen consisted of 7 days in an ETOH inhalation chamber (mean terminal BAL = 175.1 + 16.09 mg%). Saline tests 12 and 18 hrs after removal from ETOH chambers yielded 94% and 93% PTZ-lever responding, respectively.

The data confirm and expand the earlier finding of Lal and colleagues, showing that acute and chronic ETOH withdrawal produces IDS which generalize to the PTZ-induced cue. However, the data further suggest that while ETOH may mimic the CDP cue at certain doses and times of injection, ETOH does not have anxiolytic properties sufficient to block the strong "anxiogenic" effects produced by PTZ or by acute ETOH withdrawal. While these conclusions must be tempered by the dose ranges and conditions tested, ETOH's status as an anxiolytic remains questionable.

93.14 DEVELOPMENT AND MAINTENANCE OF ETHANOL TOLERANCE FOR OPERANT BEHAVIOR OF RATS: ROLE OF NUMBER AND PATTERN OF INTOXICATED PRACTICE OPPORTUNITIES AND ABSOLUTE ETHANOL EXPOSURE. F.A. Holloway, R.C. Michaelis, R.D. Harland, and J.R. Criado. Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Prior work (Bird, D.C., & F.A. Holloway, Psychopharmacol., 87:414, 1986) suggested that the long-term shifts-to-the-right in ethanol (ETOH) dose-effect curves (DECs) for operant behavior resulted from development of functional tolerance to ETOH. The mechanism for such tolerance was thought to depend on the acquisition of some compensatory behavior which counteracted the rate-reducing properties of ETOH in this task. Learning of new instrumental behaviors generally is facilitated by increases in training trials and by the pattern of those training trials. The present study sought to examine the magnitude of ETOH tolerance for operant behavior or rats as a function of: (a) magnitude of ETOH exposure; (b) the relative extent of intoxicated practice (IXP) opportunities; and (c) the pattern of IXP sessions.

Seventy-eight rats were trained to stable performance on a FR30 (fixed ratio), food-reinforced operant task. Estimated blood ethanol levels were determined by a breath/GC procedure. DECs were obtained with a cumulative-dosing procedure prior to a period of daily injections and at selected points during and/or after the chronic injection period. The animals were randomly assigned to one of thirteen experimental conditions, defined by the frequency of IXP sessions (0%, 33%, 67%, 100%), by whether the IXP sessions were spaced (S) or massed (M), and by whether the daily pre-session injections were supplemented by post-session ETOH injections (Y) or not (N). The pre-session ETOH injection period lasted 45-60 days and the dose was incremented from 1-1.75 g/kg. Where daily ETOH was held constant, the combined pre- and post-session ETOH dose was 1.75 g/kg.

All groups receiving any IXP sessions displayed shifts-to-the-right in their DECs for operant behavior but not for peak ethanol levels (p's $<.05$). Generally, the more frequent the IXP sessions, the greater the magnitude of tolerance developed. Further, supplemental post-session ETOH injections appeared to weakly facilitate tolerance acquisition, particularly in groups with less IXP (p's $<.05$). A weak trend also was apparent for distributed practice groups to develop more tolerance than massed practice groups. Finally, all groups, which acquired any degree of tolerance, displayed persistence of these effects for up to 3 months (p's $<.05$).

These data are consistent with the learning interpretation discussed above, but also suggest: (a) that the extent of IXP and absolute amount of ETOH exposure interact in affecting tolerance development; and (b) that the magnitude of tolerance (and to some extent, tolerance persistence) varies as a function of number and pattern of IXP sessions.

93.15 RAPID TOLERANCE TO THE DISRUPTIVE EFFECTS OF ETHANOL ON HIGH-SPEED REACTION TIME IN RATS. R. D. Mayfield*, M. Grant*, W. W. Spirduso[a] and T. Schallert[b]. Institute for Neurol. Sciences, Depts. of Physical & Health Education[a] and Psychology[b], Univ. Texas at Austin, Austin, TX 78712

Behavioral tolerance to ethanol (EtOH) appeared within a 5-day period of chronic EtOH treatment (20% w/v, oral intubation). F344 male rats, 8 mos of age, were shaped to perform a high-speed lever-release task until they had reached a minimum success criterion of 80% (10 - 14 days). Prior to EtOH exposure, a complete test session was given in the absence of EtOH (saline control). The first challenge with EtOH represented the start of the chronic EtOH regimen. Each test consisted of a series of 10 trials at 5, 10, 20, 45, 65 and 90 min post-EtOH on each of 5 days. EtOH was administered 3 times per day at 8 hr intervals to provide moderately high blood alcohol concentrations (BACs). The EtOH doses were increased each day to insure that the BACs were greater than or equal to the BACs of the previous test session. EtOH doses ranged from 1.6 - 1.8 g/kg EtOH on the first day to 2.0 - 2.5 g/kg by the fifth day.

The average peak BACs for the 5 test days were 105.4, 138.0, 150.3, 167.0, and 185.9 mg/dl. BACs were 0.0 mg/dl prior to each EtOH test session. Although the BACs increased each day, lever-release reactions (measured in terms of percent success and mean latency of response) improved. On the first test day, successful performance at each of the time points (5, 10, 20, 45, 65 and 90 min) was 59.4, 66.3, 48.0, 52.6, 75.4, and 80.0% of their control (pre-EtOH) values. By the fourth day, however, their performance improved to 84.6, 96.0, 93.7, 73.7, 89.1 and 89.2% of their control values. Lever-release was faster on the fourth and fifth days of testing than on the first three days even though BACs were higher. In addition, on the first day of EtOH exposure, performance was better at 65 and 90 min than at 5, 10, 20 and 45 min even though BACs were higher at 65 and 90 min. Thus, both within-test session and between-test session tolerance was demonstrated independent of metabolic factors. Subsequent experiments indicated that the tolerance was not due to exposure to EtOH *per se*, but depended on practice during intoxication. The improved performance of the rats on this highly practiced, reaction time behavior occurred with doses of EtOH that were comparable to voluntary human EtOH consumption.

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93.16 THE EXPRESSION OF A BEHAVIORAL EFFECT OF ETHANOL: NECESSITY OF THE BASAL FOREBRAIN (Ch4) CHOLINERGIC SYSTEM. J.A. Stidham and L.D. Devenport. Department of Psychology, University of Oklahoma, Norman, OK 73019.

Several considerations lead to the suspicion that ethanol's (ETOH) action on cholinergic projections to frontal and parietal cortex might be the basis of some of the drug's behavioral effects (1, 2). Ethanol disrupts eight-arm radial maze performance in predictable ways (3, 4), one of the least complicated cases being spatial dispersion among the arms of the maze (5). If animals are allowed to obtain eight rewards per trial, normal rats will visit at least 75% of the different arms on each trial, even though the rewards could be obtained by returning to previously visited arms. In contrast, animals injected with moderate doses of ETOH rarely exceed chance levels of arm visitation. This is not because ethanol exacts a spatial mapping deficit or memory impairment (6, 7). Instead, it seems to result from a strong tendency to revisit sites that recently yielded reward (4, 6, 7).

Such a task readily lends itself to a neural analysis as it requires almost no training and is insensitive to any unusual reaction to nonreward as "errors" are impossible to commit in the reward-replacement design. The effects of ETOH on this task were examined in 380 g rats pretreated with diazapam (13 mg/kg) and infused with kainic acid (1.5 ug in 1 ul saline, pH 7.6) via cannula in the Ch4 (nucleus basalis) region of the forebrain, or in animals prepared similarly but with sham lesions. After recovery from surgery, 16 daily radial maze sessions (3 trials/session) were conducted 13 min after ETOH (1.5 g/kg, i.p. as a 10% w/v solution in saline) or saline injection.

In CNS-intact rats, the results were as usual: Saline-injected animals dispersed their visits throughout the maze, injected animals restricted their movements to a few preferred arms. Ethanol's effect almost completely disappeared in acetylcholine-depleted rats. This occurred in spite of the fact that the lesion itself had no effect on radial maze performance. The Ch4 cholinergic sytem seems necessary for the expression of the particular behavioral effect of ETOH examined here. As to whether other ETOH effects rely on such an "enabling" mechanism remain to be seen.

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93.17 INTERACTION BETWEEN RO15-4513 AND ETHANOL IN MICE P.J. Syapin(1), K.W. Gee, M. Bejanian, B. Jones*, and R.L. Alkana (1)Departments of Psychiatry and Neurology, School of Medicine and Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033

The partial inverse agonist imidazobenzodiazepine Ro15-4513 has been shown to have a novel property as an ethanol antagonist in rats (Suzdak et al, 1986). This effect of Ro15-4513 appears to be mediated through its binding to brain benzodiazepine receptors. We have examined the ability of Ro15-4513 to antagonize other effects of ethanol using mice. Male C57BL/6J mice (6-8 weeks of age) were used in all experiments. We first examined the ability of Ro15-4513 to antagonize the hypnotic action of ethanol. Mice were given a hypnotic dose of ethanol (3.6 g/kg, 20% w/v in saline) and ten minutes later were injected with either vehicle (4% Tween 80 in saline) or a 20 mg/kg dose of Ro15-4513 (0.01 ml/gm body weight). All injections were made i.p. Ro15-4513 significantly antagonized the loss of righting reflex. Mice given ethanol+vehicle had a mean (± SE) sleep-time of 35.0 ± 3.6 minutes; mice given ethanol+Ro15-4513 had a mean (± SE) sleep-time of 17.8 ± 3.7 minutes ($p < 0.01$, 2-tail t-test). Blood ethanol concentrations at wake-up were also significantly higher following Ro15-4513 treatment ($p < 0.02$, 2-tail t-test) indicating that the drug was not acting by altering ethanol pharmacokinetics. In contrast, body temperature at wake-up was not significantly different between treatment groups; both groups were hypothermic upon wake-up. In a second experiment we examined further the effect of Ro15-4513 on ethanol-induced hypothermia. Mice were given either saline or 3.6 g/kg ethanol injections followed by vehicle, Ro15-4513, or Ro15-4513+Ro15-1788 injections 10 minutes later. Rectal temperature was measured for 120 minutes after the first injection. Significant and long-lasting hypothermia was seen at all time points in both the ethanol+vehicle and ethanol+drug groups when compared to either saline group, while no differences were found between the ethanol+saline and ethanol+drug groups. No significant hypothermia was seen in the saline+vehicle group, however, a small but significant hypothermia was seen in the saline+Ro15-4513 group up to 30 minutes post-saline. These results indicate that 20 mg/kg Ro15-4513 did not antagonize ethanol hypothermia. In summary, our studies show that a high dose of Ro15-4513 is effective in reducing the hypnotic, but not hypothermic, effect of ethanol in mice. These results suggests that ethanol's hypnotic, but not hypothermic, effects may be mediated through interaction with the GABA/benzodiazepine/chloride receptor complex. (Supported by NIAAA grant AA 03972 and BRSG S07 RR05792).

93.18 PHARMACOLOGICAL CHARACTERIZATION OF Ro15-4513 INHIBITION OF ALCOHOL-INDUCED BEHAVIORAL INTOXICATION IN THE RAT. S.M. Paul*, J. Crawley and P.D. Suzdak. (SPON: M. Kafka) Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892

Both biochemical and behavioral evidence suggests that that depressant/anxiolytic effects of ethanol may be mediated via the GABA/Barbiturate receptor-coupled Cl^- ion channel. Recently, the ability of ethanol to both (A) stimulate $^{36}Cl^-$uptake in vitro via the GABA receptor complex, and (B) produce anxiolytic and motor incoordination in vivo have been shown to be antagonized by the selective imidazobenzodiazepine, Ro15-4513. (Suzdak et al, Science 234, 1243 (1986)).

The present study examined the ability of Ro15-4513 to prevent, or reverse, the behavioral intoxication produced by ethanol and related short chain length alcohols in the rat. Ro15-4513 dose-dependently (0.5-10 mg/kg i.p.) prevented ethanol-induced intoxication ($IC50=1.5$ mg/kg). The ability of Ro15-4513 to prevent ethanol-induced intoxication was reversed by the central benzodiazepine receptor antagonists Ro15-1788 (10 mg/kg) and CGS-8216 (10 mg/kg). However, the ability of Ro15-4513 to prevent ethanol-induced intoxication was not mimicked by other benzodiazepine receptor antagonists or inverse agonists (FG-7142 (10-30 mg/kg) or βCCE (10 mg/kg)). Ro15-4513 (2.5-10 mg/kg), administered six minutes after the administration of ethanol, dose-dependently reversed the ability of ethanol to produce behavioral intoxication ($IC_{50}=5$ mg/kg). This effect was blocked by either Ro15-1788 or CGS-8216, but not mimicked by other benzodiazepine receptor antagonist or inverse agonists. These data suggest that the ability of Ro15-4513 to prevent, or to reverse, ethanol-induced intoxication may be mediated by binding to the central benzodiazepine receptor. The lack of effect of other benzodiazepine receptor antagonists or inverse agonists suggests that the "alcohol reversing" properties of Ro15-4513 are mediated through a unique interaction with the GABA/benzodiazepine receptor-coupled Cl^- ion channel. Thus it appears that the "alcohol antagonist" properties of Ro15-4513 may be dissociable from its inverse agonist properties.

93.19 ACUTE INTOXICATION WITH ALCOHOL IMPAIRS COGNITIVE PROCESSING OF PROSE. T. V. Petros,* B. E. Beckwith, M. Haut,* and J. Stempel.* University of North Dakota, Grand Forks, ND 58202.

The present study examined the effects of intoxication with ethanol on encoding and retrieval of prose. Intoxicated and sober subjects read three narrative and three expository passages from a computer at their own rate. Immediately after reading each passage subjects were asked to orally recall it. Reading times and the proportion of idea units recalled were the primary dependent variables. Intoxicated subjects recalled fewer idea units than sober subjects, but both intoxicated and sober subjects favored the main ideas in their recall. A multiple regression analysis of the reading time data indicated that intoxicated subjects did not modify their reading times according to the cognitive demands of the text to the same degree as sober subjects. The results suggest that ethanol impaired the efficiency of specific components of text processing.

SEROTONIN: FUNCTIONAL STUDIES I

94.1 REGIONAL EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON 5-HT-2 RECEPTORS AND PHOSPHOINOSITIDE TURNOVER IN THE RAT. P.D. Butler[1] M.R. Pranzatelli[2], A.L. Barkai[1*]. Departments of Psychiatry[1] and Neurology[2], College of Physicians and Surgeons of Columbia Univ., New York, NY 10032.

Behavioral supersensitivity (myoclonus) can be elicited in response to serotonin (5-HT) agonists from several hours to at least 12 months after 5,7-dihydroxytryptamine (DHT) injection in rats and has been attributed to denervation supersensitivity of post-synaptic 5-HT receptors. DHT lesions could induce changes in receptor ligand binding and/or second messenger activity in the post-synaptic cell. To pursue both possibilities, we measured regional 5-HT-2 receptor ligand binding and 5-HT-stimulated phosphoinositide turnover in rats with DHT lesions and their saline-treated controls. We selectively depleted 5-HT in rats with intracisternal injection of DHT after pretreatment with desipramine: 77% depletion in frontal cortex, 47% in brainstem, and 78% in spinal cord, without changes in norepinephrine (NE). In saturation studies (six concentrations from 10 to 0.1 nM) of fresh brain tissue two weeks after DHT injection, ^{3}H-ketanserin specific binding (using 10 uM methysergide to define non-specific binding) was unchanged in brainstem and spinal cord, and increased in frontal cortex (+12%) compared to controls (expressed as pmol/g brain). This represented a small significant change in Bmax but not Kd or Hill coefficient. In the same two groups of rats, cross-chopped brain and spinal cord slices were prelabeled with ^{3}H-myo-inositol. Accumulation of ^{3}H-inositol phosphates (IP) was measured in the presence and absence of 5-HT (10 uM) and NE (5 uM) to assess the function of 5-HT-2 and alpha-1-adrenergic receptors, respectively. DHT lesions did not affect basal levels of ^{3}H-IP accumulation but significantly increased 5-HT-stimulated ^{3}H-IP accumulation in the brainstem (+27%). Effects in spinal cord and cortex were in the same direction but were not significant. NE-stimulated ^{3}H-IP accumulation was not altered by DHT treatment in any of the regions studied. These data suggest a regional dissociation between changes in phosphoinositide metabolism and density of 5-HT-2 receptor recognition sites following DHT lesions. In view of the importance of the brainstem in DHT-induced myoclonus, the compensatory increase in 5-HT-stimulated phosphoinositide hydrolysis in brainstem following DHT lesions in the rat may be relevant to serotonergic behavioral supersensitivity. Further studies will assess the time course and subregional localization of these effects and their relation to myoclonus.

Supported by NIMH 33690 and BRSG 903-E813T[1], and a grant from the Myoclonus Research Fund[2].

94.2 5HT-2 RECEPTOR-MEDIATED PHOSPHATIDYLINOSITOL (PI) HYDROLYSIS IN HUMAN PLATELETS:MODULATION OF PI TURNOVER BY PROTEIN KINASE C ACTIVATORS. M. Mikuni, H. Mori*, Y. Yoshida, I. Yamashita* and K. Takahashi*(SPON:K.Satoh) Div. of Mental Disorder Res. Natl. Institute of Neuroscience, NCNP, Tokyo, 187 and Dept. of Psychiat. Hokkaido Univ. Schl. of Med. Sapporo, 060, Japan.

The metabolism of inositol phospholipids in response to 5HT was investigated in human platelets using sensitive radioisotopic method of Berridge(1983). In platelets prelabeled with ^{3}H-myo-inositol, 5HT caused a dose-dependent accumulation of inositol-1-phosphate(IP-1) during 15min incubation in Ca^{++}free HEPES buffer containing 10mMLiCl and 1μM fluoxetine. A maximal increase in IP-1 formation was observed at 30μM of 5HT and its EC_{50} value was 4μM. Ketanserin was a potent inhibitor of 5HT-stimulated IP-1 accumulation with a Ki value of 12nM, but a 5HT-1 antagonist, propranolol(10μM) failed to block the 5HT response. Metergoline, a mixed 5HT-1 and -2 antagonist caused the almost same reduction in 5HT induced IP-1 formation as ketanserin, with a Ki value of 5.2nM. Moreover, chlorpromazine and imipramine inhibited 5HT-stimulated IP-1 accumulation, with Ki value of 124nM and 2.56μM, respectively. Spiperone(1μM) inhibited completely, and clozapine(1μM) and amitriptyline(1μM) reduced partially (50-70%) 5HT-induced IP-1 accumulation, but sulpiride(1μM) failed to block the 5HT response. The potencies of these compound to inhibit 5HT-stimulated IP-1 accumulation in human platelets was positively correlated with the affinities to 5HT-2 receptor as defined by radioligand binding in rat cerebral cortex membranes.

The biologically active phorbol ester was reported to inhibit the PI turnover stimulated by 5HT in rat aorta(Roth et al,1986). We investigated, therefore, whether or not phorbol-12,13-dibutylate(PDB) and mezerein which is tumor promotor and protein kinase C activator as well as PDB, but not phorbol ester, inhibit 5HT-stimulated IP-1 accumulation in human platelets. PDB(100nM) inhibited 5HT response in human platelets, and mezerein was also found to inhibit 5HT-induced IP-1 accumulation with a IC_{50} value of 1nM. These results suggest that an activation of protein kinase C may desensitize 5HT-2 receptor-mediated PI hydrolysis in human platelets.

94.3 ICS205-930 INHIBITS HIGH AFFINITY SEROTONIN BINDING AND SEROTONIN STIMULATED ADENYLATE CYCLASE IN THE RAT SPINAL CORD. S.R. GLAUM and E.G. ANDERSON. Department of Pharmacology, College of Medicine, University of Illinois - Chicago, Chicago, IL 60680.

Dorsal root ganglion cells (DRG's) have been used in intracellular recording as pharmacological models of dorsal horn afferent terminals. Individual DRG's of the frog and rat display multiple electrophysiological responses to perfusion with serotonergic agonists, suggesting the presence of multiple receptor subtypes. (Scroggs and Anderson., NS Abst. 12,1238 1986). Serotonin (5HT) binds with nanomolar affinity to two distinct receptor families, the $5HT_1$ and $5HT_3$. The $5HT_1$ class are broadly distributed within the central and peripheral nervous system. At this time, no previous examples of central $5HT_3$ receptors have been reported. The development of a specific antagonist at $5HT_3$ receptors, ICS205-930, has revealed that many of the peripheral excitatory effects of 5HT are mediated by $5HT_3$ receptors (Richardson and Engel., TINS 9,424 1986). We now report the presence of central $5HT_3$ receptors based on ICS205-930 displacement of high affinity ^{3}H-5HT binding in the dorsal horn of male Sprague-Dawley rats. Additionally, the ability of ICS205-930 to inhibit 5HT-stimulated adenylate cyclase (AC) in the dorsal cord was determined.

^{3}H-5HT binds with high affinity to the dorsal cord (K_d =1.2nM, B_{max}=40 fmol/mg protein). ICS205-930 maximally displaces 35% of the high affinity binding, suggesting the presence of residual high affinity sites, possibly $5HT_1$. Methiothepin antagonizes ^{3}H-5HT binding to $5HT_1$ and $5HT_2$ sites, but not $5HT_3$ sites. In the presence of 1nM methiothepin, ICS205-930 displaced ^{3}H-5HT binding with an IC_{50}=20nM (nH=1). AC was stimulated in a dose dependent manner by 5HT in rat dorsal cord. Maximal stimulation (136% of basal) occurred at [5HT]=100nM. Higher concentrations reduced stimulation, suggesting low affinity 5HT sites linked to the inhibition of AC. ICS205-930 inhibited 5HT-stimulated AC at concentrations as low as $10p^M$. The potency of ICS205-930 as an antagonist of 5HT-stimulated AC is similar to the pA_2 values reported by Richardson and Engel for its peripheral effects.

These data support the existence of centrally located $5HT_3$ receptors. We are currently investigating the neuronal subtypes where $5HT_3$ receptors predominate and their possible function in the CNS. (Supported by USPHS grant NS 17834-02)

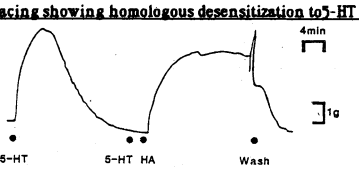
94.4 **COMPARISON OF THE AFFINITY OF SEROTONIN AGONISTS FOR THE 5-HT-1A RECEPTOR WITH THEIR POTENCY AT ELICITING A 5-HT-1A RECEPTOR-MEDIATED RESPONSE.** **S.J. Offord, T.M. DeAngelo*, H.L. Wang* and A. Frazer, Departments of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine and VA Medical Center, Philadelphia, PA 19104.**

Multiple subtypes of the serotonin-1 (5-HT-1) receptor exist in the rat brain. For 5-HT-1A receptors, the radioligand agonist 3H-8-hydroxy-2-(di-N-propylamino)tetralin (3H-DPAT) has been used to determine affinities of drugs. In general, the affinity of serotonin agonists for the 5-HT-1A receptor as measured with 3H-DPAT is lower than that reported when affinities are measured using the non-selective agonist 3H-5-HT. In the 3H-5-HT assay GTP was included as was TFMPP, to prevent 3H-5-HT from binding to 5-HT-1B receptors (Sills et al., J. Pharmacol. Exp. Therap., 231:480, 1984). In particular, RU 24969, TFMPP and m-CPP have been reported to have higher affinity for displacing the binding of 3H-DPAT (Peroutka, J. Neurochem. 47:529, 1986) than that found using 3H-5-HT (Sills et al., 1984). To study this issue further, we determined the potency of these serotonin agonists for producing a 5-HT-1A mediated response, inhibition of forskolin-stimulated (F-S) adenylyl cyclase activity in homogenates of rat hippocampus. As reported by DeVivo and Maayani (J. Pharmacol. Exp. Therap. 238:248, 1986), we found that spiperone prevented the inhibitory effect of 5-HT on F-S adenylyl cyclase with a K_D of 26±3 nM, a value in agreement with the affinity of spiperone for the 5-HT-1A receptor (73±14 nM). By contrast, the selective 5-HT-2 antagonist ketanserin (1uM) was not able to block the inhibitory effect of 5-HT, consistent with this response being mediated by 5-HT-1A receptors. The inhibitory effect of 5-HT required GTP (25 uM) and was not mimicked by dopamine. 5-HT, DPAT, RU 24969 and 5-MeODMT were full agonists, producing a maximal inhibition of about 30%. TFMPP and m-CPP were partial agonists, causing about half the maximal inhibition produced by the full agonists. EC50 (nM) values for inhibiting F-S adenylyl cyclase were: DPAT, 15±2; 5-HT, 40±1; RU 24969, 124±12; 5-MeODMT, 380±63; TFMPP, 3530±1580; m-CPP, 18000±6000. We found that affinities obtained for drugs in the 3H-DPAT binding assay agreed with values reported by Peroutka (1986). For 15 compounds, the Pearson correlation coefficient was 0.97. The Ki (nM) values for DPAT, 5-HT, RU 24969 5-MeODMT, TFMPP and m-CPP were 1±0.3, 2±0.3, 1±1, 3±1, 56±9 and 113± 11, respectively. These values are 15-160 fold lower than the EC50 values measured in the cyclase assay. Several factors could contribute to these quantitative differences. Spare receptors could affect potency in the cyclase assay but this would be expected to increase potency in this assay, not lower potency. A second explanation could be that 3H-DPAT binds to multiple affinity states of the 5-HT-1A receptor. This seems likely as this receptor is linked to adenylyl cyclase through an inhibitory guanine nucleotide binding protein. Consistent with this is our finding that 3H-DPAT binding is inhibited by GTP. These results indicate that affinities of serotonin agonists obtained using 3H-DPAT should be interpreted cautiously. **(This research was supported by USPHS Grants MH 29094, GM 34781 and research funds from the VA).**

94.5 KINETIC CHARACTERIZATION OF DESENSITIZATION OF THE RESPONSE TO 5-HYDROXYTRYPTAMINE IN AIRWAY AND VASCULAR TISSUES. R.R.Ben-Harari*, B. Dalton*, J.Kaplan* and S.Maayani†. Departments of Anesthesiology and Pharmacology†, Mt. Sinai School of Medicine, City University of New York, New York, N.Y. 10029.

Desensitization and resensitization of a response to an agonist presents difficulties both experimentally, in characterization of receptors and receptor-effector interactions, and clinically, in therapeutic efficacy. Despite its obvious importance, desensitization in intact tissues has either been reported descriptively or tissue responses have been measured at steady-state. Both desensitization and resensitization are time-dependent which cannot be accurately analyzed under steady-state conditions. Kinetic analysis with novel pharmacologic protocols (J. Pharm. exp. Ther., 236, 48, 1986) allows us to characterize these two phenomena. In comparison to the rabbit aorta preparation (half lifetime for desensitization to 5-HT is 70 min), the response to 5-HT in guinea-pig trachea shows very fast rates of desensitization (t1/2 = 5min) and thus, kinetic studies become experimentally feasible. Tissue tension in guinea-pig trachea and rabbit aorta were measured isometrically in Krebs-Henseleit buffer, pH 7.4, gassed with 95% O_2 + 5% CO_2. Under steady-state conditions (concentration-response curves), the $5\text{-}HT_2$ antagonist, ketanserin (10µM) abolished the response to 5-HT and no responses were obtained to 8-OH-DPAT or to 5-carboxamidotryptamine on tissue precontracted with histamine (HA). 5-HT therefore acts at a single homogeneous receptor population. Experiments with 4mM $NaNO_2$ demonstrated that the contractile response of the tissue to 5-HT was not necessary for desensitization to occur. Desensitization to 5-HT was homologous and was not a result of monoamine oxidase activity, removal by neuronal or extraneuronal uptake or release of an endogenous inhibitor of contraction. Rate constants for the decay of the response (desensitization) to 5-HT were: a) reversible after 3h; b) slower at 15°C than at 37°C; c) increased with increasing concentrations of 5-HT; and d) faster than to N-methyl 5-HT (a partial 5-HT agonist) at equal occupancy of the receptor. The results demonstrate that homologous desensitization to 5-HT is operationally a time, concentration, temperature and efficacy dependent loss of tissue responsiveness in a functional system. (Supported by USPH GM-34852).

Fig. Original tracing showing homologous desensitization to5-HT on trachea.



94.6 FUNCTIONAL 5-HT RECEPTORS IN BRAIN AND PERIPHERY AND THEIR RELATED BINDING SITES: ACTION OF AGONISTS AND ANTAGONISTS. D.K. Hyslop[1], F.D. Yocca[1], and S. Maayani[2], [1]Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Wallingford, CT 06492, and [2]Department of Pharmacology, Mount Sinai School of Medicine, CUNY, NY 10029

The recognition sites of two distinct functional 5-HT receptors can be selectively labeled: the 5-HT-sensitive [^{3}H]8-OH-DPAT sites are indistinguishable from the $5\text{-}HT_{1A}$ receptor that is negatively linked to adenylate cyclase activity (DeVivo and Maayani, JPET: 238, 1986) and the methysergide-sensitive [^{3}H] ketanserin site (Leysen et. al., Mol. Pharmacol: 21, 1982) or the cinanserin-sensitive [^{3}H]DOB site (Lyon et. al., Mol. Pharmacol: 31, 1987), sites which are pharmacologically congruent with the $5\text{-}HT_2$ receptor that mediates contractility in vasculature, such as the rabbit aorta preparation (Maayani et. al., JPET: 229, 1984). 8-OH-DPAT is an agonist at the $5\text{-}HT_{1A}$ receptor, while DOET and DOI (the latter being an analog of DOB) are $5\text{-}HT_2$ agonists in the rabbit aorta preparation. Affinity values of 5-HT agonists at these two sites (EC_{50} at the $5\text{-}HT_{1A}$ and K_A at the $5\text{-}HT_2$) were determined in the functional systems and compared to reported K_i values at the high affinity sites. The EC_{50} and K_A values at the functional receptors are 20-30 times higher than their related affinity for the 5-HT-sensitive [^{3}H]8-OH-DPAT or cinanserin-sensitive [^{3}H]DOB binding sites. Also, the binding to the 5-HT-sensitive [^{3}H]8-OH-DPAT sites by spiperone and methiothepin, drugs that were reported to act as competitive $5\text{-}HT_{1A}$ antagonists (DeVivo and Maayani, JPET: 238, 1986) does not obey the simple Michaelis-Menten model (Hill coefficients of 0.59 ± .05 and 0.47 ± .03, respectively). Similarly, BMY 7378 (7,9-dioxo-8-[2-(4-o-methoxyphenylpiperazinyl)ethyl]-8-azaspiro[4.5]decane dihydrochloride), a buspirone analogue with high affinity, selectivity and low intrinsic activity at the $5\text{-}HT_{1A}$ receptor in rat and guinea pig hippocampal membrane (Yocca et al., E.J.P., submitted) (K_D = 10 nM), binds in a complex manner to the 5-HT-sensitive [^{3}H]8-OH-DPAT site (Hill coefficient of 0.69 ± .03). These data may be explained by the existence of interconvertible states of the 5-HT receptors (uncoupled, high affinity and coupled, low affinity states) or by two distinct binding sites. Because the binding of antagonists to the recognition binding sites labeled with agonists (8-OH-DPAT and DOB) is complex, while the binding of agonists at these sites seems to be simple, it is proposed that these 5-HT receptors and their related binding sites exist in two interconvertible states (supported in part by USPH GM 34852).

94.7 EFFECT OF 5,7-DIHYDROXYTRYPTAMINE-INDUCED LESIONS ON THE 5-HT_{1A} RECEPTOR NEGATIVELY COUPLED TO ADENYLATE CYCLASE IN RAT HIPPOCAMPUS. F.D. Yocca, A.S. Eison, E. Ryan* and J. Torrente*. Preclinical CNS Research, Bristol-Myers Company, Wallingford, CT. 06492-7660.

The 5-HT_{1A} receptor linked negatively to adenylate cyclase in rat hippocampal membranes has been shown to be pharmacologically indistinguishable from the 5-HT-sensitive [^{3}H]-8-OH-DPAT binding site in similar preparations. (Devivo and Maayani, JPET: 238, 1986). While measurement of the functional response to this receptor has proven to be a powerful tool for characterizing drug responses and identifying structural requirements necessary for activation of the 5-HT_{1A} receptor (Yocca et. al., Soc. Neurosci: 12, 1986), little is known about its location (pre vs. post-synaptic) or whether this receptor can demonstrate plasticity. To answer these questions, a study was performed which involved selective lesioning of central serotonergic neurons by 5,7-di-hydroxytryptamine (5,7-DHT), with the aims of 1) removing 5-HT terminals in the hippocampus (where these receptors may reside) and 2) denying putative postsynaptic 5-HT_{1A} receptors a source of 5-HT to determine if the phenomena of "receptor supersensitivity" can be induced.

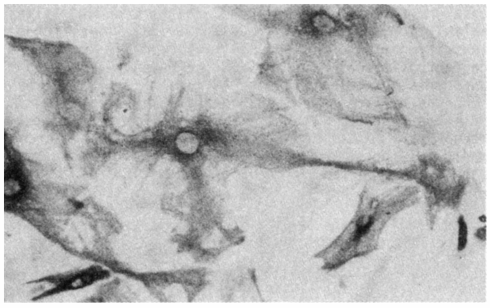
Lesions of 5-HT neurons were performed (Eison et. al., PBB: 24, 1986) five days after surgery when polyethylene cannula were inserted into the lateral ventricles of male Sprague-Dawley rats. Prior to lesioning, rats were pretreated with 25 mg/kg desipramine followed by 15 mg/kg pentobarbital treatment, after which 5,7-DHT (150 ug/20ul) was slowly infused (10ul per side). Seven and fourteen days after administration of the neurotoxin, rats were sacrificed and the hippocampus homogenized so as to measure 5-HT_{1A} inhibitory cyclase and 5-HT levels from the same preparation.

5,7-DHT lesions significantly depleted hippocampal 5-HT levels seven (88%), and fourteen (93%) days after administration of the toxin. The functional status of the hippocampal 5-HT_{1A} receptor was assessed using the potent agonist 5-carboxamidotryptamine. When compared to sham-operated controls, the functional parameters generated in lesioned hippocampi (EC_{50} = 5-10 nM; intrinsic activity=1.0) were not statistically different at any time point tested. These findings are consistent with the idea that the 5-HT_{1A} receptor is not located on presynaptic 5-HT terminals in the rat hippocampus. Furthermore, the lack of change in the sensitivity of the receptor 14 days after i.c.v. administration of 5,7-DHT suggests that this receptor may lack the ability to adapt to changes in synaptic 5-HT concentrations. Continuing investigations with partial 5-HT_{1A} agonists which exhibit a range of potencies and intrinsic activities may provide insight into this apparent lack of plasticity.

94.8 AUTORADIOGRAPHIC AND FUNCTIONAL STUDIES OF HIGH AFFINITY SEROTONIN RECEPTORS ON ASTROGLIAL CELLS. P.M. Whitaker-Azmitia and E.C. Azmitia. Dept. of Psychiatry, SUNY, Stony Brook, NY and Dept. of Biology, New York University, New York, NY.

Using neuronal cultures, we have shown that high affinity serotonin receptors modulate the development of serotonergic neurons (Neurosci. Letters 67:307, 1986). Some of these receptors occur on astroglial cells and their number varies with the age of the cultures and the region of brain from which the astroglial cultures originate (J. Neurochem. 46:1186, 1986). Together, these observations led us to investigate in more detail the characteristics of these receptors on astrocytes and how they might function in neurodevelopment.

Primary astroglial cultures were derived and maintained as previously described. For production of growth factors, cells were exposed to serum-free media or media containing serotonin, and the conditioned media added to cultures of dissociated serotonin neurons. These studies showed the presence of factors in the media from treated cultures which regulated neuronal growth. For autoradiographic studies, lightly fixed cells were rinsed and incubated for thirty minutes with 5-8 nM radiolabelled serotonin. Non-specific binding was determined in the presence of 1,000 nM serotonin. In some cases, cells were stained immunocytochemically with an antibody raised against GFA. Slides were dipped in emulsion and developed after two to three months. These studies showed that serotonin receptor labelling is not uniform over a cell. Moreover, not all cells within the culture were labelled with serotonin, although they were labelled for GFA. An example of this work, shown in a culture stained for GFA, is reproduced below.



94.9 RAPID DETECTION OF THE STIMULUS PROPERTIES OF 5-HYDROXYTRYPTAMINE (5-HT) AGONISTS. I. Lucki, J.A. South* and R. Berger*. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Drug discrimination studies have shown that rats distinguish the stimulus properties of 5-HT agonists that are selective for different receptor subtypes (see R.A. Glennon: J. Med. Chem., 1987, 30:1-12). Establishing this discrimination generally takes extensive training procedures lasting 6 weeks or longer. However, using a discriminated conditioned taste aversion procedure, rats were trained to recognize the stimulus properties of 5-HT agonists after only 8 conditioning sessions.

Fluid-restricted rats were given access to 0.25% saccharin 30 min each day for 18 conditioning sessions. On 9 drug days, separate groups of rats (N=8) were administered one of the training compounds, either the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)-tetralin (DPAT; 0.4 mg/kg) or the 5-HT_{1B} agonist trifluoromethyl-phenylpiperazine (TFMPP; 0.8 mg/kg), 15 min prior to drinking saccharin. Saccharin drinking was immediately followed by administration of .15 M LiCl (1.8 mEq/kg). On 9 alternate nondrug days, 0.9% NaCl was administered instead of the 5-HT agonists and the LiCl. Two additional groups of rats (N=8) receiving either DPAT or TFMPP prior to saccharin without LiCl served as unconditioned controls.

Associating the injection of DPAT with LiCl, but not with NaCl, produced a discriminated conditioned taste aversion after only 4 pairings, as indicated by significant differences in drinking between NaCl and DPAT. During the last 3 acquisition sessions, rats drank 19.6 $\pm$ 1.7 ml (mean $\pm$ 1 SEM) following NaCl but only 4.8 $\pm$ 2.3 ml after DPAT (75% reduction). In generalization studies, DPAT produced a dose-dependent inhibition of saccharin drinking (ED50 = 0.25 mg/kg) in LiCl conditioned animals at doses that did not reduce drinking in unconditioned controls.

Rats trained to discriminate the 5-HT_{1B} agonist TFMPP from NaCl showed similar rapid acquisition of the discriminated aversion after only 4 pairings. During the last 3 acquisition sessions, conditioned rats drank 21.6 $\pm$ 2.1 ml after NaCl but only 6.4 $\pm$ 2.6 ml after TFMPP (70% reduction). TFMPP inhibited saccharin drinking dose-dependently in LiCl conditioned rats (ED50 = 0.31 mg/kg) at doses that failed to alter drinking in unconditioned controls.

Drug substitution studies were conducted with other agonists selective for either 5-HT_{1A} or 5-HT_{1B} receptors. The stimulus properties of DPAT were similar to the effects of other 5-HT_{1A} compounds, such as buspirone or ipsapirone. The stimulus properties of TFMPP were similar to the 5-HT_{1B} selective agonist m-chlorophenylpiperazine. This agrees with previous studies. Thus, discriminated conditioned taste aversion appears to be a useful method for establishing the rapid acquisition of the stimulus properties of selective 5-HT agonists in rats.

Supported by USPHS grants MH 36262 and GM 34781.

94.10 CHARACTERIZATION OF SYNTHESIS MODULATING SEROTONIN (5HT) AUTORECEPTORS IN BRAIN SLICES, M.P. Galloway, E.A. Novak*, and B.N. Mathews* Lafayette Clinic and Depts. Psychiatry and Pharmacology, Wayne State Univ. Sch. Med., DETROIT, MI 48207

Autoreceptor control of neuronal function is known to occur at the level of impulse flow, transmitter release, and transmitter biosynthesis. In serotonin (5-HT) containing neurons, relatively little is known about the pharmacological or physiological nature of autoreceptors that modulate tryptophan-5-monooxygenase (TRYP-H) activity. To determine the role of 5-HT autoreceptors in the regulation of 5-HT synthesis, we have measured 5-HTP accumulation (in the presence of NSD-1015) in either cortical or striatal brain slices (0.3 mm^2).

Basal 5-HT synthesis in cortical slices was inhibited by the 5-HT-1B agonist TFMPP (1uM) but not by the 5-HT-1A agonist 8-OHDPAT (10 uM). After activation of TRYP-H in the presence of 30 mM K, TFMPP and m-CPP dose dependently decreased 5-HT synthesis with a greater potency and efficacy when compared to effects on basal synthesis. In contrast, 8-OHDPAT had no significant effect on activated TRYP-H. Similar findings were obtained in striatal slices except that basal striatal 5-HT synthesis was unaffected by agonists. Methiothepin, propranolol and quipazine shifted dose response curves of 5-HT agonists to the right.

The results suggest that nerve terminal synthesis modulating 5-HT autoreceptors are of the 5-HT-1B subtype with little 5-HT-1A character. We, and others, have previously shown that 8-OHDPAT is an extremely potent inhibitor of 5-HT synthesis in vivo, an effect that can be prevented by axotomy of ascending 5-HT fibers. Earlier studies by others suggest that 5-HT autoreceptors modulating release also appear to be 5-HT-1B, however somatodendritic autoreceptors appear to be exquisitely sensitive to 5-HT-1A agonists such as 8-OHDPAT. Taken together, the results support the following model: Under normal conditions, nerve terminal TRYP-H is primarily regulated by impulse flow (thus 5HT-1A cell body ARs) and only under conditions of TRYP-H activation are terminal 5HT-1B autoreceptors recruited. This circumstance differs considerably from autoregulation of tyrosine hydroxylase activity in dopamine neurons. Moreover, the results underscore the utility of this slice preparation for measuring 5-HT-1B function and further suggest that 8-OHDPAT induced suppression of 5-HT synthesis in vivo may be a biochemical measure of somatodendritic autoreceptor stimulation. Supported by MH-41227 and DA-04120 (MPG) and the State of Michigan-DMH.

94.11 EFFECT OF TRYPTOPHAN AVAILABILITY ON RELEASE OF ENDOGENOUS SEROTONIN FROM RAT HYPOTHALAMIC SLICES, Judith D. Schaechter and Richard Wurtman (SPON: W.J.H. Nauta) Dept. Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

Brain serotonin (5HT) synthesis is influenced in vivo by brain tryptophan levels, and thus by the plasma [tryptophan]/[large neutral amino acid] ratio. This study examines the relationship between 5HT release and the level of tryptophan available to brain neurons in vitro, using a superfused rat hypothalamic slice preparation. The efflux of 5HT and its chief metabolite, 5-hydroxyindole acetic acid (5HIAA), from these slices is measured under basal conditions and with electrical stimulation; tissue tryptophan, 5HT, and 5HIAA contents are also measured.

Increases in brain tryptophan content (20-600%) caused by adding tryptophan to the superfusing medium caused elevations in tissue 5HT (10-30%) and 5HIAA (20-200%) levels. Spontaneous and evoked 5HT release were augmented in parallel to these elevations: When rat hypothalamic slices were superfused in the presence of 1, 2, 5, or 10uM l-tryptophan, the spontaneous release of 5HT increased in a concentration-dependent manner, by 10.82 ± 4.72%; 21.61 ± 6.42%; 48.79 ± 10.16%; and 150.61 ± 7.28% respectively, over a period of 80 minutes. Spontaneous 5HT release as a percent of final tissue 5HT content was independent (0.08%/minute) of tissue tryptophan levels. Electrical field-stimulation of the slices (1400 bipolar square pulses, 5 Hz, 2ms) caused 3.62 ± 0.012% of the tissue 5HT to be released over a 10 minute period. While the fraction of tissue 5HT released with electrical stimulation did not change when tryptophan was added to the medium, the rate of 5HT release was clearly elevated with increased tryptophan availability; superfusing the brain slices in the presence of 1, 2, 5 or 10uM tryptophan augmented electrically evoked 5HT release by 8.43 ± 2.04%; 25.73 ± 4.94%; 39.54 ± 8.46; and 61.09 ± 11.43% respectively. There were also corresponding increases in the rate of 5HIAA efflux, i.e., by 28.50 ± 5.52%; 60.38 ± 11.41%; 109.27 ± 31.31%; and 177.39 ± 45.40% respectively.

These data demonstrate that the release of 5HT from brain neurons, both spontaneously and during electrical stimulation, parallels the tryptophan concentration in the extracellular space of the brain. Moreover, the amount of 5HT released spontaneously and with neuronal depolarization is proportional to brain 5HT levels. It is noteworthy that the increase in brain tryptophan (20-150%), caused by superfusing slices with low (1-2uM) tryptophan concentrations is within the range that occurs physiologically after consumption of a carbohydrate-rich, protein-free meal. Our data thus provides additional evidence that food consumption can modify 5HT release.

94.12 K^+-EVOKED RELEASE OF [^{3}H]5-HT FROM THE GUINEA PIG FRONTAL CORTEX. M.E. Bremer* and D.N. Middlemiss* (SPON: J. Farah). Searle Research & Development, Chesterfield, MO. 63198.

The release of [^{3}H]5-HT from preloaded slices of rat brain tissue has been extensively reported but only limited studies have been carried out in other species. The present study has investigated the characteristics of the K^+-evoked release of [^{3}H]5-HT from slices of the guinea pig frontal cortex. Slices of frontal cortex were loaded with [^{3}H]5-HT, superfused and exposed to Krebs solution containing elevated K^+ ions and 10 µM fluvoxamine (Middlemiss, D.N., Naunyn-Schmiedeberg's Archives of Pharmacology, 327:18, 1984). Elevated K+ ions (10-50 mM) caused a concentration related release of tritium. Exposure of the slices to 30 mM K^+ Krebs increased the release of tritium from 0.42 +/- 0.02% tissue stores/min (mean +/- SEM, n=6) to 1.86 +/- 0.05%. This K^+-evoked release was reduced to basal levels by the exclusion of Ca^{++} ions from the Krebs solution. Thus the stimulated release of [^{3}H]5-HT from guinea pig frontal cortex slices bears the expected characteristics of Ca^{++} dependent neurotransmitter release.

5-HT (30 to 300 nM) caused a concentration related inhibition of 30 mM K^+-evoked [^{3}H]5-HT release with a maximum inhibition observed at 300 nM of 48 +/- 3% (mean +/- SEM, n=5). This inhibition was attenuated by methiothepin (pA_2 = 8.23), cyanopindolol (pA_2 = 6.47), and mesulergine (pA_2 = 6.20), but not by ICS 205-930 (1 µM). 8-OH-DPAT (30 nM to 1 µM) did not inhibit K^+-evoked release of [^{3}H]5-HT.

These findings indicate that the pharmacological specificity of the terminal autoreceptor in the guinea pig does not correspond to that of the rat (Engel et al. Naunyn-Schmiedeberg's Archives of Pharmacology, 332:1, 1986).

94.13 INTERACTIONS OF THE 5HT$_1$ ANTAGONISTS QUIPAZINE, SPIPERONE, AND MESULERGINE, AT THE SEROTONIN (5HT) NERVE TERMINAL AUTORECEPTOR IN THE RAT SPINAL CORD. L.M. Brown, G.M. Williams, J.B. Amedro*, and D.J. Smith. Depts. of Pharmacology/Toxicology and Anesthesiology. West Virginia University Medical Center, Morgantown, WV 26506.

The selective $5HT_{1B}$ antagonist quipazine, the $5HT_{1A}$ antagonist spiperone (Peroutka, J. Neurochem. 47:529, 1986) and the $5HT_{1C}$ antagonist mesulergine (Hoyer et al., Eur. J. Pharmacol. 118:13, 1985) were used to confirm that the nerve terminal 5HT receptor (autoreceptor) responsible for modulating the release of 5HT in the rat spinal cord corresponds to the $5HT_{1B}$ binding site. A superfused synaptosomal preparation of rat spinal cord tissue labelled with ^{3}H-5HT was used. In this preparation the non-selective agonists 5HT (30-100nM) and lysergic acid diethylamide (LSD) (0.1-1µM) and the $5HT_{1B}$ selective agonists 1-(m-trifluoromethylphenyl) piperazine (TFMPP) (0.3-1µM) and 1-(m-chlorophenyl) piperazine (mCPP) (1-3µM), (Peroutka, 1986; Sills et al., J. Pharmacol. Exp. Ther. 231:480, 1984) inhibited the K^+ (15mM)-evoked release of ^{3}H-5HT in a concentration-dependent manner. On the other hand, the $5HT_{1A}$ agonists 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (1µM) (Middlemiss and Fozard, Eur. J. Pharmacol. 90:151, 1983) and 5-methoxy-N, N-dimethyltryptamine (5-MEODMT) (1µM) (Peroutka, 1986) were ineffective.

Only quipazine (1µM), the $5HT_{1B}$ antagonist, was capable of reversing the effects of the autoreceptor agonists. This antagonist reversed the depressions in ^{3}H-5HT release induced by 5HT (30nM) and LSD (0.1µM) (73 and 83% of control to approximately 90 and 96% of control, respectively). In a similar manner the depressions in ^{3}H-5HT release caused by TFMPP (1µM) (79% of control) and mCPP (3µM) (75% of control) were reversed to 95 and 89% of control, respectively. Spiperone and mesulergine were both ineffective in reversing the effect of either 5HT or other autoreceptor agonists tested. Therefore, it appears that the rat spinal cord nerve terminal 5HT autoreceptor is of the $5HT_{1B}$ subtype. Supported by NIH grant 5 T32 GM07039 and the WVU Med. Corp.

94.14 SPECIES DIFFERENCES IN SEROTONIN AUTORECEPTORS. J. Schipper*, J.A.M.van der Heyden*, B.Olivier (SPON: L.D. Bradford). Dept. Pharmacology Duphar B.V., P.O. Box 2, 1380 AA Weesp, The Netherlands.

The release of serotonin (5-HT) from nerve terminals is frequently studied by measuring the [^{3}H]-5-HT overflow from rat brain slices in vitro. Administration of exogeneous 5-HT results in inhibition of 5-HT release, indicating the presence of a negative feedback via presynaptic 5-HT autoreceptors. Pharmacological characterisation with 5-HT agonists and antagonists has indicated that the 5-HT autoreceptor in rats resembles the $5\text{-}HT_{1B}$ receptors site as defined by receptor binding studies (Engel et al. N.S. Arch. Pharm. 332: 1, 1986). Recently however, it has been reported that the $5\text{-}HT_{1B}$ site is not present in human and pig brain tissues (Hoyer et al. Eur. J. Pharm. 118: 13, 1985; Heuring et al. Eur. J. Pharm. 122: 279, 1986). Therefore we were interested to see whether 5-HT autoreceptors were present in species, which lack the $5\text{-}HT_{1B}$ site.

Cortical tissue was obtained from rats, mice, guinea pigs, rabbits, pigs, rhesus monkeys and immediately chilled in cold Krebs-Ringer buffer (KRB). Slices (0.25x0.25x2mm) were prepared with a tissue chopper and prelabelled with [^{3}H]-5-HT (30 nM) during 15 min at 37°C under $95\%O_2+5\%CO_2$ atmosphere. Slices were transferred to a superfusion apparatus and superfused with oxygenated KRB containing 10 µM fluvoxamine (5-HT uptake inhibitor) at a rate of 0.2 ml/min. After 45 min presuperfusion, 15 min fractions were collected. Release was stimulated by a 5 min exposure to 20mM K^+. Drugs were added 15 min before stimulation. Effects on stimulated release were corrected for basal release and expressed as percentage of control.

In all species studied (including those which lack the $5\text{-}HT_{1B}$ site) 5-HT induced a dose dependent inhibition of K^+ stimulated 5-HT release. 8-OHDPAT ($5\text{-}HT_{1A}$ agonist) was inactive in all species studied. Trifluoromethylphenyl piperazine ($5\text{-}HT_{1B}$ agonist) was active in rat and mouse cortical tissue, but inactive as an agonist in the other species. Cyanopindolol (0.1µM), a $5\text{-}HT_{1B}$ antagonist, completely blocked the 5-HT effects in rat and mice, but was inactive in the other species. Methiotepine (1µM), a $5\text{-}HT_1$ antagonist, blocked the 5-HT effects in all species, whereas mianserine (0.1µM), a $5\text{-}HT_{1C}$ antagonist and ketanserine (0.1µM), a $5\text{-}HT_2$ antagonist were inactive.

In conclusion, serotonin autoreceptors are present in cortical tissue of all species studied, but the pharmacological characteristics differ between species. In rat and mouse the 5-HT autoreceptor is of the $5\text{-}HT_{1B}$ type. In the other species the 5-HT autoreceptor is a $5\text{-}HT_1$ like receptor but not of the 1_a, 1_b or 1_c type.

94.15 THE EFFECTS OF CHRONIC ETHANOL AND ETHANOL/LYSINE COMBINATION ON SEROTONIN RELEASE FROM PARIETAL CORTICAL BRAIN SLICES.
Hoau-Yan Wang*, Hing Char* and Eitan Friedman* (Spon: Wagner Bridger) Division of Neurochemistry, Departments of Psychiatry and Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Changes in brain serotonin transmission have previously been reported to alter alcohol-induced narcosis and tolerance and to reverse the cognitive dysfunction caused by ethanol ingestion. In the present investigation we have examined the effects of 2 or 7 weeks of treatment with a liquid diet containing 5% ethanol on K^+-stimulated ^{3}H-serotonin release from preloaded parietal cortical brain slices. Furthermore we examined the effect of treatment with l-lysine, which has previously been shown to prevent the hypnotic effect of ethanol (Toxicol. Appl. Pharmacol. 22, 422, 1972), on ^{3}H-serotonin release and on its interaction with chronic ethanol administration. Male Sprague-Dawley rats (100-125g) were divided into four groups. Two groups were fed Liquidiet with ethanol and two received the control diet. One of each of these groups also received oral l-lysine (1.5g/kg)daily. At the end of 2 or 7 weeks, 16 hr prior to killing, the respective diets were given by gastric intubation (3ml/100g body weight). Cortical slices were incubated with ^{3}H-serotonin, and superfused with oxygenated physiological solution. Depolarization was elicited by a brief (30 sec) pulse of 65mM K^+. Stimulated release and basal efflux of ^{3}H-serotonin were calculated as the % fractional release of the tissue content at the time of stimulation. The results show that 2-weeks of treatment with ethanol(a) diminished K^+-induced ^{3}H-serotonin release and basal efflux by about 30%, (b) the co-administration of l-lysine completely prevented the effect of ethanol on serotonin release and (c) in alcohol-treated animals the release-inhibitory response to d-LSD was significantly greater than that found in control or in alcohol plus lysine treated animals. Animals treated with ethanol for 7 weeks (a) exhibited an increase (17%) in ^{3}H-serotonin release which was also prevented by the co-administration of l-lysine and (b) this treatment period resulted in a decreased responsiveness to d-LSD which was not different in the ethanol/lysine treated group. The results suggest that chronic ethanol ingestion results in presynaptic serotonin autoreceptor and serotonin release changes. Furthermore, some of these effects may be prevented by the co-administration of the l-lysine.

94.16 SYNTHESIS AND SECRETION OF A TRANSFERRIN-LIKE PROTEIN BY RAT CHOROID PLEXUS. M. Tsutsumi,* M.K. Skinner,* and E. Sanders-Bush. Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, TN 37232

Serotonin (5HT) via the 5HT-1c receptor increases phosphoinositide hydrolysis 5- to 10 fold in choroid plexus (CP) (Conn et al., Proc. Natl. Acad. Sci. USA. 83, 1986). Interestingly, 5HT is the only known substance to induce this effect. Autoradiographic studies have shown that the 5HT-1c receptor is localized to the epithelial cells of the CP (Yagaloff and Hartig, Mol. Pharmacol. 29:120, 1986). Evidence also suggests that the receptor is under the influence of cerebrospinal fluid(CSF)-borne 5HT; however, the physiological significance of the receptor is not understood.

It is generally accepted that the CP has two major functions; 1) CSF production and 2) the blood/CSF barrier. Data suggest that the CP not only functions as a gateway for the transport of various substances between the plasma and CSF, but also synthesizes proteins that are selectively enriched in the CSF. Dickson et al. have shown that high levels of mRNA for transthyretin, transferrin, and ceruloplasmin are found in rat CP (see e.g., J. Biol. Chem. 261: 3475-3478, 1986). Furthermore, a significant portion of the total protein synthesized and secreted by rat choroid plexus is transthyretin.

In an attempt to better understand the function of CP and to elucidate a possible role of the 5HT-1c receptor, we have cultured isolated cells from CP of 20-day old male, Sprague-Dawley rats. The cultured cells retain functional 5HT-1c receptors as evidenced by enhanced phosphoinositide hydrolysis. We examined the profile of the proteins synthesized and secreted by radiolabelling the cells with various amino acids (^{3}H-glycine, 35S-methionine, 35S-cysteine) for 48 hours in medium lacking glycine, methionine, and cysteine. Proteins present in the medium were separated by gradient polyacrylamide gel electrophoresis and fluorographed. Preliminary results show that 4 major proteins with approximate molecular weights of 17k, 35k, 70k, and 130k Da are synthesized and secreted by these cells. Furthermore, the 70k Da protein can be specifically immunoprecipitated with rabbit anti-rat transferrin antisera, suggesting that these cells produce and secrete transferrin into the medium. If transport proteins such as transthyretin and transferrin are normally synthesized and secreted, this would suggest that CP plays a crucial role in maintaining the homeostasis of the environment of the central nervous system. (Supported by USPHS Grants MH-34007 and HD-20583).

CONTROL OF POSTURE AND MOVEMENT II

95.1 Modification of Responses to Postural Disturbances in Leg Muscles by Transcranial Motor Cortex Stimulation in Man. C. Van derLinden*, M.R. Dimitrijevic, W.J. Eaton*, A.M. Sherwood. Section for Restorative Neurology and Clinical Neurophysiology, Dept. of Rehabilitation, Baylor Coll. Med., Houston TX 77030.

Stretching the triceps surae (TS) muscle by dorsiflexion at the ankle while standing on a rotatable platform induces several EMG responses in the TS and its antagonist the tibial anterior (TA) muscle. The first response (M1, latency ~45 msec) in the TS muscle is comparable to the monosynaptic stretch reflex. A medium latency response (M2, latency ~70 msec) in the TS muscle and a long latency response (M3, latency ~130 msec) in the TA muscle are suggested to be mediated through a long loop circuitry. The M2 response destabilizes and the M3 response stabilizes posture. Transcranial electrical stimulation of the scalp at the vertex with high voltage (~300 V) short duration (100 us) pulses evokes motor EMG responses (MEPs) in bilateral distal and proximal leg muscles (with a latency of ~30 msec in TA and TS muscles). We studied the interaction between the postural responses which are primarily mediated through bulbospinal pathways and the MEPs which are primarily mediated through direct corticospinal pathways in four neurologically healthy subjects.

A platform on which the subjects stood was rotated with an initial posterior deflection of 10 degrees with a velocity of 100°/sec. M1, M2, and M3 responses were recorded in addition to the anterior-posterior (A-P) body sway. An electrical stimulus at the vertex was triggered at various delays after the onset of the posterior deflection of the platform. M1, M2, and M3 responses and MEPs were recorded in bilateral TA and TS muscles with surface electrodes.

MEPs recorded without platform rotation were more polyphasic and of larger amplitude in the TA Muscles than in the TS muscles, accompanied by an induced body sway in the forward direction. When the cortical stimulus was delayed, after the onset of the posterior deflection, so that the latency of the MEP was comparable to that of the M2 response, the M2 response was only partially suppressed and the A-P sway was not significantly altered. However, when the latency of the MEPs was comparable to that of the M3 response, the M3 response was completely suppressed and the A-P sway in the forward direction was significantly increased.

One possible interpretation of this study is that postural reflexes under bulbospinal control are suppressed by the more potent corticospinal system, and that activation of the TA Muscles in humans is predominantly under corticospinal control, whereas, the TS Muscles, may be more under bulbospinal control.

95.2 LOAD EFFECTS ON CORTICAL SOMATOSENSORY EVOKED POTENTIALS DURING POSTURE AND ACTIVE MOVEMENT.
B. Dimitrov*, M. Hallett and J. N. Sanes. Human Motor Control Section, Medical Neurology Branch, NINCDS-NIH, Bethesda, MD 20892.

Cerebral cortical somatosensory evoked potentials (SEPs) are generally depressed in amplitude during the execution of voluntary movement in comparison to SEPs evoked at rest. It has been reported that SEPs, evoked by cutaneous stimulation of nerves in the leg, are suppressed by voluntary movement and by tonic activation of leg muscles without joint displacement. The present study attempted to determine if this phenomenon also occurred with finger movements. Thus, we investigated the magnitude of SEPs during finger movements and when different constant loads opposed postural maintenance.

Humans had the right index finger coupled to the axle of a torque motor, permitting abduction-adduction movements about the metacarpophalangeal joint. Subjects maintained a steady position of 0° ±1° of abduction while using a video screen for visual guidance. Two somesthetic stimuli were delivered: a positional ramp of 20° towards adduction, 100 msec duration, or an electric shock, at 3x sensory threshold, delivered to the proximal phalanx of the index finger. In the first condition, the somesthetic stimuli were presented during maintenance of posture against no load or a load of 0.15 Nm opposing abduction. In the second condition, subjects performed a 20° abduction movement of the index finger, from -10° to 10°, in 200 msec. The somesthetic stimuli were delivered 10° into the movement in an unpredictable fashion on one-third of the trials. As with the posture experiment, movements were performed with or without load. The EEG was recorded from the scalp 2 cm in front and behind C3, digitized at 500 Hz, and epochs of 200 msec preceding and 300 msec following the stimulus onset were averaged off-line. Four major components were analyzed: P40-55 (P1), N85-115 (N1), P170-190 (P2), and N220-240 (N2).

The SEP components were affected differently when evoked during postural maintenance or active movement and with or without load. During postural maintenance, the ramp evoked a larger P1 than the shock, whereas the shock evoked a larger P2 and N2 than the ramp. Maintenance of posture against a constant load potentiated the ramp-evoked SEP for the P1 and N1 pre-central components and the N1 and P2 post-central components, but did not affect the shock-evoked SEPs. The SEPs evoked by either the ramp or shock stimuli during movement were generally smaller in comparison to SEPs evoked during postural maintenance. The suppressive effects of active movement were greatest for the shock-evoked SEPs and were observed most readily for the P2 and N2 components in the pre- and post-central recordings. Imposition of load during active movement did not affect the magnitude of the SEP.

These data demonstrate that SEPs related to deep and superficial somesthetic inputs are differentially sensitive to imposition of a constant load during postural maintenance, but are generated similarly during active movement. In particular, a constant load potentiated SEPs evoked by the ramp stimulus, whereas SEPs were uniformly suppressed during active movement. This suggests that a gating mechanism selectively enhances deep inputs to the cerebral cortex when humans tonically activate motor neuron pools, but that both deep and superficial inputs are suppressed equally by phasic activation of motor neuron pools.

95.3 HUMAN BODY SWAY IN AN OPTICAL FLOW. M. Flückiger* (SPON: M. Woollacott). Exp. Psych. Lab. of FPSE, Univ. of Geneva, 1211 Geneva 4, Switzerland.

Postural adjustments of standing adults were studied using a textured optical flow pattern projected on the ground and walls of a darkened room. The flow pattern was structured to correspond to textures encountered in open outdoor settings. The optical flow texture consisted of circular spots 9 cm in diameter, distributed at intervals of .5 meters. Body sway was determined by measuring both changes in vertical force on a platform on which the subject stood and by measuring the displacement of a diode attached to the chest. 16 adults (aged 19-30 years) participated in the study.

The experiment consisted of the successive presentation of 5 optical conditions of 20 seconds each. Conditions 1,3 and 5 consisted of a motionless texture, measuring baseline postural sway. Condition 2 consisted of an optical texture approaching the subject, while condition 4 consisted of a flow pattern receding from the subject. The velocity of the flow was 1.39 m/sec.

Results indicate that an approaching optical flow texture causes backward postural inclination, while a receding texture gives forward inclination. The backward sway, at the onset of an approaching texture, was also less important than the forward sway with a receding flow. In addition, at the offset of image motion, there is a large inverse resetting of body position extending beyond the initial equilibrium position. 75 % of both onset and offset sway responses occurred within the first 3 second interval of an experimental condition. As each 20 sec exposure condition progressed, the oscillations tended to decrease. These experiments provide a more precise approach to the analysis of visually driven postural sway in humans.

95.4 MECHANICAL AND COGNITIVE CONSTRAINTS ON STANDING INFLUENCE POSTURAL ADJUSTMENTS AND MAXIMAL FORCES DURING PULLING. W.A. Lee, M.W. Rogers*. Phys. Ther., Northwestern Univ., and Rehabil. Inst. of Chicago, Chicago IL 60611.

This study describes how biomechanical and EMG correlates of postural adjustments (PAs) which precede the onset of voluntary bilateral upper limb movement vary with the length of the support base under the feet and with cognitive constraints on posture.

Nine subjects exerted maximal force pulses under self-paced conditions by pulling against a rigid handle fixed at elbow height. The length of the support base and instructional set were varied. Support bases equaled 100%, 80%, 60%, 40% and 20% of foot length. For the first instructional set, subjects were told to maximize the pulling force and to keep balanced. Body movement (hip angle) was unconstrained. For the second set, subjects were told to maximize the pulling force, keep balanced, and also to keep the body straight. Each subject performed 10 trials for each combination of set and base length. We measured hip and trunk displacements in the sagittal plane, pulling force, and EMG activity in the gastrocnemius, biceps femoris, tibilais anterior, and quadriceps muscles. Hip angle was computed from displacement records. The force impulse (from force onset to peak force) and the change in hip angle (from before body movement to force onset) were measured.

Preliminary analysis of ensemble averaged records have yielded four major findings. (1) All subjects kept the body straighter when instructed to do so, but hip flexion always increased before initiation of the force impulse. Thus, instructions about posture influenced the PA pattern of hip movement quantitatively, not qualitatively. (2) About half of the subjcts exerted higher forces when body movement was unconstrained. The other subjects exerted higher forces when the body was straighter. (3) All subjects had complex, bi-phasic EMG patterns of PAs in the leg muscles. The first phase (P1) preceded movement of the hips, which led the force impulse by up to 1.0 sec. The second phase (P2) of the EMG pattern immediately preceded the onset of the pulling force. The P1 component accelerated the body away from the center of the support base and presumably helped to maximize the force impulse. Some qualitative differences between EMG patterns were noted for pulls made under the two instructions. (4) The force impulses produced by subjects generally decreased with base length, with a 50% decrement in force between the 100% and 20% base lengths. More complete analysis of individual trials is in progress.

The results show more complex patterns of PAs than have been previously reported and implicate a role for some PAs in generating voluntary force rather than in controlling balance. The mixed effects of support base and hip flexion on force magnitude suggest that dynamic aspects of PAs contribute to the maximal forces which subjects can produce.

95.5 THE EFFECT OF LEARNING ON THE ELECTROMYOGRAPHIC AND KINEMATIC PATTERNS IN A PERTURBED ARM MOVEMENT. C. Dugas and R.G. Marteniuk, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, N2L 3G1, CANADA

There is evidence to show that motor learning and mental set or strategy can produce changes in reflex responses. Both factors can lead to facilitation, inhibition or even suppression of the medium and long latency reflexes to stretch, if this modulation is appropriate to the demands of the task. The purpose of this study was to investigate how reflex modulation and voluntary motor commands are integrated in order to achieve effective goal directed movements. The strategy used by the subjects was manipulated by changing the number of perturbed trials that were presented in each training session. Four independent groups, with four subjects in each group, were trained with 0%, 20%, 50% or 100% of perturbed trials on two consecutive days with 100 trials of practice per day. Subjects were instructed to perform a 70 degree forearm extension movement in 900 ms to a visual target. A perturbation in the flexion direction (90 ms, 13 Nm peak torque) was introduced 500 ms after the onset of movement on specific trials following a pseudorandom order in each group. Arm position and acceleration were recorded along with surface EMG's from the triceps and biceps muscles for specific blocks of trials on each day.

Average data and their variability for trajectories, acceleration and EMG profiles were used to assess the effects of learning and strategy in each group. The behavioral results from all four groups showed that there was a 15% improvement in performance, in terms of successful trials (when the target was reached), over days. The phase-plane trajectory data revealed that for all subjects there was a decrease in mean within subject trajectory variability with learning and that variability was affected by the level of uncertainty the subjects were facing. The associated EMG profiles showed changes with learning, in terms of decreases in the mean level of average EMG (AEMG) and mean within subject variability for agonist and antagonist muscles. More specifically there was a significant decrease of the AEMG in the M3 component (90-150 ms after onset of perturbation) over days for the groups with 20% and 50% of perturbed trials on each day. This decrease allowed subjects to change the speed of the movement after the perturbation in order to reach the target. Subjects seemed to be overcompensating for the disturbance on the first day, and with training they were able to modify the trajectory of the movement and accommodate to the demands of the task. In the 100% group, the subjects developed an anticipatory strategy that optimized the resistance to the perturbation. Subjects increased the stiffness of the limb 150 ms before the onset of the perturbation and decreased the M3 response in order to minimize the effect of the disturbance in the movement.

95.6 **THE ROLE OF GEOMETRICAL CONSTRAINTS IN THE CONTROL OF MULTI-JOINT POSTURE AND MOVEMENT.** F.A. Mussa-Ivaldi*, N. Hogan* and E. Bizzi (SPON: J.S. Barlow). Dept. of Brain and Cognitive Science, MIT, Cambridge, MA 02139.

The purpose of this investigation was to gain some understanding of the relative role of the mechanical and geometrical properties of the musculo-skeletal system in the control of multi-joint posture and movements. Recent work has indicated that muscles, in vivo, behave like tunable springs. A convenient way to represent the net spring-like behavior of the arm muscles at a given hand position was developed by Mussa-Ivaldi et al.(1985). Briefly, while subjects maintained a hand posture, small displacements of the hand along different directions were imposed. While the hand was maintained in the displaced position, the elastic restoring forces were measured. By relating the restoring forces to the displacement vectors, the net stiffness of the hand was derived. At each position of the workspace, the hand stiffness was then graphically represented as an ellipse characterized by three geometric parameters: size (the area), shape (the ratio of the major and minor axes) and orientation (the direction of the major axis). It was found that the postural hand stiffness followed a very consistent pattern as the hand location changed in the workspace: i.e., the major axis was oriented in a polar direction passing through the hand and the shoulder and the shape became less isotropic (more elongated ellipses) as the hand approached the distal boundary of the workspace. When the hand posture was maintained against externally imposed disturbances acting in different directions we observed that, while size increased, shape and orientation remained unchanged.

In the experiments reported here we investigated whether these invariances can be explained on the basis of a change in the pattern of neural input to the muscles or as a consequence of the geometrical properties of the musculoskeletal system. To this end we measured the hand stiffness as constant forces with different amplitudes and directions were applied to the hand. From the measured values of hand stiffness we derived, by geometrical transformations, the contributions to the hand stiffness from three muscle groups: muscles acting only on the shoulder, muscles acting only on the elbow and two-joint muscles. Finally we compared the actual variabliity observed in the hand stiffness parameters to the simulated variability of the same parameters computed from the muscle contributions in the hypothesis that these could vary independently of each other (e.g. with no coordination) within the measured range of values.

Our results indicate that the variations of shape and orientation of the hand stiffness field which were derived by randomly combining the measured stiffnesses from three muscle groups are of comparable magnitudes to the observed variations of measured hand stiffness. Hence, we conclude that the orientation and shape of the hand stiffness is poorly sensitive to changes in muscle stiffness. It follows that the CNS tuning of muscle properties is not a crucial factor in the expression of these invariances.

The significance of this finding became clear through simulations which show that the invariance of the pattern of hand stiffness shape and orientation has the effect of simplifying the generation of hand movements. This simplification is a consequence of the fact that the dynamic interactions of the spring-like forces with limb inertia and viscosity are more predictable if the muscle activity can affect the joint stiffness terms only by a common scaling factor.

To sum, our results are consistent with the view that biomechanical constraints arising from the geometrical structure of the arm instead of representing a problem for the motor controller provide simpler solutions for generating and representing multi-joint arm movements. (Research supported by NIH grants AM26710 and NS09343)

95.7 INFLUENCE OF LIGHT TOUCH INPUT FROM THE HAND ON POSTURAL SWAY. M.K. Holden, J. Ventura*, and J.R. Lackner. Ashton Graybiel Spatial Orientation Lab., Brandeis Univ., Waltham, MA 02254.

We have examined the possible contribution of light touch (and associated upper extremity proprioceptive) input from the fingers in the control of postural sway. That such input may be important is suggested by the informal observation that some patients with balance disorders can improve their standing and walking by simply lightly touching a wall.

Subjects(n=12) were tested under three conditions of support: no support(NS), touch support(TS), and pressure support(PS), with eyes open(EO) and closed(EC). A Kistler force platform measured reaction forces during unilateral stance over trials of 30s duration. Three measures of postural sway were derived from center of pressure coordinates: mean sway amplitude (MSA), path length (PL), and average velocity (AV). In the TS and PS conditions, subjects placed one index finger on a laterally adjacent metal bar. The amount of applied force was monitored via strain gauges. In TS trials, subjects were limited to a force of <100 g; in PS trials subjects could use as much force as desired.

The results showed a significant main effect on MSA, PL and AV for support and vision, and an interaction between the two factors ($p<0.001$). The reduction in MSA for TS vs NS with EC was significant ($p<0.05$); the reduction in MSA for PS was not significantly different from TS. For PL and AV, TS produced a significant ($p<0.05$) decrease vs NS for both EO and EC conditions. For PL and AV, TS was greater than PS. The mean force applied by the finger during TS trials was 39g and during the PS trials, 644g.

Although applied force was much greater in PS than TS trials, the concomitant decreases in sway measures relative to the TS trials were not impressive. The sensory input provided by TS decreased the sway associated with NS and EC by a magnitude similar to the decrease produced by allowing vision in the NS condition (about 60%). PS provided only about an additional 15% attenuation. These findings suggest that the decreases in MSA, PL and AV during the TS compared to the NS trials are not attributable to mechanical factors alone, and that sensory and motor information about arm position can play a significant role in the control of postural sway.

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95.8 VISUAL INFLUENCES ON BODY SWAY IN STRABISMICS AND NORMALS. CR Fox, GT Petito*, C Mason*. SUNY Optometry and Veterans Administration.

Strabismics provide an opportunity to examine the unique contribution of both retinal and oculomotor factors to visual control of postural equilibrium. Binocular viewing in the strabismic does not imply fusion and the eye position information from the oculomotor system for the two eyes will not necessarily correspond. Therefore, the contribution of each eye may be significantly different for visual processing, this is particularl. true in constant, unilateral strabismics. Further, suppression, fixation nystagmus, and ARC may be present. The consequences of this for postural equilibrium are unknown. Few studies have been performed relating strabismus and postural equilibrium and they have major flaws which do not allow examination of postural sway control. None have attempted to assess the effect of eye position or movement which makes any attempt to examine the effect of fusio or lack of fusion merely speculative. None of these studies have attempted to separate the individual relative contributions of eac eye and because of a lack of proper clinical evaluation and control, the interpretation of the data presented is questionable.

With the present state of our knowledge, there is no reason to assume a difference in equilibrium between normals and non-neurologically induced strabismics when the eyes are closed. However, with eyes open, binocular viewing would be expected to have less effect on sway in the strabismic than the normal observer due to the fact that the strabismic has weakly or abnormally correlated retinal and/or oculomotor information between the two eyes. This should result in minimal stabilization with binocular vision. This is particularly true in constant strabismics, due to the semi-independence of the two eyes and lack of normal fusion. The degree to which the binocular condition attenuates sway in the strabismic should be related to the degree of independence between the two eyes.

This experiment examined visual control of postural equilibrium with specific attention to the effects of strabismus. The observers were either normal visioned, constant unilateral strabismics (both ARC and NRC), or constant alternating strabismics (both ARC and NRC). Sway was measured during quiet standing in a stable visual surround (ie, a static environment) which was either fully illuminated and structured or dark while the subject fixate a small, stationary target monocularly with each eye and binocularly. Sway with eyes closed and eyes open in complete darkness was also measured. Eye movements were objectively monitored throughout all postural measurements when the eyes were open.

Results are discussed in terms of retinal, oculomotor, and spatial abilities in the strabismic.

95.9 VESTIBULAR AND NECK PROPRIOCEPTIVE INPUTS MODIFY THE COORDINATE TRANSFORMATION OF VISUAL TARGET INTO BLIND POINTING POSITIONS. D.Ott* and R.Eckmiller (SPON: G.Q. Fox). Division of Biocybernetics, University of Düsseldorf, FRG.

Pointing to visual targets (presented sequentially at positions along a horizontal line in head coordinates) without seeing the pointing arm (blind pointing), which was studied in four healthy subjects yields a highly reproducible blind pointing characteristic (BPC). Whereas the BPC branch contralateral to the pointing arm is almost parallel to the line of targets, the ipsilateral BPC branch is typically rotated upwards relative to the target line by an angle τ ($0<\tau<20$ deg) thus indicating a dichotomy of the BPC.

The brain functions required to generate these blind pointing movements are assumed to include the following sequence of neural coordinate transformations (CT): CT_1 projects points in physical space onto a neural eye position map representing horizontal and vertical coordinates of eye position (while fixating the target) relative to head position. CT_2 projects points from the eye position map onto an internal space map (for neural internal representation of physical space). CT_2 is assumed to process multimodal signals regarding the head tilt angle. CT_3 projects points from the internal space map onto a pointing motor map representing desired pointing positions in physical space. CT_3 is assumed to be modified by neck proprioception proportional to the head-trunk tilt angle. Finally, CT_4 executes pointing movements in physical space based on points represented in the pointing motor map.

Results:

1) The sequence of coordinate transformations CT_1 to CT_4 is modified following stepwise lateral tilt (tilt angle 20 deg) of body, head, or trunk only in the ipsilateral pointing hemispace as evidenced by dynamic changes in angle τ. 2) For blind pointing with the right arm, body tilt (left ear down) causes a steplike increase of τ. Trunk tilt (right shoulder down) gradually decreases τ (with an average time constant of 5 min.), trunk tilt (left shoulder down) gradually increases τ. Head tilt (left ear down) leeds to a transient increase of τ, which then decreases again below the pre-tilt value (with an average time constant of 6 min.).

Conclusions:

A) The dichotomy of the BPC indicates a hemispherical subdivision of the underlying neural sensori-motor coordinate transformation process. B) The dynamic tilt-induced changes of τ seem to be caused by vestibular-otolith (body tilt), neck proprioceptive (trunk tilt), or combined (head tilt) stimulation, indicating a side-specific dynamic input of these afferents. C) Due to its high reproducibility and its reversible tilt dependance this paradigm is a powerfull tool to evaluate sensori-motor coordinate transformation performance in normal subjects and patients.

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95.10 BIOMECHANICAL STRATEGIES FOR POSTURAL CONTROL IN HUMANS. J.F. Yang and D.A. Winter*. Department of Physiology, University of Alberta, Edmonton, Alberta, and Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada.

A computer simulation model, representing the dynamics of a standing human, predicted specific joint torque strategies necessary to compensate for mechanical disturbances (J.F. Yang et al., Soc. Neurosci. Abstr., Vol 12, Part 2, p.1301, 1986). These joint torque strategies were represented as a solution space. The model suggested that an output principle in the form of proportional joint torque combinations could be used to control posture over a wide range of disturbance types. Three model predictions were experimentally validated: (1) the joint torque strategies in response to a knee-forward disturbance, (2) the change in response produced by a greater disturbance force, and (3) the change in response produced by a different starting posture. An apparatus capable of generating impulsive force disturbances was designed and constructed. The disturbance force was a triangular waveform of 20 ms, applied to the leg just below the knee. Film, force plate and surface electromyogram (EMG) from the rectus femoris, vastus lateralis, medial hamstrings, tibialis anterior, soleus and medial gastrocnemius on the right side were recorded from five subjects. Three experimental conditions were presented in a random order: a straight starting posture with a light disturbance force (170 N), a straight starting posture with a medium disturbance force (235 N), and a 20 degree knee flexed posture with a medium disturbance force. Subject joint torque responses were calculated using standard inverse dynamic solution methods, for the early response time period 40 to 125 ms from the disturbance onset. The EMG responses were averaged over five trials. Subjects responded with joint torques which fell within the solution space, in spite of intersubject differences in the individual joint torques and muscle combinations used. Different disturbance forces and different starting postures were responded to with a gain change, as predicted by the model. While the individual joint torques varied between subjects, a consistent total limb strategy was evident. The model was able to account for the different subject responses, and thus appears more general than those reported by others.

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95.11 TASK DEPENDENCY OF MUSCULAR SYNERGIES EVOKED BY PERTURBATION OF COMPLEX MOVEMENT. W.E.McIlroy and J.D.Brooke, Human Biology /Biophysics, College of Biol.Sci., Univ. of Guelph, CAN. N1G 2W1.

Contrasting patterns of muscular synergies have been evoked at medium and long latencies following perturbation of rhythmic leg movement (McIlroy & Brooke Brain Res., 407:317-326, 1987). The two patterns were suggested to reflect pre-volitional and volitional outputs, respectively. This was based on the response latencies and the relationship between evoked whole limb movement and task requirements. The present study was designed to quantify the contribution made by volitional sources (including preparatory set and evoked volitional discharge), both which would be altered by task. Four subjects participated in the study. EMGs from six muscles of the right leg were sampled; soleus (SOL), lateral gastrocnemius (LG), tibialis anterior (TA), vastus medialis (VM), rectus femoris (RF) and semitendinosus. Subjects pedalled an ergometer at a rate of 0.8 Hz against a resistance of 15 N. A mechanical brake on the flywheel rapidly increased resistance. The brake was activated between 60° and 80° past top of the right crank. Subjects were instructed to either maintain movement velocity ('resist') or to relax the limb ('no resist'), with perturbation. The first experiment sampled 15 trials of both these conditions per subject. Perturbations were presented randomly (3 to 20 cycles apart). All subjects demonstrated inhibition of LG and SOL, and excitation of TA, VM and RF in the 'resist' condition at medium latencies (range 70-110 ms). Longer latency responses (range 140-180 ms) included excitation of SOL and LG. The longer latency response was also associated with a large increase in pedal force. In the 'no resist' condition a similar pattern of medium latency responses was identified, although with significantly smaller duration and magnitude. The longer latency responses were absent, consistent with the instruction set. This indicated that volitional contribution, by prior setting of CNS or by descending discharge in response to the perturbation, was modulating characteristics of both patterns. A second study was conducted in an attempt to distinguish between the two sources of volitional input. A choice reaction time task was used in the paradigm. Subjects were to resist when they saw a green light and not resist with a red light. For the initial study, the lights were triggered at the onset of perturbation, in an attempt to delay the preparation of instruction set. It is noteworthy that correct responses were only observed in 60% of the trials. Most responses evoked, correct or incorrect, did not differ from the data recorded in experiment 1. There were a few trials which appeared to differ, suggesting subjects did attend to the lights. However the small number of such samples limited quantification. (Supported by NSERC Grant #A40025).

95.12 KINEMATICS OF CEREBELLAR INCOORDINATION STUDIED WITH A 3-DIMENSIONAL MOVEMENT ANALYSIS SYSTEM R.G.Lee, W.J.Becker, B.L.Morrice*, D.G. White*. Department of Clinical Neurosciences, University of Calgary, Calgary, Canada, T2N 4N1

Lesions of the cerebellum result in errors in the trajectory, velocity and accuracy of target-directed limb movements. To quantitate these abnormalities and to investigate mechanisms responsible for incoordination, we studied four patients with cerebellar damage and four normal subjects using a Watsmart 3-dimensional movement analysis system.

Infrared emitting diodes (IREDS) were attached to the tip of the index finger, the wrist, the elbow and the shoulder. From a starting position with the arm at the side and the elbow flexed to 90°, subjects were required to move the finger tip to a target located at shoulder height and arm's length in front of them. This task required a movement of almost 90° at both the shoulder and elbow joints. Two infrared cameras recorded the position of each IRED at a rate of 200/sec. The results were converted into 3-dimensional coordinates which were used to generate plots of movement path, velocity, and joint angles.

In normal subjects the path followed by the tip of the index finger was a relatively straight line which remained fairly constant for trials made at a number of different velocities. In the cerebellar patients the paths were non-linear and highly variable from trial to trial. Part of the reason for this could be seen in the joint angle plots which revealed uncoupling of the normal precise relationship between elbow angle and shoulder angle. For example cerebellar patients would often begin the movement flexing the shoulder through a number of degrees before starting to extend the elbow, causing the finger to move vertically rather than directly towards the target. In addition, the symmetrical Bell-shaped velocity profiles which are a feature of normal movements of this type were distorted in the cerebellar patients.

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95.13 VENTROFLEXED RIGHTING PRODUCED BY MEDIAN RAPHE' LESIONS. R. M. Chesire. Psychology Dept., Univ. Hawaii at Manoa, Honolulu, HI 96822.+

Electrolytic lesions of the median raphe' nucleus (MRN) can produce several forms of postural and locomotor abnormalities, including locomotor hyperactivity. Many such symptoms resemble those produced by lesions of the nucleus reticularis tegmenti pontis (NRTP; 1-3). This report describes a form of surface righting displayed by MRN-damaged rats that approximates the form of righting shown by NRTP-damaged rats. Because NRTP-damaged rats have been shown to be partially serotonergically controlled with respect to posture and locomotion, and because the MRN is a major serotonin-containing nucleus, (2-4), the results suggest a transmitter-mediated rather than a locus-mediated control for pronation from supination in rats with MRN or NRTP damage.

Twenty male Long-Evans hooded rats were given electrolytic lesions of the MRN (1 ma for 20 sec.). When locomotor hyperactivity developed (1-3 days postoperatively), they were tested for the ability to pronate from a supine position by being placed gently on a flat surface in the supine position. The form of pronation (e.g., ventro- or lateroflexion was videotaped). Ten undamaged rats and 4 sham-operated animals were tested in the same manner. Sham-operated and control animals righted to a prone position using a lateral rotation of the head and shoulder girdle. Rats with MRN lesions ventroflexed the upper torso (especially the head), and often did not complete the righting sequence. When pronation occurred in MRN-damaged rats, they rolled forward in the ventroflexed position rather than in the normal lateral rotation.

The ventroflexion displayed by MRN-damaged rats is displayed by NRTP-damaged rats. The results suggest that there is significant serotonergic mediation of the symptomatology displayed by the MRN and NRTP preparations.

95.14 TONIC VIBRATION REFLEXES ARE GRAVITOINERTIAL FORCE DEPENDENT. J. R. Lackner, P. DiZio, and J. Fisk. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Tonic vibration reflexes (TVRs) can be elicited by mechanically vibrating the body of a muscle to activate the spindle receptors within it. The abnormally high level of spindle activity leads to reflex contraction of the vibrated muscle through activation of the alpha-motor neurons innervating it (Hagbarth & Eklund, 1966). If the action of a TVR is resisted by restraining the limb controlled by the vibrated muscle, illusory motion of the limb will be experienced in the direction that would be associated with lengthening of the vibrated muscle (Goodwin, McCloskey, & Matthews, 1972).

We have studied the influence of gravitoinertial force level variations on the illusory motion evoked by resisting TVRs. The experiment was conducted in a Boeing KC-135 aircraft that performed parabolic maneuvers to generate alternating periods of free fall, 0 G, and of high force, 1.8-2.0 G peak, each lasting approximately 20 s. Vibration of the restrained right arm (elbow angle=135°) began in 1 G and continued through 0 G or 1.8-2.0 G. Subjects (n=6) tracked with their left arm the apparent position of their right forearm during separate vibration (120 Hz) of the right biceps brachii and triceps brachii. An electrogoniometer on the left arm and a linear accelerometer permitted correlation of apparent limb position and force level.

The findings indicate that changes in apparent limb position associated with resisting TVRs are virtually eliminated in free fall and greatly increased in 1.8-2.0 G relative to straight-and-level flight, 1 G. The mean apparent displacements (in degrees) and ranges are presented below.

	0 G	1 G	1.8-2.0 G
Biceps Brachii Vibration (Apparent Extension)	2.9 (0-8.2)	16.6 (9.1-22.3)	36.1 (24.6-45.0)*
Triceps Brachii Vibration (Apparent Flexion)	1.3 (0-5.7)	7.4 (3.6-12.3)	11.7 (8.8-16.1)

* Two subjects reported hyperextension of the forearm beyond the physiologically possible limits; the maximum possible goniometer signal however was only 45°.

The G-force dependence is virtually immediate, being manifest generally within 1 s. These findings point to immediate vestibulo-spinal influences, and possibly proprio-spinal and somatosensory spinal, influences on postural tonus as a function of background gravitoinertial force levels.

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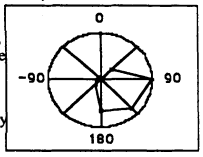
95.15 **DIRECTIONAL PREFERENCES OF HUMAN NECK MUSCLES DURING HEAD STABILIZATION.** E. A. Keshner, D. Campbell* and B. W. Peterson. Dept. of Physiology, Northwestern University Med. School and Sensorimotor Performance Program, Rehabilitation Institute of Chicago, Chicago IL 60611, USA. Supported by grants NS22490 and 1T32 NS07243.

The head-neck motor system is a highly complex musculoskeletal linkage composed of multiple joints, and more muscles than there are degrees of freedom of motion. Thus it is theoretically possible that a given movement could be produced by many different muscle patterns. However, animal studies have indicated that the CNS chooses a single muscle pattern for each movement. To see if this is also true in humans, w e measured the EMG response patterns of four muscles in the neck during isometric stabilization of the head. Surface electrodes were placed over the palpable areas for the right semispinalis capitis, splenius capitis, trapezius, and sternocleidomastoid muscles. Twelve seated subjects were given visual feedback of head position and instructed to hold the head still and upright at all times. A weight was lowered in 16 different directions within the frontal plane (including pitch and roll) from the top of a helmet worn on the head. Equal weights were also placed on either side of the helmet so that one pulled forward and the other pulled backward in order to produce rotational torques (yaw).

EMG responses of each muscle to the unidirectional forces suggested an orthogonal pattern of activation where the muscle was activated maximally in one specific direction and silenced in the orthogonal direction. A distinct pattern of activation was observed for each muscle, thereby supporting our supposition of having measured four different neck muscles. These preferred patterns of reciprocal activation to small unidirectional forces were consistent across the subjects and quantifiable. As seen in the figure, the right sternocleidomastoid muscle produced its greatest EMG activity when the subject stabilized against a leftward extensor force by pulling into rightward roll (90°) with some head flexion(135°). There was gradually diminished activity between the forces of pure head flexion (180°) and pure roll; no activation occurred in the extension (0°) and leftward roll directions. Subsequent recordings with bipolar needle electrodes confirmed that the results were representative of activity in individual muscles. When forces applied to the head were increased, the amplitude of the reciprocally activated response increased linearly in the preferred direction. Eventually, coactivation of the muscles appeared even in the null direction. Torsional forces increased the amplitude of response as much as threefold, producing cocontraction patterns with greater activation in one or the other direction of head turning. Our results show that each muscle has clearly defined spatial properties with consistent patterns of minimal and maximal activation for each muscle. The size of the response and its appearance in the null direction were altered when cocontraction rather than reciprocal activation occurred. Since the preferred directions do not align with the pulling directions described in anatomy texts, we suggest that the human neck muscles may be programmed like those in the cat where preferred activation and pulling directions differ (Peterson et al., Neurosci Abstr, 11: 83).



95.16 SPRING ACTIVATED HUMAN TILTING PLATFORM FOR THE STUDY OF BIPEDAL BALANCE CONTROL IN MAN. E.V. Lo Monaco*, C.W.L. Chan and D.G. Watt (SURG: G. Mandl). School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada, H3G 1Y5.

Evidence from human and animal studies indicates the presence of interactions between visual, vestibular and somatosensory afferent discharges in the control of posture and balance. However, the actual neural interactions and organizations of these sensory modalities in coordinated activation of lower limb muscles for the maintenance of normal human standing against external perturbation is yet to be fully understood. This in part arises from difficulties in discerning the specific contribution of a particular sensory modality during the regulation of bipedal balance. In normal human stance, the body behaves as a multi-link inverted pendulum with rotation at the ankle, knee, hip and neck. Experimental investigation of the direct action and interaction of vestibular and ankle somatosensory inputs in balance control therefore requires a technique, whereby the body can be modelled as a single link pendulum rotating only about the ankle joint. Accordingly, a tilting apparatus has been developed to produce vestibular stimulation alone, or in combination with ankle somatosensory stimulation, during rapid fore-aft tilts of the head and body about an axis colinear with the ankle joint.

The apparatus is composed of a tilting device attached to a structural base, and provides rotation in the antero-posterior plane. Mechanical rotation of the tilting device is achieved by a spring-activating system mounted at its base. Subjects are secured to the device's vertical support by a body and leg harness system with the head immobilized. Simultaneous application of dynamic vestibular and ankle somatosensory inputs are induced by rotating only the vertical back support, whereas vestibular input alone results from the concurrent rotation of both back support and standing surfaces. The maximum excursion of the back support is 15 degrees from the vertical midposition, with magnitudes of acceleration ranging from 0.1 to 0.5g as measured by a linear accelerometer mounted on a dental bite. During the testing session, electromyographic recordings will be obtained from the lower limb muscles bilaterally, with the subjects blindfolded and wearing earphones to minimize auditory stimulation.

This experimental technique will make possible the elucidation of neurophysiological mechanisms underlying the close functional relationship between vestibular and somatosensory reflexes in the regulation of standing balance. Such knowledge will provide a basis for further studies relevant to the understanding of equilibrium disorders.

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95.17 **THE EFFECT OF FORCE ON THE PERCEPTION OF FINGER MOVEMENT.** L.A. Jones and I.W. Hunter. Faculty of Medicine, McGill University, Montreal, Canada H3G 1Y5

The perception of static limb position has recently been shown to be influenced by the forces generated as the limb moves from one location to another (Watson et al., 1984). Errors in the perceived position of a limb occur when subjects are required to generate a changing isometric force against a device moving the limb, the magnitude of these errors being proportional to the amplitude of the forces produced at the end of the imposed movement (Rymer & D'Almeida, 1980). Consistent with these findings, Roland and Ladegaard-Pedersen (1977) have demonstrated that the perceived amplitude of voluntary limb movements is affected by the external forces against which the limb has to move, but the precise relation between force and the perception of limb movement has not been determined.

The objective of the present experiment was to examine the influence of the force exerted during the course of a voluntary finger movement on the perception of movement amplitude. The hand was positioned in such a manner that the wrist was immobilized and the tip of the index finger rested on the end of a lever protruding from a galvanometer operating in a constant-force servo mode. Subjects were required to track a reference movement that was presented on a screen by flexing and extending the proximal interphalangeal joint of the index finger. Three different movement amplitudes were used ranging from 5 to 10 mm. Immediately after the finger returned to the rest position the subject was asked to reproduce the movement in the absence of visual feedback. The forces against which the reference and matching movements were made, varied from trial to trial and ranged from 0 to 4 N. Finger position was measured using a capacitive angular position transducer. The sequence of trials and the displacement of the finger were recorded on-line by a MicroVAX computer.

The results indicated that force had a small but consistent effect on the perceived amplitude of the reference movement. Although there was some inter-subject variability in the accuracy with which movements were reproduced when the forces in the two conditions were equal, when the force against which the matching movement was made was considerably greater (e.g. 3 N) than that of the reference trial there was a tendency to overestimate the amplitude of the reference movement. These findings indicate that the forces exerted during the course of voluntary limb movements can influence both the perceived amplitude of the movement and the perceived position of the limb. They also suggest that under some conditions sensory signals arising from muscles are unable to provide accurate information about the position of the limb.

(Supported by NSERC)

95.18 ELECTROMYOGRAPHIC RESPONSES TO LOAD PERTURBATIONS IN A MULTI-JOINTED LIMB. J.F. Soechting and F. Lacquaniti, Laboratory of Neurophysiology, Univ. Minnesota, Minneapolis, MN 55455.

A force applied to the human forearm leads to angular motion at the elbow and shoulder. If the direction of force in the sagittal plane is varied, different combinations of angular motions can be induced (e.g. flexion at the elbow with flexion or extension at the shoulder) as can different combinations of changes in torque at the two joints (e.g. shoulder flexion with shoulder flexor or extensor torque). It thus becomes possible to ask the question: to which if any of the four variables (kinematic and dynamic) are the electromyographic responses to load perturbations related?

Force perturbations consisting of pseudo-random pulse trains were applied to the forearm and electromyographic responses of shoulder and elbow flexors and extensors were computed by cross-correlation with the input. It was found that the average response to a single pulse consisted of two components, an 'early' one 40-80 ms after pulse onset and a later one occurring with a latency of 80-120 ms. In any given muscle, the sign of these two components could differ and during these intervals, the sign of the response could also differ in the three main elbow and shoulder flexors examined. Thus, biceps activation could be accompanied by a depression in anterior deltoid or brachioradialis activity.

The amplitude of the 'early' components was found to be best correlated with the angular velocity of the limb resulting from the perturbation: for mono-articular muscles, the angular velocity at that particular joint and for bi-articular muscles, a combination of shoulder and elbow angular velocity. By contrast, the amplitude of the 'late' components was best related to changes in elbow and shoulder torque, with a weighting which could be predicted from results obtained under static conditions. Finally, a principal component analysis of the correlations among the activities in different muscles gave no evidence of a set of fixed synergies in the activation of shoulder and elbow flexors and extensors. The patterns of activity in each muscle resulting from a load perturbation depends in a unique manner on the kinematic and dynamic parameters describing the motion of the limb.

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95.19 QUANTIFICATION OF PRECISION GRASP CAPABILITIES IN HUMANS. S.M. Drucker* and J.M. Hollerbach. Dept. of Brain and Cognitive Sciences, Massachussetts Institute of Technology, Cambridge, MA 02139.

Many attempts have been made to characterize human grasps. In particular it has been proposed that grasps can be classified primarily into two categories, precision grasps and power grasps. There exists, however, very little quantitative data on the exact capabilities of precision grasping in human hands. In robots hands, analyses have been performed to determine what capabilities are necessary to grasp and manipulate arbitrary objects. These studies have determined theoretically the grasp forces that ensure secure grasps, and mapped out primary and secondary work spaces for multifingered hands. In robotics, it is theorized that for fine motion control (which is necessary for interfacing grasped objects to the geometry of the environment), the robot must be able to impart arbitrary incremental displacements in six dimensions. This is equivalent to generating arbitrary force-torque vectors on an object.

Our hypothesis for this study is that during precision grasps humans can generate arbitrary force-torque vectors. To examine this, we use a 6-axis force-torque sensor in the shape of a sphere to collect data from subjects in a variety of manipulation tasks using three fingers. Initially the subject is directed to attempt to achieve certain target force-torque combinations. The subject receives constant feedback via computer graphics about what forces and torques are actually being applied to the sensor. The subject is then directed to manipulate the sphere in arbitrary directions in an attempt to characterize the natural workspace of human grasps. The forces and torques are recorded and analysed.

An attempt is made to compare the data collected to existing characterizations and theories on both human and robot hands.

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95.20 THE EFFECTS OF VISUAL INFORMATION, OBJECT MOTION AND SIZE ON REACHING AND GRASPING KINEMATICS. C.L. MacKenzie & J. Van den Biggelaar*. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The unified act of grasping requires processing information about the spatial location and specific features of the object such as shape, size, weight and texture. The motor system(s) must transport the limb to the appropriate location and control grip formation for effective prehension. Jeannerod (1981,1984) labelled these the transport and manipulative components, and suggested parallel visuomotor channels for their control. The purpose of this research was to evaluate visual information, object motion and target size on the transport trajectory and grip aperture.

Five right-handed adults were seated and instructed to grasp and lift small (3 cm) or large (6 cm) wooden disks 27 cm in front of the body midline. There were 3 grasping conditions: 1) "stationary central" - subjects looked at the target placed 27 cm in front of them; 2) "moving central" - subjects watched the target as it approached on a moving treadmill from left to right; 3) "moving peripheral" - subjects focussed on a point marked on the treadmill frame (body midline) and saw the target moving left to right in peripheral vision. Treadmill (target) speed was 53 cm/s. The WATSMART system produced 3 dimensional position coordinates for markers placed on the wrist, index and thumb (sampled at 200 Hz, filtered at 10 Hz). Differentiated wrist data indicated the transport component and the distance between thumb and index markers indicated grip aperture over the course of the movement.

For transport, results over both disk sizes indicated peak speed was fastest and most variable over 10 trials for moving peripheral, then moving central, then stationary central conditions. Aperture results showed larger and more variable maximum apertures in peripheral than in central vision conditions, regardless of whether the object was moving. Consistent with previous work, maximum apertures were larger for large than small disks. Thus the transport component was affected by whether the target was moving and type of vision, but not disk size. In contrast, the manipulative component was affected by type of vision and disk size, not whether the object was moving. Although instructed to grasp the disk between index and thumb, almost all subjects tried to use additional fingers in the moving peripheral condition.

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95.21 **THE INFLUENCE OF MULTIDIMENSIONAL STIFFNESS AND VISCOSITY ON TRAJECTORY FORMATION AND CONTROL OF A MODEL HUMAN ARM.** S. A. Ellias* (SPON: W. Richards) Massachusetts General Hospital, and Massachusetts Institute of Technology, Cambridge MA 02139.

As a prelude to experiments on the influence of shoulder and elbow stiffness and viscosity on arm movement control and trajectory formation, numerical simulations were conducted on a model human arm executing planar point to point movements. The following experimentally relevant issues were addressed: Can the trajectories and velocity profiles of the end point [tip] of a two-link arm be used as discriminators of the orientation of tip stiffness and viscosity during an arm movement? (The stiffness orientation is defined as the direction of the greatest stiffness at the tip). For example, could stiffness and viscosity orientation vary in a way that would leave many trajectories unchanged in spite of large variations in the direction of maximal stiffness during a trajectory? Are there directions of trajectories which are more sensitive to these parameters?

Design issues for movement controllers with a moving "equilibrium point" and no inverse dynamics were studied: e.g., what are the advantages of feedback control laws which cause the tip stiffness to be isotropic, polar (directed from shoulder to the tip) or arbitrarily directed.

The planar nonlinear dynamical equations for a model two-link arm executing fast point to point movements were numerically integrated. The model was governed by a linear feedback controller with a straight line minimum-jerk reference trajectory in cartesian space. Two different control strategies were compared: constant stiffness and viscosity at the tip versus constant stiffness and viscosity at the joints.

These simulations suggest that if the tip orientations of stiffness and viscosity are kept constant during the trajectory, then the shape of the trajectory can be a very sensitive indicator of the stiffness orientation. This is true if the trajectories are oriented obliquely to the direction of maximal tip stiffness and if the tip viscosity orientation is not perpendicular or parallel to the stiffness orientation. However, a coordinated variation of tip stiffness and viscosity orientation may make the trajectory and velocity profiles insensitive to stiffness orientation. The straightest trajectories with constant tip stiffness and viscosity occur with isotropic values for both. Though, trajectories passing close to the shoulder may still be curved.

If the joint stiffness and viscosity orientation are kept constant (yielding variable tip parameters), then many trajectories are yet closer to the reference trajectory - even for trajectories passing close to the shoulder. Extremely straight trajectories have been obtained when the stiffness and viscosity orientations were polar. Thus, for point to point movements there are advantages to a control system or mechanical geometry which can create an almost constant relationship between the polar direction and the orientation of tip stiffness and viscosity: an arm with a linear feedback controller can create good straight line trajectories without computing detailed inverse dynamics.

This work is supported by NIH Grants K07-NS00883 and NS09343.

96.1 COORDINATION OF THE HEAD AND BODY IN STANDING POSTURE IN NORMALS AND PATIENTS WITH BILATERALLY REDUCED VESTIBULAR FUNCTION. C. L. Shupert*, L. M. Nashner, F. B. Horak, and F. O. Black. Neurological Sciences Institute of Good Samaritan Hospital, Portland, OR 97209.

Control of body and head position during stance is achieved by combining sensory information from the somatosensory, visual, and and vestibular systems. Although these sensory systems all provide similar information about head and body motion, none of the three can, in isolation, completely specify the motion of the body's center of gravity. The task of correctly interpreting information from multiple sensory systems would be simplified, however, if the head's inertial sensors were kept at a constant angle with respect to gravity during body motion.

In order to determine whether a constant head angle with respect to gravity is maintained during postural adjustments, 5 normal subjects aged 30 to 56 were subjected to 3.6 cm backward translations of their support surface at 15 cm/sec with eyes open or closed. Subjects were tested both standing on a flat support surface, which typically elicits forward ankle sway, and standing crosswise on a 4-inch beam, which typically elicits forward hip sway. A group of 4 adults aged 49 to 69 with bilaterally reduced vestibular function (absent caloric responses bilaterally, and gains of less than .15 for .05 Hz and .2 for .2 Hz sinusoidal rotations) were also tested. Head, hip, and ankle angle were recorded during sway using a Watsmart system, and leg, trunk, and neck EMG were also recorded. Angular head velocity was recorded using a rate sensor attached to a snugly fitting helmet.

For translations on the flat surface (ankle sway), both normals and patients with bilaterally reduced vestibular function initiated postural responses before any measurable linear or angular changes in head position had occurred. During the active postural response to the induced forward sway, the head was rotationally displaced briefly, and was then restabilized. For ankle sway in normals and patients, neck EMG activity was minimal, but the small active neck response counteracted the inertial effect of upper body motion on the head. Finally, the rotational head stabilization of the patients was as complete, if not more complete, than that of the normals. However, none of the patients were able to use hip motions, which were required to maintain balance on the beam. For normals on the beam (hip sway), the earliest neck EMG appeared in the neck flexors with a latency of about 100 msec, and the subjects achieved good rotational head stabilization by coordinating the motions of the hip and neck. Finally, the results of both patients and normals were largely equivalent for eyes open and eyes closed. (Supported by grants NS-12261 to LMN and NS-19222 to FOB.)

96.2 MOVEMENT CONTROL IN THE ELDERLY. W.G. Darling and J.D. Cooke, Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

In previous studies we have suggested that the relationship between velocity and position (phase plane trajectory) is controlled throughout simple limb movements. Recently it was shown that motor control processes of elderly subjects may differ from young adults in that they exhibit asymmetric movement profiles. In the present study we examined trajectory variability of movements made by young adults and elderly subjects to determine whether there is an effect of aging on movement-to-movement trajectory control.

Eight elderly subjects (aged 68-95 years) and six young adults (aged 21-24 years) performed elbow flexion and extension movements in a visual step tracking paradigm. Movement amplitudes ranging from 10° to 80° were performed under two instructions - "own speed" and "fast and accurate". In a second experiment 5 elderly subjects practiced 30° movements for a total of 180 flexion and 180 extension movements. Instructions were to make movements smoothly and accurately and to attempt to increase speed while maintaining accuracy during practice.

Trajectory variability increased with both movement amplitude and speed as expected from previous work. Trajectory variability was greater in the elderly subjects for both the acceleratory and deceleratory phases of the movements. With practice, the elderly subjects showed large decreases in trajectory variability with little change in movement speed.

Analysis of EMG patterns showed that although the first agonist burst appeared normal in elderly subjects, there was greater agonist-antagonist cocontraction. Control of the antagonist was abnormal. There were usually not clear antagonist bursts, or if present, their timing was inappropriate. With practice, there was little change in the overall EMG pattern, although a clear antagonist burst developed in a few subjects. Variability in agonist-antagonist EMGs was, however, clearly decreased with practice.

The results show that movement trajectories are less accurately controlled in elderly subjects. Substantial reductions in trajectory variability can, however, be attained during practice by elderly subjects. As indicated from the observed EMG patterns, motor control processes of the elderly are not the same as those used by young adults. In spite of these differences, the ability to improve performance with practice is clearly not lost in elderly subjects.
Supported by the Physician's Services Inc. of Ontario.

96.3 IMPAIRMENT OF MOTOR PROGRAMMING AND TRAJECTORY CONTROL IN A DEAFFERENTED PATIENT. J. Gordon, M. Iyer*, & C. Ghez. Ctr. for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY

Human subjects use a pulse height control policy to vary the amplitudes of targeted force impulses: the peaks of the time derivatives of force (dF/dt and d^2F/dt^2) are proportionally scaled to the target force while rise time is regulated around a preset value. Rapid force trajectories are controlled by alternating contractions in agonist and antagonist muscles. Peak force and the peaks of the derivatives of force are scaled to the magnitude of a first EMG burst in the agonist (AG1); termination of the rising force depends on a reciprocal burst in the antagonist (ANT-R) during the agonist silent period. Accurate control over peak force therefore requires the precise timing and scaling of commands to opposing muscles. In order to determine the role of somesthetic information in calibrating these feedforward commands, we have examined the control of isometric force trajectories in a 41 y.o. woman with a severe sensory neuropathy of unknown etiology involving the arms. Muscle strength is normal, and both muscle biopsy and clinical EMG studies show no evidence of motor involvement.

The patient was required to produce rapid uncorrected impulses of elbow flexor force to accurately match the amplitudes of target shifts of different sizes displayed on an oscilloscope along with visual feedback of her force. Her accuracy in this task was severely impaired: peak forces were highly variable and the range of peak forces constricted. Peak d^2F/dt^2 was poorly scaled to the target and was less predictive of peak force achieved ($r=.68$) than in normals (mean $r=.94$). Although mean force rise time was similar to that of normals and rise time was independent of peak force, its variability was twice as great in the patient. Thus, the patient's inaccuracy resulted from an inadequately programmed initial output and a lack of stereotypy of her trajectories. Although the patient used an alternating pattern of agonist (biceps) and antagonist (triceps) contractions, the EMG bursts were abnormal. AG1 magnitude was poorly related to trajectory variables. Whereas AG1 is normally truncated at the time of peak dF/dt by a silent period, in the patient it was twice as long in duration, lasting beyond peak force. Moreover, the agonist EMG never showed an initial pause or late bursts, as were always observed in the fastest force impulses of normal subjects. ANT-R was abnormally large and prolonged in duration. Its time of onset was highly variable and not, as in normals, precisely timed to the peak dF/dt.

Thus, the patient was able to produce isometric force trajectories at the elbow with a normal range of rise times, and she could program alternating contractions of opposing muscles. However, the accuracy of her control and the precision with which she could time muscle activation were severely impaired. These deficits have not improved with 6 sessions of practice. Our findings demonstrate the importance of somesthetic information for the calibration of motor programs that determine the size and timing of agonist and antagonist contractions. (Supported by NS22715)

96.4 REACTION TIME PROCESSING FOR NORMALS AND PARKINSONIANS (PKs) MAKING REACHING MOVEMENTS AVOIDING OBSTACLES IN VISUAL SPACE. M.C. Verrier* and W.G. Tatton. Departments of Rehabilitation Medicine and Physiology, University of Toronto, Toronto, Canada M5T 2S8.

Reaching movements to pseudo-randomly located parafoveal targets which avoid obstacles randomly-placed along the direct path to the targets show increases in mean reaction time (RT) of 48 msec compared to movements without obstacles when the targets and obstacles are simultaneously illuminated to cue the movements (Thompson et al., 1986). At least part of the increase can be attributed to the sensory processing and integration of the novel obstacle locations relative to those of the target and the kinesthetic information concerning initial limb and body position.

In the present studies subjects fixated on a yellow LED and 40 cm reaching movements were made from a microswitch to a suddenly-illuminated green (4 cm diameter) spherical target pseudo-randomly located within a $\pm$ 20 degrees visual angle. On random trials, one to four 4 cm diameter red spheres placed along the direct path to the target were illuminated with a target. The studies were carried out in a dark room so that target and obstacle position were not discernible prior to their illumination.

Limb trajectories were monitored, with LEDs on the shoulder, elbow, wrist, 5th metacarpalphalangeal, and 1st index finger, using a Selspot kinematic system. Studies were conducted in young PKs (<45 years $n=5$) and age-matched normal subjects. Although normal subjects showed increased RTs for the obstructed reaching as opposed to the unobstructed reaching, PKs showed similar mean increases in comparison to normals for both the obstructed and the unobstructed movements. Increased RTs were not related to number of obstacles in either PKs or normals. Vector velocities in PKs were decreased ($\bar{x}$ = 1.1 m/sec) compared to normals. In PKs vector velocities maintained a single maxima in both obstructed and unobstructed trials similar to normal subjects. However, analysis of individual X, Y, and Z velocity profiles demonstrated marked discontinuities in the Y (vertical) profiles. Position/velocity profiles describing the instantaneous vector velocity in extracorporeal space showed highly "stereotyped" movements. Therefore, the Y velocity discontinuities and the stereotyped nature of the the movements were the only features which distinguished the normal from the PK reaching movements.

These findings will be described in the context of a model proposing "defective" processing of sensory information relating the location of the target and obstacles to limb and body positions. The model suggests that PKs are not able to use previously acquired position information to "plan" either obstructed or unobstructed reaching movements.

96.5 ALTERATIONS IN MOTOR UNIT FIRING BEHAVIOR DURING ISOMETRIC CONTRACTIONS IN OLDER ADULTS. G. Kamen, D. Stashuk*, C.J. De Luca. NeuroMuscular Research Center, Boston University, Boston, MA 02215 USA.

We conducted an investigation to determine whether alterations in motor unit behavior accompany age-related changes in the neuromuscular system. Motor unit firing patterns were studied in the first dorsal interosseus (FDI) and the tibialis anterior (TA) muscles in aged (>65 yrs) individuals with no known neuromuscular disorders. Each individual performed a series of muscle contractions at 40-60% MVC, each contraction lasting approximately 20 secs. Motor unit activity was recorded by a specialized quadrifilar needle electrode and stored on FM tape. The signal was later digitized off-line at 50 khz. A previously-described decomposition algorithm was used to identify motor unit firing times, yielding 4-6 motor unit action potential trains during each contraction. A comparison with previously-published data using younger subjects revealed many similarities. For example, of the 70 motor units studied to date, there was a tendency toward higher peak firing rates in the FDI than in TA. Cross-correlational analysis of motor unit firing rates in both FDI and TA produced high correlations (.4 to .9) around a lag of 0 $\pm$10 ms, suggesting a common drive linkage between motor unit firing rates, as reported earlier. However, some highly unusual behavior was observed in both FDI and TA, particularly in high-threshold motor units. Some late-recruited units tended to continually increase in firing rate, even when force declined to very low levels. While peak firing rate is usually inversely related to recruitment threshold, there were some high-threshold units which fired steadily at high firing rates. While these results are still preliminary, they seem to support the idea that in order to produce contractions of moderate intensity requiring force feedback, older adults may use an alternative strategy to compensate for peripheral neuromuscular changes.

This research was supported by a grant from the National Institute for Aging (NIA NRSA 1 F33 AG05405-01).

96.6 ABNORMAL ISOMETRIC ARM RESPONSES IN PARKINSON'S DISEASE. M.M. Wierzbicka*, A.W. Wiegner, E.L. Logigian*, R.R. Young. Clin. Neurophysiology Lab., Mass. General Hospital & Harvard Medical School, Boston, MA 02114

Force impulses produced during isometric quick flexion of the elbow were studied to gain insight into the pathophysiology of the abnormally slow movements (bradykinesia) characteristic of Parkinson's disease.

Four normal subjects and five Parkinsonian patients were seated with the shoulder abducted and elbow flexed at 90 degrees. The forearm was immobilized while a load cell measured force produced across the elbow. Each subject was asked to produce "brief, rapid force pulses" to match targets of 4, 8 and 12 kg presented on a CRT display. Surface electromyographic activity (EMG) was recorded from biceps and triceps. Twenty trials were acquired for each target. In addition, each subject's maximum voluntary contraction force was measured over a 2-second period.

All normal subjects were able to match all 3 targets by producing consistent, smooth force pulses with short rise times (65 $\pm$ 10 ms). In contrast, force records from patients (which showed large variability from subject to subject as well as within the same subject) ranged from nearly normal behavior to abnormally slow, staircase-like force patterns. Each force step within the staircase was produced with a brief rise time and corresponded to an EMG burst in the agonist (and usually a co-contraction burst in the antagonist). For a given target, the force increment per step varied among patients but was usually consistent for any one patient. Frequency of EMG bursting varied somewhat from trial to trial; most often bursts were separated by clear silent periods, but occasionally bursts merged and the resulting force was smoother. Average force rise times in all patients except one were substantially increased for all targets. This abnormally long rise time was approximately equal to the normal rise time multiplied by the number of force steps made. For one patient, rise times were within the normal range but his coefficient of variation was twice as large as normal. This patient's maximum voluntary force was only 15% less than normal (31 $\pm$ 1.8 kg) whereas other patients were only able to produce about half the normal maximum strength.

Slowness and segmentation of movements observed in patients with Parkinson's disease appear to be explained by their inability to produce smooth muscle contractions. It does not appear that the fast motor program has been replaced by the slow motor program (in which the antagonist is not activated and there is a longer agonist burst). Rather, there is failure to match the magnitude of the initial response to the required task.

96.7 THE EFFECTS OF BODY WEIGHT SUPPORT ON THE LOCOMOTOR PATTERN OF NORMAL AND SPASTIC PARAPARETIC SUBJECTS DURING TREADMILL WALKING. M. Visintin*, L. Finch*, N. Wainberg* and H. Barbeau. School of Physical and Occupational Therapy, McGill University, Montreal, P.Q., H3G 1Y5.

Neurological patients are unable to adequately bear weight through the affected lower extremities during ambulation resulting in gait abnormalities which often persist following conventional treatment strategies. An alternative approach might be to decrease body weight through the lower extremities by supporting the trunk while retraining gait. Animal studies have shown that the adult spinal cat could recover a near normal gait pattern following interactive locomotor training where the hindquarters were supported while walking on a treadmill (Rossignol S et al. In Development and Plasticity of the Mammalian Spinal Cord. Goldberger M et al (eds), III:323-346, 1986). During each training session the animals were given just the amount of weight they were able to support on the hindlimbs. Progressively the animals walked on the treadmill bearing their full weight. This training strategy has been proposed to retrain gait in neurological patients. Before validating this approach, the effects of decreasing body weight on the EMG, temporal distance and kinematic parameters of normal and neurological gait have to be determined.

In 7 normal subjects, 0, 30, 50 and 70% of body weight was mechanically supported by an overhead harness during treadmill walking. Maximum comfortable speed decreased with increasing body weight support (BWS) and % BWS trials were therefore compared to full weight bearing trials (0% BWS) at comparable speeds. Electromyographic (EMG) activity of Medial Hamstrings (MH), Vastus Lateralis (VL), Tibialis Anterior (TA) and Gastrocnemius (GA), footswitch and kinematic data were simultaneously collected. The results demonstrated significant decreases ($p<0.01$) in % stance, total double support time (TDST) and maximum hip and knee flexion angles with increasing BWS. At all BWS there was a significant decrease in mean burst amplitude for GA and an increase for TA. VL was unaffected by BWS and MH showed a significant decrease only at 70%.

In a preliminary study 6 spastic paraparetic subjects walked on a treadmill at 0, 20 and 40% BWS at a constant speed (mean=0.29 $m \cdot s^{-1}$). Data were collected as for the normal subjects with the addition of gluteus maximus (GM). A significant increase ($p<0.01$) in cycle duration was noted for all subjects from 0 to 40% BWS. A more symmetrical gait pattern was evident in 4/6 at 40% BWS as shown by a decrease in % stance difference between the two limbs ranging from 2.4% to 10.5%. 3/6 demonstrated an increase in single limb support time for the more involved lower extremity. TDST decreased in 2 of the 3 subjects who were able to walk independently on the treadmill. Qualitatively, 4/6 demonstrated a smoother locomotor pattern at 40% BWS. In 3 of these subjects this was manifested as an increase in knee flexion angle at toe-off (5° to 15°) and/or initial swing (5° to 10°). 5/6 demonstrated greater knee extension at initial foot-floor contact (5° to 12°) and/or at midstance (10° to 21°) during single limb support. An improvement in trunk alignment, with less forward flexion, was also noted. Analysis of EMG activity with increasing BWS revealed an overall trend of a decrease in peak amplitude for GA, MH, VL and GM while TA showed an increase in amplitude for peak activity. Some muscles with BWS produced an EMG profile with more appropriate phasing in relation to the gait cycle. 5/6 were able to walk at higher comfortable speeds with BWS with increases ranging from 0.06 to 0.1 $m \cdot s^{-1}$ at 20% BWS and 0.06 to 0.17 $m \cdot s^{-1}$ at 40% BWS.

It appears that in normal subjects BWS at levels below 70% modifies gait parameters without producing an abnormal gait pattern. However, decreasing body weight in spastic paraparetic subjects may elicit a more normal gait pattern as evidenced by the changes in EMG, kinematics and temporal distance parameters studied. The effects of BWS on the locomotor pattern is presently being investigated in subjects stratified according to the severity of spasticity. (Supported by Canadian MRC. H.B. is a FRSQ scholar.)

96.8 THE EFFECTS OF CLONIDINE ON CLINICAL SPASTICITY AND IN MODULATION OF THE LOCOMOTOR PATTERN IN CHRONIC SPASTIC SPINAL CORD PATIENTS. J.E. Stewart*, H. Barbeau, and S. Gauthier MD. School of P. & O.T., McGill University, Mtl, P.Q., H3G 1Y5 & M.G.H., 1650 Cedar Ave., Mtl, H3G 1A4.

Recent studies have shown that the noradrenergic agonist, clonidine, modulates locomotor function and reduces cutaneous excitability in chronic spinal cats (Rossignol et al. in: Development and Plasticity in the Mammalian Spinal Cord, Goldberger et al.(eds.), Spoleto, III:323-346, 1986). Based on these findings, a double-blind crossover study involving 6 spastic paraplegic and 3 paraparetic subjects, was developed to investigate the effects of clonidine (oral administration, dosage range: 0.15 - 5.0 mg/day) on spasticity and in modulation of the locomotor pattern of chronic spinal cord patients. Clinical measures of spasticity included the degree of evoked tonic stretch reflex (TSR) and foot/ankle clonus, as well as the use of a visual analog scale to record patients' perceived levels of spasticity. Locomotor function was evaluated as the subjects walked on a treadmill at 0.26 $m \cdot s^{-1}$ with bipolar surface electrodes recording the electromyographic activity of Vastus Lateralis (VL), Medial Hamstrings (MH), Tibialis Anterior (TA), Gastrocnemius (GA), and Gluteus Maximus (GM). Footswitches recorded the temporal parameters of gait. The subjects were supported in an overhead harness which allowed partial or complete support of body weight. The walking movements were passively manipulated in the paraplegics, and assisted in the paraparetics as necessary. Overground locomotion was also assessed in the paraparetics.

While on clonidine, TSR was diminished in 5/6 paraplegic subjects, whereas clonus was unchanged. Perceived levels of spasticity were reduced in 4/6 paraplegics. During treadmill locomotion, a reduction in the clonic discharge in GA during stance, or in the stretch reaction induced in MH during swing phase was observed in 4/6 paraplegics, with no evidence of drug induced locomotor pattern generation. Two examples are illustrated below: Ia) GA (placebo), Ib) GA (clonidine), IIa) MH (placebo), IIb) MH (clonidine). Arrows indicate the stance/swing transition. The decrease in spasticity was not reflected by an improvement in daily function except in 1/6 paraplegic. Moreover, 4/6 paraplegics experienced side-effects (eg. hypotension).

Among the paraparetic subjects (S.Q., S.H., M.H.), only S.H. demonstrated a reduction in TSR or clonus while on clonidine, however all reported a decrease in perceived levels of spasticity. S.H., initially non-ambulatory due to excessive spasticity, gained the ability to take 5-10 steps, while for S.Q. and M.H., already capable of overground locomotion, minimal changes in gait parameters were noted. During treadmill locomotion, all paraparetics demonstrated improved stance/swing ratios ($p<0.05$). Moreover, S.H. and S.Q. showed more appropriate phasing in the knee muscles (VL and MH). In addition, S.Q. and S.H. reported improvement in daily functioning while on clonidine, especially in locomotor abilities and transfers. All of the paraparetics experienced negative side-effects.

In conclusion, the effect of clonidine in the paraplegic patients was a decrease in stretch activation due to spasticity, without the generation of a locomotor pattern. In the paraparetics, clonidine had the effect of diminishing spasticity in addition to modulating the locomotor pattern. However, in light of the side effects, and the inconsistent functional benefits, further investigation is warranted into alternative methods of administration, or the combination of clonidine with substances acting on other descending systems. (Supported by Canadian MRC and Boehringer-Ingelheim Canada. H.B. is an FRSQ scholar.)

96.9 A DYNAMIC EMG PROFILE INDEX TO QUANTIFY LOCOMOTOR SPASTICITY. J. Fung[1] and H. Barbeau (SPON:P.McKinley).School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, H3G 1Y5.

Spasticity is a complex phenomenon characterized by hypertonia, hyperrefleria, clonus and impaired control of voluntary movement. Existing clinical and physiological measures of spasticity have mainly focused on the evaluation of clonus and reflexes. Subjected to the limitation of testing in a resting position, the results may not necessarily reflect the extent of functional impairment caused by spasticity. To evaluate spasticity in a dynamic,voluntary movement such as walking, a more objective and task-specific approach is essential. We therefore propose an index, 'I', as a functionally relevant measurement of spasticity in locomotion. Surface electromyography (EMG) in four lower limb muscles, simultaneous footswitches and video recordings were made from 5 normal male subjects and 5 spastic male patients of spinal origin during treadmill walking. The EMG signals were full-wave rectified, linear-enveloped and normalized to the gait cycle. The mean ensemble average amplitude ranges over 10 cycles were normalized to the peak activity for inter-subject comparison. The profiles were then divided equally into 2 'on' and 'off' domains defined from normal gait profiles (Winter D., The biomechanics and motor control of human gait, 1987). For the Vastus Lateralis (VL), the 'off' bin was chosen as 35% to 85% of the gait cycle; Medial Hamstring (MH), 30% to 80%; Tibialis Anterior (TA), 20% to 70% and Gastrocnemius (GA), 0% to 20% and 70% to 100%. Integral areas under the profiles were calculated for each bin (eg. a_1, a_2, a_3 in fig.D). 'I' was defined as the ratio of the area in the 'off' bin(s) to that in the 'on ' bin(s), i.e. $I=a_2/(a_1+a_3)$.

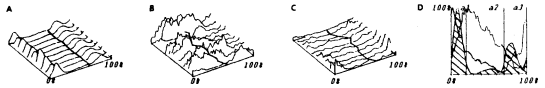

Fig.A depicts the ensemble average segments of a normal TA while the hatched area in fig.D illustrates the area under the mean profile of the same muscle. The 'I' of this muscle is 0.13. Fig.B shows a spastic TA with an 'I' value of 1.95 and fig.C, the same muscle after cyproheptadine administration, which drastically reduced the 'I' to 0.36. The superimposed profiles are shown in fig.D. The following table summarizes the 'I' range in each muscle in the normal and spastic group of subjects and the change of 'I' with two types of intervention, cyproheptadine and body weight support (BWS). Even with a small N (5) in each group, the data clearly demonstrate that the range of 'I' in spastic muscles falls completely outside that of the normal.

Group/Intervention	VL	MH	TA	GA
Normal (N=5)	0.10 - 0.21	0.10 - 0.16	0.10 - 0.21	0.07 - 0.13
Spastic (N=5)	0.48 - 1.24	0.35 - 2.99	0.52 - 1.95	0.17 - 0.89
Medication (pre→post)	1.02 → 0.57	2.99 → 0.79	1.95 → 0.36	0.86 → 0.70
BWS (0%→40%)	1.24 → 0.57	0.74 → 0.48	0.81 → 0.51	0.89 → 0.49

The marked decrease in 'I' observed in one subject after administration of an antispastic medication (cyproheptadine) corresponds to a decrease in spasticity and improvement in functional outcome. The effect of cyproheptadine on spastic paraparetic gait has been reported as a case study in the same subject (Wainberg M., Barbeau H. and Gauthier S., J. Neurol. 233: 311-314, 1986). 'I' is also sensitive to the change in muscle profile seen when a percentage of BWS is provided during walking, which is associated with a subjective and objective improvement in locomotion. We are now in the process of validating this index with respect to the severity of spasticity as well as the effect of different therapeutic interventions.

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96.10 METHODS FOR ANALYSIS OF HUMAN MOVEMENT: DIGITIZED VIDEO IMAGES. BM Myklebust, JB Myklebust, JOB Greaves*. VA Medical Center and Medical College of Wisconsin, Milwaukee, WI and Motion Analysis Corp., Santa Rosa, CA.

Previous studies have identified myotatic reflex variations in children and adult subjects with cerebral palsy (Myklebust *et al.*, 1982), and some elderly subjects with spinal stenosis (Myklebust *et al.*, 1986); these subjects also have gait abnormalities by clinical examination. We are currently conducting objective studies in these subjects to determine if there are correlations between myotatic reflex abnormalities and the profiles of joint angles and velocity during gait. Kinematic analyses of human walking previously have been performed by hand-digiziting the movement of limb segments recorded on 16 mm film, videotape, or sheet film (using stroboscopic techniques). Alternatively, light-emitting diodes placed on the joints can be tracked under computer control.

We are currently using a computer-based system for digitizing limb movements recorded on videotape as the subject walks along a 15 ft walkway (Motion Analysis Corp., Santa Rosa, CA). A single camera is used to videotape (60 fields/s) trunk and limb motion in the sagittal plane (*i.e.*, flexion and extension movements in the plane of progression). Furthermore, using an overhead mirror system (Murray *et al.*, 1967), we can measure anterior-posterior and medial-lateral displacements of the head, shoulder, trunk, arm, and feet. To identify body landmarks for digital processing, 3-dimensional retro-reflective markers are secured to standard locations on the lateral aspect of the body.

The movement of the markers is digitized and analyzed to determine the paths of the targets as a function of time. From the path information, motion variables (*e.g.*, joint angles of the hip, knee, and ankle, and velocity profiles for each walking trial) are calculated and plotted with respect to time. External scaling is used to transform pixel (video) data to real-world dimensions. Stick figures are plotted to represent the movement of limb segments along the walkway. Gait patterns among subjects are compared using such study variables as walking speed, stride length, duration of the walking cycle, duration of the stance phase and swing phases, flexion-extension patterns of the hip, knee, and ankle, and the vertical trajectory of the heel and toe.

We will present data to compare myotatic reflex patterns and gait parameters in normal adult subjects, young normal children, children who walk on their toes, healthy elderly subjects, and adult patients with neurologic disease, including spinal cord injury, spondylotic myelopathy, and multiple sclerosis.

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Murray MP: Gait as a total pattern of movement. Am J Phys Med 46:290-333, 1967.

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96.11 KINEMATIC PROPERTIES OF MOTOR SKILL ACQUISITION IN CHILDREN. T.J. Pincince* and K.L. Kerman*. (SPON: J.A. Anderson). Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge MA 02139, and Pediatric Neurology Division, Massachusetts General Hospital, Boston MA 02114.

We examined the development of motor skill acquisition in children by studying the kinematic properties of visually guided planar arm movements. Right-handed boys and girls, ranging in age from 30 months to 14 years, were asked to execute a series of continuous arm movements from a start point to an end point through an intermediate via point. The start point and end point were located in front of the subject at the midline, and the via point was located 20 centimeters to the right, at the midpoint between the start and end points. Position and time information were collected during the movement and analyzed off-line to recover velocity, acceleration and higher derivitives. The children were instructed to make the movement as quickly and accurately as possible. Previous studies suggest that adults perform this type of movement using a strategy that minimizes jerk.

Our analysis indicate that children employed three age-dependent strategies. The first strategy (ages 2-6) was characterized by a multi-segmented path and a velocity profile with many small peaks. The second strategy (ages 6-10) was characterized by a bi- or tri-segmented path, composed of long straight movements. The velocity profile for this strategy was composed of two or three large peaks separated by zero-velocity troughs. The third strategy (starting at age 10) was equivalent to the minimum jerk strategy of adults. This strategy was characterized by a smooth, arced path and a velocity profile with two large peaks separated by a non-zero trough. Movement path lengths and total trajectory time shorten, while the mean and peak velocity increase with age.

The multi-segmented path strategy suggests that movement is closed loop, employing continuous visual and kinesthetic feedback. The bi- and tri-segmented path strategy is composed of two or three chained movements, with feedback used only at the end of these smaller movements. The minimum jerk strategy may be seen as a single, open loop movement.

All children improved their performance across repeated trials, using one of two learning methods. Children using the first learning method always made movements of a single strategy type, but improved within the strategy criteria. The second learning method was seen in children who initially made movements employing one strategy that, across repeated trials, developed into another strategy.

These data imply that as children get older they learn to more fully plan complete trajectories while optimizing a number of movement parameters. This improvement in movement planning may arise from the development of an increasingly accurate representation of body kinematics.

96.12 DEVELOPMENT OF INDEPENDENT SITTING IN INFANTS. C.A. Giuliani, R.T. Harbourne*, and J.C. Mac Neela*. Motion Analysis Lab., Dept. of Medical Allied Health, Sch. of Med., University of North Carolina, Chapel Hill, NC 27514 USA.

The development of postural control for independent sitting is poorly understood. The purpose of this study was to describe kinematic and EMG variables during the development of independent sitting in normal infants. Ten normal children were tested at three stages of sitting development. Children were tested at Stage I (2-3 mo. old and unable to sit), at Stage II (sits propped forward on extended arms), and again at Stage III (sits without support 30 sec. or more).

Infants were supported at the trunk in an erect sitting position. As the examiner released support, muscle activity and trunk movement responses were recorded by surface EMG and on videotape, respectively. Angular displacement and velocity of trunk movement were measured from an erect sitting position to the position of maximum trunk displacement. EMG from the upper paraspinals, lower paraspinals, gluteus maximus, hamstrings, abdominals, and quadriceps were recorded on FM tape along with an event marker to synchronize EMG and video events.

Significant differences were seen between Stages I and II in velocity of trunk movement and trunk displacement ($p < .05$). Muscle activation was inconsistent in Stage I, but by Stage II the infants showed a significantly greater percentage of organized muscle responses, and by Stage III a clear paraspinal-hamstring muscle pattern emerged ($p < .05$). The gluteus maximus and abdominal muscles did not participate during postural response.

Our data suggest that the paraspinal-hamstring muscle synergy may be necessary for the development of independent sitting posture. The increasing occurance of this synergy related to the child's ability to sit suggests that strategies of postural control emerge during the development of independent sitting. Identifying characteristics of postural control in the normal development of sitting may increase our understanding of control problems in infants with delayed motor development.

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96.13 IMPAIRMENT OF BIMANUAL CO-ORDINATION IN PATIENTS WITH UNILATERAL SMA LESIONS. F. Viallet*, J. Massion**, R. Massarino** and R. Khalil*. *Service de Neurologie CHU La Timone, 13005 MARSEILLE and **LNF, CNRS, 13402 MARSEILLE CEDEX 9, FRANCE.

Unilateral lesions of supplementary motor area (SMA) result in an impairment in bimanual co-ordination. According to BRINKMAN (Brinkman, C., J. Neurosci., 4: 918, 1984), the effect of lesion in the monkey depends on the role played in the task by the contralateral hand: when it is acting as the "postural" hand, the co-ordination is impaired; whereas, when it is the "active" hand, the co-ordination remains rather preserved but compensatory strategies are observed.

A model of bimanual co-ordination has been tested in humans (Hugon, M. et al., Pflüg. Arch., 393: 292, 1982). In this task, one postural forearm, held in a horizontal position while supporting a 1 kg weight, was unloaded either by the experimenter's hand (imposed unloading) or by the subject's other hand in response to a tone burst (voluntary unloading). The variables recorded were reaction time, movement time, force changes and elbow angle. The EMG activity from the elbow flexors and extensors were recorded. The two arms were tested alternately as to which arm was "postural" and "active". In normal subjects, the voluntary unloading performed by the "active" arm was accompanied by a phasic decrease in EMG activity of the flexors of the "postural" arm. This anticipatory postural adjustment minimized the amplitude of the elbow rotation of the unloaded arm.

This bimanual task was tested in five patients with unilateral lesions including the SMA as confirmed by CT scan and/or NMR imaging. The results showed, that in all but one case, the co-ordination was impaired when the "postural" arm was contralateral to the SMA lesion. This disorder consisted in an increased elbow rotation of postural arm after voluntary unloading. By contrast, the co-ordination remained unimpaired when the "postural" arm was ipsilateral to the SMA lesion. In the same way, in two patients with spastic hemiparesis, no co-ordination was observed when the "postural" arm was the spastic arm. A specific role of the corpus callosum in this co-ordination could be excluded since a split-brain patient showed a normal co-ordination on both sides. It is suggested that in a bimanual task where one arm is active and the other is postural, the SMA contralateral to the "postural" arm, together with other parts of the motor-premotor areas, play a specific role in the control of the co-ordination between the two arms.

96.14 CHARACTERISTICS OF POSTURAL CONTROL ACCOMPANYING VOLUNTARY MOVEMENT IN THE ELDERLY. J.S. Frank, A.E. Patla and J.E. Brown*, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Performance on standard balance tests, e.g., the Rhomberg test, have demonstrated that balance control declines with adult aging (cf. Fernie et al., Age and Aging, 11:11, 1982). However, few investigations have examined age-related changes in postural control when attempting to stabilize the body during a perturbation. Perturbations can arise from external forces, as well as, voluntary movements of the limbs and trunk. The purpose of this investigation was to examine the organization of postural control during movements of the right arm in young (n=9, $\bar{x}$ age = 22 years) and older (n=9, $\bar{x}$ age = 75 years) adults. The tasks performed included rapid arm flexion, pulling on a stiff lever and pushing on a stiff lever. Surface emg was recorded from 6 muscles: tibialis anterior (TA), lateral gastrocnemius (LG), rectus femoris (RF), biceps femoris (BF), anterior deltoid (AD) and posterior deltoid (PD). For each task, 10 trials were ensemble averaged prior to response analysis. The organization of the postural response was examined with respect to a) which of the 4 postural muscles was activated first and b) the onset time of that muscle with respect to activation of the focal (arm) muscle, i.e., the postural-focal latency (P-F latency).

Young subjects demonstrated a consistant pattern of postural control in all 3 tasks. Postural muscles were always activated prior to focal muscles. The first postural muscle activated and its P-F latency was: BF, 40 $\pm$ 20 ms for the arm raise task (8 subjects), LG + BF, 69 $\pm$ 38 ms for the pull task (6 subjects), and TA + RF, 54 $\pm$ 60 ms for the push task (7 subjects). In contrast to young adults, the organization of postural control was more variable for older adults. A small number of subjects displayed a postural control pattern similar to young adults; however the P-F latency increased. The results were: 6 subjects, 69 $\pm$ 43 ms for the arm raise task, 3 subjects, 110 $\pm$ 71 ms for the pull task, and 155 $\pm$ 95 ms for the push task (3 Ss).Remaining subjects displayed a change in the ordering of postural muscle activation, tonic co-contraction of postural muscles and/or activation of postural muscles following focal muscle activation.

In addition to the above findings older adults displayed longer reaction time, later onset of centre of pressure shift and smaller centre of pressure displacements for the 3 tasks. These results demonstrate that postural muscle coordination changes with age and may contribute to poorer balance performance by older adults.

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96.15 MOVEMENT RELATED PHASIC EMG ACTIVITY IN HUMANS: RELATIONS WITH MOVEMENT ACCELERATION-DECELERATION CHARACTERISTICS. S.H. Brown[1] and J.D. Cooke, Dept. of Physiology, Univ. Western Ontario, London, Ontario, Canada.

We have investigated the relation between the magnitudes and durations of the various components of the triphasic EMG pattern and the accelerations/decelerations of the resulting movements. Utilizing phase plane tracking, subjects performed movements in which the amplitude, duration, peak velocity, temporal profile and acceleration/deceleration characteristics were explicitly controlled.

Initial agonist burst (AG1) - AG1 duration increased linearly with acceleration duration. Above mean accelerations of 400-500 deg/sec2, AG1 magnitude increased linearly with mean acceleration. This linear range corresponded to acceleration/deceleration duration ratios of less than approximately 0.8 which is the value seen in normal subjects performing step tracking movements. Below accelerations of 400-500 deg/sec2, there was little change in AG1 magnitude with acceleration magnitude.

Antagonist burst (ANT1) - The time of onset of ANT1 was linearly related to the duration of the acceleratory phase. ANT1 duration was relatively constant across all conditions. ANT1 magnitude, however, increased linearly with mean decelerations above about 500 deg/sec2. Below this level of deceleration, little change occurred in ANT1 magnitude.

Second agonist burst (AG2) - AG2 duration increased with increasing duration of the deceleratory phase. AG2 magnitude increased with deceleration magnitude for decelerations greater than 400-500 deg/sec2.

The data shows that the components of the triphasic EMG pattern are linked to the acceleration/deceleration characteristics of the movement rather than to the classic kinematic variables as movement amplitude and duration. Previously determined relations between EMGs and movement kinematics are explainable on the basis of changes in accelerations and decelerations associated with varying amplitude and/or durations of movements having a constant temporal profile. The data thus suggests that the triphasic EMG pattern, rather than being 'hard wired' centrally, is a consequence of the choice of a particular movement profile, a choice which is presumably based on energetic considerations.

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96.16 AN EMG STUDY OF SCRATCHING IN CHICKS. M.B. Smith*, N.S. Bradley, and A. Bekoff (SPON: D. Whitlock). Dept. EPO Biology, University of Colorado, Boulder, CO 80309.

Scratching, a rhythmic grooming behavior, has been studied in both normal and spinal transected frogs, cats, and turtles. Here, we describe the behavioral and EMG characteristics for scratching in chicks 1 to 7 days after hatching.

Selected leg muscles are surgically implanted with chronic EMG electrodes in White Leghorn chicks. Muscles studied include the right gastrocnemius lateralis (GL, ankle extensor), tibialis anterior (TA, ankle flexor), sartorius (SA, hip flexor-knee flexor), and femorotibialis (FT, knee extensor). Recordings are made 24 hrs following implantation. To verify electrode placement prior to and between tests for scratching, EMG activity is also recorded during runway walking. Final position of the electrodes is determined by dissection at termination of the experiment. Computer-assisted methods are used to quantify EMG characteristics for scratching and include: cycle period, based on the onset of consecutive bursts in the reference muscle (GL); phase relationships of non-reference muscles; and burst durations for each muscle.

Scratching is most readily elicited by placing tape on or near the right external ear. To initiate scratching, a chick first shifts its weight to the contralateral leg. Occasionally, chicks abduct and extend the contralateral wing, while some also lower the wing toward the ground. The ipsilateral leg flexes to position the foot near the ear. During scratching, the leg then alternately flexes and extends in attempts to remove the tape. Younger chicks occasionally lose their balance during the alternating limb motions, but this does not always terminate scratching.

Preliminary findings indicate scratching is characterized by reciprocal activation of leg flexors and extensors. Some aspects of muscle activation resemble those for chicks during walking; for example, FT exhibits two bursts, one co-active with TA, the other with GL (Bekoff, A., *et al., J. Neurosci.,* in press). However, phase relationships of non-reference muscles appear shifted from those seen in walking. Also, activation of ankle antagonists (GL and TA) is asymmetric; extensor bursts are shorter in duration than flexor bursts. This pattern is the reverse of that for walking in chicks, but is similar to that for scratching in decerebrate cats (Deliagina, T.G., *et al., Brain Res.*, 100:297, 1975). Because scratching is ballistic (≤10Hz) and repetitive in the chick, it may prove a useful behavior to study to further understanding of neural-mechanical interactions in motor control. Funded by NIH grant NS 20310.

96.17 KINEMATIC ANALYSIS OF WALKING, SWIMMING AND AIRSTEPPING IN CHICKS. R.M. Johnston* and A. Bekoff (SPON: J. Werner). Dept. EPO Biology, University of Colorado, Boulder, CO 80309.

The present study was undertaken to compare the range and phasing of joint motions involved in intralimb coordination during walking, swimming and airstepping. Walking was chosen because it is the most common locomotor behavior in chicks. Swimming and airstepping were selected because sensory information due to weight bearing does not occur in either behavior. However, the conditions under which the latter two behaviors occur are different. That is, in swimming the buoyant chick floats and the leg movements occur in water. In airstepping, the chick is suspended, the legs are pendent and the leg movements occur in the air.

Kinematic analysis of free walking on a runway was performed in 1- to 7-day old chicks using video recordings. This was compared to swimming in a clear plastic pool and airstepping while supported by a ring glued to the chick's back. Black dots were placed on the lateral aspect of the right leg to mark the hip, knee, ankle and foot. The dots were digitized, and hip, knee and ankle joint angles were calculated for each behavior using a computer program. A cycle was defined from peak ankle flexion to peak ankle flexion for all three behaviors.

The results confirm that certain kinematic characteristics distinguish each behavior. Walking has the longest average cycle period, while airstepping has the shortest. The range of joint angular excursions and maximum joint angles also differ among the three behaviors. On the other hand, some features that are shared by swimming and airstepping are not seen in walking. For example, the flexion and extension phases within a cycle are similar in duration in both swimming and airstepping. In contrast, during walking, extension is longer than flexion. This suggests that the relative lengthening of the extension phase seen during walking is related to sensory information due to weight bearing (Bekoff, A., *Soc. Neurosci. Abstr.*, 12:880, 1986).

Finally, we also identified some features of the leg movements that were shared among the three behaviors. For example, knee extension (or flexion) always preceded hip and ankle extension (or flexion). This similarity may result from constraints imposed by the underlying CPG circuitry, supporting the idea that these movement patterns share common circuitry elements. Alternatively, this similarity may result from mechanical constraints present in the hindlimb of the chick. Supported by NIH grant NS 20310.

96.18 COMPARISON OF WALKING AND AIRSTEPPING IN CHICKS: HINDLIMB NEUROMUSCULAR PATTERNS. T.N. Anderson and A. Bekoff. EPO Biology Dept., University of Colorado, Boulder, CO 80309.

The purpose of this study was to examine the effect of sensory feedback due to weight support on stepping in chicks. Previously, a comparison of walking, swimming, and embryonic motility in chicks suggested that the relationship of extensor and flexor burst duration to cycle period is regulated by sensory information related to weight support (Bekoff, A., *Soc. Neurosci Abstr.*, 12:880, 1986). Sensory feedback due to weight support is eliminated during swimming and embryonic motility. However, sensory feedback resulting from increased resistance due to the aqueous medium and turbulence caused by the movements may be added. Sensory feedback due to weight support is also eliminated during airstepping, but the medium through which the limbs move during the flexion phase is the same as in walking. Therefore, a comparison of the activation pattern of hindlimb muscles in the chick during airstepping versus walking will further examine the effects of sensory feedback due to weight support.

The neuromuscular activity patterns of walking and airstepping were quantified using electromyogram (EMG) recordings in 0- to 4-day old chicks. Chicks spontaneously walked on a runway and airstepped when suspended by a shielded cable attached to their backs. Bipolar hook electrodes were surgically implanted in extensor and flexor muscles at the hip, knee, and ankle. Parameters used to quantify the neuromuscular activation patterns were: cycle period of the reference muscle, gastrocnemius lateralis (GL), phase relationships for non-reference muscles, and burst durations for all muscles.

A comparison of walking and airstepping in cats with thoracic spinal transections demonstrated that extensor burst durations are shorter during airstepping than during treadmill walking while flexor burst durations remain unchanged (Giuliani, C.A. and J.L. Smith, *J. Neurosci.*, 5: 1276, 1985). The role of feedback due to weight support cannot be readily studied in intact cats, however, as they only airstep during the first two postnatal weeks. At this time in development, kittens do not have the postural stability to maintain a mature walking gait (Bradley, N.S. and J.L. Smith, submitted). In contrast, both airstepping and mature walking can be elicited in intact chicks immediately after hatching. Therefore, the chick may be a good model for studying the regulation of neuromuscular patterns by sensory information related to weight support in vertebrates. Supported by NIH grant NS 20310.

96.19 INVOLVEMENT OF RED NUCLEUS GABAERGIC SYSTEM IN MOTOR PERFORMANCE IN THE CAT.

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Injections of a GABA agonist (muscimol) and an antagonist (bicuculline) were made in the area of Red Nucleus (RN), in 3 cats. Each cat was trained to press a pedal with the contralateral forelimb and to release it after a randomly delivered go-signal with Reaction Times (RTs) less than 400ms. Prior to RTs testing, pressure injections (0.5 or 1ul in 1.5 or 3min) were made with an injection needle placed in a cannula implanted above the RN.

Injection of saline in the RN had no effect on RTs. Injection of 25ng of muscimol dissolved in saline increased the RTs (up to 25%), with force changes occurring later and more slowly after the go-signal. There was no effect on the tonic force measured at the go-signal. Higher doses (up to 200ng) were required to produce comparable effects for injections slightly above the RN. When 200ng of muscimol was injected directly in the RN, RT performance was blocked for hours and the blockage was associated with a permanent contralateral head torsiflexion. Surprisingly, injection of 200 ng of bicuculline in the RN also produced an increase of RTs (up to 25%) with delayed and prolonged force changes and, again, no effect on the tonic force. Higher doses of bicuculline (300ng) produced a transient blockade of RT performance associated with anxiety signs but no abnormality of head posture.

In one cat, the rubrospinal output was monitored with an electrode implanted in the medulla. An evoked potential was recorded in response to the go-signal with a constant latency of 10 ms and an amplitude correlated with the RTs. The rubrospinal response was unchanged by injection of saline in the RN, but was markedly decreased by injections of muscimol or bicuculline which produced an increase in RTs.

The known inhibitory action of muscimol on rubral activity is consistent with the depressant effects of muscimol injections on RT performance and rubrospinal output. As for bicuculline, its depressant effects could possibly be explained by a depolarization-block resulting from the suppression of the GABAergic hyperpolarizing influence which normally interacts with the strong depolarizing cerebellar control over rubrospinal neurons. In conclusion, the disruption of RT performance and rubrospinal output observed after injections in the RN of both a GABA agonist and an antagonist in the RN suggests that the triggering of a motor response after a go-signal requires a well-balanced rubral GABAergic activity.

96.20 DIFFERENTIAL TRANSNEURONAL LABELING OF SPINAL INTERNEURONS WITH WGA-HRP DURING DIFFERENT FORELIMB TASKS IN THE CAT. E. Sybirska*, J. Martin, J. Brennan*, C. Ghez. Ctr Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., New York, NY 10032

Behavioral and physiological studies in the cat suggest that corticospinal (CS) and rubrospinal (RS) commands for reaching are transmitted to forelimb motoneurons (MNs) through C3-C4 propriospinal neurons (PNs) (Alstermark et al.,1981). The purpose of the present study was to determine if these PNs also participate when the cat performs a forelimb tracking task in which the CS and RS systems play critical roles (Martin and Ghez,1986). This question was addressed by injecting a 5% solution of WGA-HRP into a nerve to a forelimb extensor muscle (lateral head of triceps), and determining the locations of neurons showing retrograde and activity-dependent transneuronal uptake (Harrison et al.,1984). Two groups of cats were examined. One group (tracking cats) performed the forelimb tracking task used in the prior study and made 400 forelimb extensor responses per day. A control group (walking cats) walked 1.5 km per day. In both groups, the dorsal columns were transected two weeks before injections to exclude anterograde labelling of PNs via ascending collaterals of afferents in the injected nerves. Four days (100 h) after WGA-HRP injection the cats were anesthetized and perfused with saline and 4% paraformaldehyde. The spinal cord was then removed, sectioned and processed with a modified TMB method (Gibson et al.,1984).

Approximately equal numbers of MNs showed intense retrograde labelling in both tracking and walking cats. However, marked differences in both the longitudinal and the laminar distribution of transneuronally labelled neurons were present between groups. Whereas in the walking cats only scattered neurons were labelled in the upper cervical segments, in the tracking cats numerous PNs were seen from C2 through C4 . These PNs were located both ipsi- and contralaterally. Ipsilateral PNs were seen both laterally and medially within lamina VI-VIII; contralateral PNs were found only in the medial portions of the these laminae.

In both groups of cats the density of labelled segmental interneurons (INs) was highest ipsilaterally, but some contralaterally labelled INs were also present. The laminar distribution of labelled INs differed in the two groups of animals. In the tracking cats ipsilateral INs were located within laminae VI and the dorsal part of lamina VII, whereas in the walking cats the labelled INs extended ventromedially through lamina VII into lamina VIII.

Our findings indicate that PNs projecting monosynaptically to triceps MNs are preferentially active during the performance of simple targeted limb movements and not during walking which also requires activation of the triceps motoneurons. The specific task conditions that are associated with activation of PNs remain to be determined. Retrograde transneuronal labelling with WGA-HRP is a very useful tool for functional identification of different populations of neurons during different motor activities in awake animals. Supported by NS 19205.

99 SYMPOSIUM. PROTO-ONCOGENES IN THE NERVOUS SYSTEM. M.R. Hanley, MRC Molecular Neurobiol. Unit (Chairperson); J. Feramisco*, Cold Spring Harbor Lab.; J. Brugge*, SUNY at Stony Brook; W.E. Wille, Univ. of Cologne; J. Morgan*, Roche Inst. Mol. Biol.; S.P. Hunt*, MRC Molecular Neurobiol. Unit.

Genetic probes derived from acutely-transforming viral oncogenes have demonstrated that homologous genes, "proto-oncogenes", are present in normal vertebrate genomes. To date, the roles of proto-oncogenes have been considered in relationship to normal and aberrant growth. Many proto-oncogenes are now recognised to be expressed in high levels in developing and mature brain, and to exhibit both acute and long-term changes in expression in response to external stimuli. Thus, proto-oncogenes may have a wider significance in neural cell function than in control of proliferation. This symposium will introduce three major classes of proto-oncogenes; GTP-binding proteins, tyrosine kinases, and nuclear proto-oncogenes, and discuss their neural expression and possible functions.

Dr Hanley will introduce the topics by a brief discussion of the identified oncogenes and their relationship to proto-oncogenes. Dr Feramisco and his colleagues have been studying the roles of the GTP-binding proteins, ras p21, in cellular proliferation and neuronal differentiation. Results from micro-injection of ras and its antisera, and single-cell functional studies will be discussed. Prof. Brugge will describe the cellular src gene product; a membrane associated tyrosine specific protein kinase that is expressed in high levels in neural tissue. The factors regulating the neuronal expression and kinase activity of the c-src gene product, and the possibility that c-src may play a regulatory role in neuronal cell function will be considered. Prof. Wille will present results on expression of the nuclear proto-oncogenes, c-myc and c-fos, in developing and mature brain; emphasising hippocampus and cerebellum. Data on in situ hybridization, sensitivity of c-fos to injury induction, specific c-fos stimulation in cerebellar neurones by taurine treatment, and the intracellular localization of the c-fos gene product will be discussed. Dr Morgan will review recent evidence for regulation of c-fos expression in cultured neurones and in vivo. These results will then be discussed within the framework of a larger model of transcriptional regulation in neurones. It will be proposed that activation of a family of immediate early genes, such as c-fos, by extracellular stimuli presents one phase of a previously unsuspected signal transduction cascade that may be involved in long-term neuronal responses. Dr Hunt will focus on observations that expression of immunoreactive c-fos protein can be induced in neurones of adult nervous system by a variety of stimuli, but that synaptic induction of c-fos expression has so far been found only within neurones of the spinal cord following sensory stimulation. The relationship between induced c-fos expression and neuronal plasticity will be considered.

100 **SYMPOSIUM. PEPTIDE-MONOAMINE INTERACTIONS: FROM MOLECULAR MECHANISMS TO BEHAVIOR.** T.Reisine, Univ. Pennsylvania (Chairperson); R.A.North, Inst. Adv. Biomed. Res.; P.Magistretti, Univ. Geneve; M-F.Chesselet, Med. Col. Pennsylvania; J.Crawley, Clin. Neurosci. Branch, NIMH.

Neurotransmitters have been shown to interact in the brain to regulate neuronal activity. The functional role of the interaction of neuropeptides with classical transmitters, such as monoamines, in the brain has been carried out at various levels of resolution, ranging from single cell to behavioral analysis. This symposium is a multidisciplinary approach to examine the recent advances in peptide-monoamine interactions in the brain. **Dr. North** will present recent electrophysiological findings on the interaction of the peptides, somatostatin and the opiates, with the monoamines, norepinephrine (NE), dopamine (DA) and serotonin. He will discuss the molecular mechanisms by which these transmitters regulate potassium conductance channels. **Dr. Reisine** will speak on the biochemical interaction of somatostatin and NE receptors in electrically excitable cells. This talk will emphasize the role of GTP binding proteins in coupling receptors to multiple effector systems such as adenylate cyclase and ionic conductance channels. **Dr. Magistretti** will speak of the interaction of VIP and NE in the cerebral cortex. He will discuss the convergence of neurons containing these substances in areas of the cortex and how these transmitters act in a synergistic fashion to regulate cell activity. Specifically, he will discuss the mechanism through which NE stimulates alphal-receptors to potentiate VIP-induced cAMP formation. **Dr. Chesselet** will speak about the interaction of DA neurons originating form the substantia nigra with striatal peptidergic interneurons and efferents. She will present evidence that the interaction of these neurons is topographically specific in the striatum. This will be shown by in situ hybridization studies revealing the effect of alterations of DA transmission on the levels of mRNA's coding for peptides in the striatum. Furthermore anatomical and pharmacological evidence supporting the existence of a dual control of DA neurons and peptide neurons in the substantia nigra and presynaptically, in the striatum will be discussed. **Dr. Crawley** will speak of the behavioral implications of the coexistence of CCK and DA in the mesolimbic pathway of rodents and primates. She will present studies in which microinjection of CCK into the nucleus accumbens, but not into the striatum, potentiates DA-induced hyperlocomotion in the rat. The ability of selective antagonists of the CCK receptor to block this facilitatory behavioral action on the DA system will be discussed in terms of the potential development of novel antipsychotic treatments.

VISUAL CORTEX II

101.1 SOME NEURONS IN THE ADULT CAT WHITE MATTER ARE SUBPLATE NEURONS. J.J.M. Chun and C.J. Shatz. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Subplate cells are the first postmitotic neurons of the neocortex, in the cat born on embryonic days 24-30 (gestation is 65 days) based on ^{3}H-thymidine studies. These cells are present in high density within the subplate during development, but then decrease in density during the first 2 postnatal months. During fetal and postnatal life, all ^{3}H-thymidine labeled subplate neurons are immunoreactive for the neuron-specific protein microtubule associated protein 2 (MAP2), as well as for GABA and the neuropeptides NPY, somatostatin and CCK. These immunophenotypes suggest that adult white matter cells observed in previous peptide immunohistochemical and Golgi studies may be the adult counterpart of the subplate neuron population.

To address this possibility, ^{3}H-thymidine was injected into three littermates at E27 to label those subplate neurons born on this day. Brains were then studied at postnatal day 49, 178 or 401 by using immunohistochemistry with the antisera mentioned above combined with autoradiography to identify the birthdated subplate neurons. At each age, all ^{3}H-thymidine labeled cells were MAP2 immunoreactive. These double-labeled cells were located in the subjacent white matter of all cortical areas examined. Some cells were also located at the base of cortical layer 6. No double-labeled (or ^{3}H-thymidine labeled) cells were seen in layer 1. The morphology of double-labeled cells varied from simple fusiform to more elaborate, multi-processed types. MAP2 immunostaining also revealed processes within white matter beneath gyri, some of which were probably dendrites belonging to the subplate neurons.

Somatostatin and GABA immunoreactive cells were also ^{3}H-thymidine labeled at each age. GABA double-labeled cells were the most common, occurring throughout the white matter and lowest extent of layer 6. Somatostatin double-labeled neurons were less frequently observed and tended to be located deep within or immediately subjacent to layer 6. No NPY or CCK double-labeled cells were found at any of the three ages studied, probably due to problems associated with sampling from these sparse populations.

These results confirm the suggestion that at least some of the adult white matter neurons are subplate neurons that persist into adulthood and show somatostatin or GABA immunoreactivity. At least some of the white matter neurons immunoreactive for NPY and CCK are also likely to be subplate neurons. These results further suggest that these earliest, peptide-immunoreactive neurons of the neocortex can exert their influence during development and into adulthood. We thank Drs. Evans, Luca, and Vallee for antibodies. Supported by NIH grants EY02858 to CJS and GM07365 to JJMC.

101.2 **INFUSION OF AN NMDA-RECEPTOR ANTAGONIST BLOCKS VISUALLY DRIVEN CORTICAL ACTIVITY IN CAT VISUAL CORTEX.** K.D. Miller, B. Chapman* and M.P. Stryker. Dept. of Neuroscience, Stanford University, Stanford, CA 94305, and Depts. of Neuroscience and Physiology, Univ. of California, San Francisco CA 94143.

Several lines of evidence from other laboratories suggest that the N-Methyl-D-Aspartate (NMDA) receptor (a subtype of glutamate receptor) may be involved in activity-dependent synaptic plasticity. Biophysical studies indicate that the receptor opens a conductance that passes Ca^{++} only in response to conjoint presynaptic activity and local post-synaptic depolarization. This suggests a possible molecular mechanism for a "Hebb"-type synapse in which conjoint pre- and post-synaptic activation (or depolarization) provides a signal resulting in potentiation of synaptic strength. In addition, studies in hippocampal slices have shown that NMDA receptor blockers will block some forms of long-term potentiation without apparent effect on the post-synaptic responses to single-pulse electrical activation of afferents.

To study the possible role of NMDA receptors in plasticity of visual cortex, we first chose to examine the effect of NMDA receptor blockers on normal visual activity. Cannulae (33 gauge; 200 μm diameter) were implanted in visual cortex of either adult cats or four-week old kittens. The cannulae were attached to minipumps containing 50 mM D,L-APV, pumping at a rate of 1 $\mu l/hr$. Multi-barrel glass electrodes were glued to tungsten recording electrodes. With this arrangement, we were able to assess the visually-driven activity, the threshold to NMDA iontophoresis, and the threshold to KA (kainic acid) iontophoresis at each recording site. Using the normal, untreated hemisphere as a control, we were able to ascertain in each animal whether the APV treatment had successfully and specifically blocked NMDA receptors without raising threshold to KA stimulation.

In animals studied 1-2 days after cannula implant, we have found that, in regions of cortex extending 2 mm and in some cases as far as 4 mm anterior to the cannula, such a specific block is achieved, and cortical activity is profoundly and often completely blocked. Partial blockades were evident in some cases at 5-6 mm. The activity block appeared to be lifted somewhat in animals studied after several days of APV exposure.

This result helps suggest an alternative interpretation of the role of NMDA receptors in cerebral cortex. Previous studies in other laboratories have shown that NMDA receptor activation results in a slow epsp; that APV, by blocking this slow epsp, can attenuate responses to high-frequency electrical stimulation in hippocampal slices; and that APV blocks responses to physiological, but not electrical, stimulation of afferents in rat ventrobasal thalamus. Together with our results, this suggests that a slow epsp mediated by NMDA receptors may be necessary for post-synaptic activation by the relatively weak, high-frequency, fast epsps associated with physiological afferent activity. Thus, NMDA receptors may play a role in regulating the temporal patterns of afferent activity that can successfully activate cortical cells. This hypothesis, if true, would not negate the hypothesis that passage of Ca^{++} through NMDA receptor-coupled channels provides a signal for plasticity; however, separating a specific plasticity signal from a more general effect on activity may present a difficult technical challenge.

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101.3 RESTORATION OF BINOCULAR VISION RETURNS GABA IMMUNOSTAINING TO NORMAL IN AREA 17 OF MONOCULARLY DEPRIVED ADULT MONKEYS. E.G. Jones and S.H.C. Hendry. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Monocular deprivation or enucleation in adult macaques reduces the density of GABA immunostained neurons in area 17. The reduction is most prominent in layer IVC of columns dominated by the deprived eye (Hendry and Jones, 1986; Nature 320:750). In this study, monocular deprivation was produced in young adult monkeys (Macaca fascicularis) either by suturing the lids of one eye closed for 4 months (one monkey) or by injecting the sodium channel blocker, tetrodotoxin (TTX) into one eye for 2 or 3 weeks (two monkeys). The monkeys were then anesthetized and a biopsy, approximately 1 cm^2 wide and 3 mm thick was surgically removed from the occipital operculum and fixed by immersion in 2% paraformaldehyde and 0.2% glutaraldehyde. The visual deprivation was terminated by re-opening the eyelids or by halting the TTX injections, after which the monkeys were allowed to survive for 4-6 weeks. They were then sacrificed by barbiturate overdose and transcardial perfusion with the same fixative as above. Sections through area 17 from the biopsies and from the brains later fixed in situ were stained histochemically for cytochrome oxidase (CO) and immunocytochemically for GABA. In the biopsies, CO staining reveals alternating light and dark bands in layers IVA and IVC corresponding to columns dominated by deprived and non-deprived eyes respectively. The difference in CO staining between columns is greater in the TTX-injected monkeys. GABA immunostaining in layer IVC follows precisely the pattern of CO staining, with deprived-eye columns containing a low density of immunostained somata and processes and non-deprived-eye columns containing a normal, high density of immunostained somata and processes. In all three monkeys, there was a return to normal CO staining and GABA immunostaining after restoration of binocular vision. The CO staining in layer IVC is uniform and a high density of GABA immunostained elements is found uniformly throughout this layer. Preliminary quantitative analyses indicate that the density of immunostained somata after restoration is normal. These results indicate that the effects of monocular deprivation on GABA immunostaining in area 17 of adult monkeys can be reversed by restoration of binocular vision. The data suggest that the levels of GABA within cortical neurons are dependent upon activity: deprivation reduces the GABA concentration to a level where it is not detectable by immunoctyochemistry, but a return to binocular vision quickly increases the GABA concentration and GABA immunostaining to normal levels.

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101.4 ACTIVITY DEPENDENT REGULATION OF TACHYKININ-LIKE IMMUNOREACTIVITY IN VISUAL CORTICAL NEURONS: INCREASED AND DECREASED IMMUNOSTAINING IN THE CYTOCHROME OXIDASE PATCHES OF MONOCULARLY APHAKIC MONKEYS. S.H.C. Hendry, E.G. Jones and N. Burstein*. Departments of Anatomy and Neurobiology and Ophthalmology, University of California, Irvine, CA 92717.

Monocular deprivation or enucleation reduces GABA and glutamic acid decarboxylase (GAD) immunostaining in neurons of adult monkey area 17 (Hendry and Jones, 1986; Nature 320:750). Many of the GABA neurons also display immunostaining for tachykinin-like substances (Jones and Hendry, 1985; Soc. Neurosci. Abstr. 11:83) and this staining is reduced by manipulations that either reduce the levels of light entering the eye or eliminate retinal activity (Hendry and Jones; Soc. Neurosci. Abstr. 11:16). In the present study, removal of the crystalline lens was used to impair pattern vision in one eye without reducing the level of light falling upon the retina. Three weeks, 3 months or 6 months following the surgical removal of one lens, monkeys (Macaca fascicularis) were sacrificed and blocks of area 17 were prepared for histochemical localization of cytochrome oxidase (CO) and immunocytochemical localization of tachykinins with a monoclonal antibody that recognizes substance P and other members of this neuropeptide family. Monocular aphakia produces little effect on CO staining of layer IVC but the rows of CO patches in layers II-III, IVB and V-VI exhibit marked changes, with rows at the centers of both the deprived and non-deprived eye columns being affected. In every other row, dominated by the aphakic eye, the patches shrink and become paler; in the alternating rows, dominated by the normal eye, the patches are no longer discrete, but widen and fuse to form expanded and continuous rows. These findings suggest that in non-deprived eye columns, neuronal activity is elevated within the regions that normally lie between CO patches. Tachykinin immunostaining is normally densest within the CO patches and equal staining is seen in each row of patches. However, in aphakic monkeys the immunostaining in the two sets of rows is uneven and follows precisely the pattern of CO staining. In rows of patches dominated by the aphakic eye, tachykinin immunostained somata and processes are restricted to the shrunken CO-stained patches, while in rows of patches dominated by the normal eye, high densities of immunostained somata and processes are present along the full length and width of the expanded patches. Quantitative analyses indicate the density of tachykinin somata is reduced by 60% in the deprived-eye rows and increased by 30% in the expanded rows; the density of the total (thionin-stained) population of neurons is not affected in either row. These results suggest that tachykinin-like immunoreactivity within single cortical neurons can be up- and down-regulated by activity.

Supported by NIH Grants EY 06432 and NS 21377.

101.5 DISTRIBUTION OF MAJOR NEUROTRANSMITTER RECEPTORS IN THE VISUAL CORTEX OF MONKEYS DEVOID OF RETINAL INPUT FROM EARLY EMBRYONIC STAGES. P. Rakic, M. Kritzer and D. Gallager, Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT 06510.

Recently, we demonstrated that ten major neurotransmitter receptors in areas 17 and 18 of adult rhesus monkeys display laminar and regional binding properties that in some instances correlate with the pattern of cellular layers and synaptic input-output relationships in these areas (Rakic et al., '87, J. Neurosci.; Kritzer et al., '87, J. Comp. Neurol.). In the present study, we examined the binding distributions of these same receptors in the visual cortex of animals that were binocularly enucleated at embryonic (E) days E63 and E81, prior to the ingrowth of geniculocortical fibers to the developing cortical plate (Rakic, '76, Nature, 261:467), delivered at term (E165) and sacrificed at 2 months. In vitro receptor autoradiography was performed using ligands selective for alpha-1, alpha-2 and beta adrenergic, serotonergic ($5HT_1$ and $5HT_2$), cholinergic, dopaminergic (D-2), GABAergic (GABA-A; muscimol and benzodiazepine) and cholecystokinin (CCK) binding sites. Nonspecific binding was assessed in the presence of appropriate unlabeled agonists or antagonists, and autoradiograms were examined using computer-assisted densitometry.

In both enucleates and age-matched controls each ligand demonstrated a unique laminar pattern, which changed abruptly at the border between areas 17 and 18. Consistent with cytoarchitectonic findings (Rakic and Williams, '86, Abst. Soc. Neurosci.), the pattern of labeling characteristic for area 17 occupied significantly less surface area in enucleates than in age-matched controls. Laminar specificity of ligand binding in area 17 was basically similar to that observed in controls. Although some blurring of stratified binding was observed among sublaminae of layer IV in autoradiograms generated with ligands selective for CCKergic, serotonergic, and alpha-1 adrenergic receptors, preservation of the basic laminar pattern was found in remaining cortical layers of area 17 and all layers of area 18. Thus, while a certain level of fine tuning for some receptor types in thalamorecipient layers may depend on normal or functionally active thalamic input, regional differences and characteristic laminar distribution of receptors in areas 17/18 develop in basically a normal fashion despite the absence of information from the periphery. These results are in harmony with our findings of normal cortical thickness, patterns of cellular laminae (Rakic and Williams, '86, Abst. Soc. Neurosci.), and normal synaptic density in early enucleated animals (Bourgeois and Rakic, '87, Abst. Soc. Neurosci.). Supported by NS22807 and EY02593.

101.6 DEVELOPMENTAL STUDIES OF SUBSTANCE P AND NEUROPEPTIDE Y NEURONS IN MONKEY VISUAL CORTEX. R. Mehra* and A. Hendrickson. Depts. Ophthalmology and Biological Structure, Univ. Washington, Seattle WA 98195

The localization pattern of two neural peptides, substance P (SP) and neuropeptide Y (NPY) was followed during development of Macaca monkey striate cortex, using light and EM immunocytochemistry. Ages studied ranged from 90 fetal days (F90) through the first 12 postnatal (PN) wks and the adult. Both neural antigens were localized to perikaryal cytoplasm, dendrites, and axonal processes including synaptic terminals. Although both peptides were present at all ages, considerable differences existed between neurons in different laminae as to the time of appearance of the antigens.

SP+ cell bodies were found deep to the cortical plate at F90; these were vertically-oriented, immature-appearing neurons, probably in the process of migration to superficial cortical layers. From late gestation to early PN ages, SP+ cells were found in II/III and V. At the border between layers V and VI, a well defined SP+ fiber plexus containing SP+ cell bodies was seen up to the first PN wk. SP+ axons of many of these cells could be traced to superficial, cortical layers, while dendrites arborized mostly in the neighborhood of the perikaryon. This layer V plexus became progressively less intensely stained after birth, until it was nonreactive by 12 PN wks. In contrast, SP+ cells in the white matter are not stained until after birth; these increase in intensity up to 12 PN wks and remain in the adult brain. Double-labeling experiments indicate many SP+ neurons in II/III, V, and white matter also contain GABA in PN cortex.

NPY+ neurons are located in layer I and deep to the cortical plate at F90. Layer I cells lose their immunoreactivity by 1 PN wk. NPY+ neurons appear in II/III by F113, and continue in this position to adult. Typical horizontal NPY+ neurons in the white matter appear in late gestation. By 1 PN wk, NPY+ axons from layer II/III cells descend to deeper cortical layers, giving collaterals at different sites, while axons of NPY+ white matter horizontal cells send their axons to superficial cortical layers. Double-label experiments show that both NPY+ populations contain some cells which also stain for SP+ in the adult. Hendry et al ('84) has previously shown that most NPY+ neurons also are GABAergic.

The functional significance of transitory or delayed peptide expression is not understood. Experiments are underway to determine whether these transient populations die, or remain but stop synthesizing peptides. Possibly these peptides have a trophic or modulatory influence on surrounding neurons during development; in particular the early appearance of both peptides long before significant numbers of synapses occur suggests such a role. EM studies are underway to determine the pattern of connections formed by SP+ and NPY+ neurons during cortical development.

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101.7 NEUROPEPTIDE Y-CONTAINING NEURONS ARE SITUATED PREDOMINANTLY OUTSIDE CYTOCHROME OXIDASE-POSITIVE BLOBS IN LAYERS II-III OF MACAQUE STRIATE CORTEX. R.O. Kuljis and P. Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510-8001.

Neuropeptide Y (NPY) is present in a small population of local circuit neurons in the striate cortex. In layers II-III, NPY-containing somata (NPYcs) tend to aggregate in loose clusters of 3-6 cells (Hendry et al., J. Neurosci. 4: 2497; Wahle et al., Exp. Brain Res. 61: 364). We analyzed whether the distribution of NPYcs was related to cytochrome oxidase (CO)-containing parcellations in layers II-III of the opercular region.

Four macaque monkeys were anesthetized and perfused with 4% paraformaldehyde, alone or with 0.08% glutaraldehyde and 15% saturated picric acid added. NPY-like immunoreactivity was revealed by the avidin-biotin-peroxidase technique (Hsu et al., J. Histochem. Cytochem. 29: 577). A histochemical procedure was used to reveal CO activity (Wong-Riley, Brain Res. 171:11). Computer-assisted plots of adjacent sections labeled for NPY or CO - and sections double-labeled for NPY and CO - were used to perform 3-dimensional computerized reconstructions of the distribution of 606 NPYcs (6 series of 6-20 serial sections). NPYcs display a low average density in layers II-III (30 cells/mm^3), with no apparent pattern in their spatial distribution. Most cells were found **outside** the CO-containing blobs (n= 513; 84.7%); 77 cells (12.7%) were found in the boundary between CO-rich and CO-poor zones. Only 16 NPYcs (2.6%) were found in the CO blobs, which comprise 35% of the volume of layers II-III. The uneven distribution of NPYcs in relation to CO compartments is highly significant (X^2= 366; A= <0.001; df= >30).

Most cells in the CO-rich blobs of layers II-III are color-coded, while CO-poor regions - where NPYcs are situated - contain many orientation-selective cells (Livingstone and Hubel, J. Neurosci. 4: 309). The present findings indicate that the circuits supporting orientation vs color selectivity may differ not only in their input-output relationships, but also in the transmitter/modulators their local circuit neurons contain.

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101.8 **THE BIPOLAR CELL IN RAT VISUAL CORTEX.** A. Peters. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

The bipolar cell of rat visual cortex has achieved prominence because it is visualized by a number of antibodies to neuroactive compounds, including antibodies to vasoactive intestinal polypeptide (VIP), cholecystokinin, choline acetyltransferase, and to α-aminobutyric acid (GABA). Reports in the literature show that the axon terminals labelled by these antibodies form symmetric synapses. Yet earlier Golgi-electron microscopic studies of bipolar cells in rat cortex showed them to have axons forming asymmetric synapses (Peters and Kimerer, J. Neurocytol. 10:921). In an attempt to resolve these conflicting data, bipolar cells in rat visual cortex have been examined further by using additional cells some prepared for Golgi-electron microscopy and others reacted with antibodies to VIP.

Additional Golgi-electron microscopic studies show that there are two types of bipolar cells. As previously described one type has axons forming asymmetric synapses, and these neurons most commonly synapse with dendritic spines. The other bipolar cell type has axons forming symmetric synapses, and these neurons preferentially synapse with dendritic shafts. This latter population must include these bipolar cells which label with the above mentioned antibodies, for the axons of some VIP labelled bipolar cells have been traced from cell bodies and shown to form symmetric synapses. Like the Golgi impregnated bipolar cells forming symmetric synapses, dendritic shafts are the most common postsynaptic element for VIP positive axon terminals, although some VIP labelled terminals synapse with cell bodies. Analysis of tissue labelled with VIP antibody shows that about 50 per cent of bipolar cells are VIP positive, and since about 80 per cent of all bipolar cells are GABA-positive, it is suggested that many VIP positive bipolar cells also contain GABA. The transmitter used by those bipolar cells with axons forming asymmetric synapses is unknown. Supported by NIH research grant NS 07016.

101.9 GABA RECEPTOR COMPONENTS IN VISUAL CORTEX OF CAT AND MACAQUE. D. Parkinson and E. Coscia* Dept. Cell Biol. & Physiol., Washington University Medical School, St. Louis, Mo

GABA is one of the major inhibitory neurotransmitters in the cerebral cortex. In visual cortex, GABAergic mechanisms contribute to the orientation and direction specificity of visually driven cells, as well as regulation of binocular input. Most of these conclusions are based on work with the GABA-A antagonist, bicuculline. Two types of GABA receptors have been identified. The GABA-A receptor is made up from three major components;the agonist binding site, the BZD binding site, and the chloride channel. The GABA-B receptor modulates adenylate cyclase activity and is selectively activated by GABA or baclofen. We used binding assays to measure the abundance of GABA receptor components and then receptor autoradiography to visualise their distributions across cortical layers.

[3H]Muscimol was used to label GABA-A agonist sites. Binding was saturable, of high affinity (Kd=9nM) and was potently inhibited by GABA and bicuculline, but not Ro 15 1788 or picrotoxin. The binding of the BZD antagonist, [3H]Ro 15 1788, was also saturable, of high affinity (Kd=1nM) and inhibited by clonazepam but not GABA. Binding of [35S]TBPS to the chloride channel required the presence of chloride ion, was saturable, of high affinity (Kd=12nM) and was inhibited by GABA and picrotoxin but not bicuculline. Bicuculline antagonised the inhibition seen with GABA but not with picrotoxin. Binding of [3H]baclofen was saturable (Kd=50nM), calcium-dependent and inhibited by GABA. The maximum number of sites for these ligands was measured. GABA-B sites were the least abundant (0.09-0.14 pmoles/mg.prot.). The concentrations of GABA-A sites and BZD sites were similar at 1.2-1.9 and 1.0-1.4 pmoles/mg. prot., respectively. The number of TBPS sites was about one third of these levels (0.3-0.6 pmoles/mg. prot.). The autoradiography patterns seen with [3H]muscimol and [3H]Ro 15 1788 were very similar. In cat visual cortex, both sites were present in all layers, highest in layers I-IV, intermediate in layer VI and lowest in layer V. In macaque, the pattern was different; specific label was seen in all layers, highest in layer IVc, intermediate in layer VI and a band composed of layers I-III. [35S]TBPS sites were seen in layers IV and VI in cat, but not in layers I-III. In macaque, the highest labelling was in layer VI and to a much lower level in layer IVc. No evidence was seen in macaque of association of these sites with "patches".

These results show that the three components of the GABA-A receptor complex are all present in cat and macaque visual cortex, but may not always be associated in a GABA-A/BZD/chloride ionophore complex. Whether there are complexes composed of GABA-A and BZD receptors, that can modulate neuronal activity, remains to be determined. Supported by NIH grant EY5904

101.10 Morphologically Distinct Subsets of GABAergic Neurons in Cat Area 17 Have Unique Patterns of Glycosylated Molecules On Their Surfaces. J. R. Naegele, L. C. Katz and C. J. Barnstable. Lab of Neurobiology, The Rockefeller University, NY, NY. 10021

The GABAergic neurons in the visual cortex form a morphologically and chemically heterogeneous group. A subpopulation of GABA cells were recently identified by their unique surface molecules, recognized by monoclonal antibodies VC1.1 and VC5.1 (J. Neurosci. 7: 1250, 1987; Soc. Neurosci. Abst. 12: 582, 1986). Here, we have asked whether the expression of particular cell surface molecules is related to characteristic morphological properties in these neurons.

Monoclonal antibodies and plant lectins were used to identify subsets of GABAergic neurons in cat area 17; these included VC1.1 and the lectin, VVA. VC1.1 recognizes a triplet of polypeptides with M_r 95-105 kd, 145 kd and 170 kd on immunoblots of polyacrylamide gels. VVA shows highly specific binding to terminal N-acetylgalactosamine residues. Both molecular probes revealed a meshwork pattern which enveloped the cell bodies and dendrites of a subpopulation of nonpyramidal neurons scattered through layers II-VI of Area 17. Immunocytochemical double-labeling with an antiserum to GABA, revealed that nearly all the VC1.1 or VVA-stained neurons were immunoreactive to GABA. VC1.1 positive cells were morphologically heterogeneous and included bitufted and multipolar varieties. VVA positive cells were a more homogeneous group and closely resembled the large Basket cell class of cortical interneurons. Neither reagent labeled axons which limited more precise identification of their morphological subclasses.

To obtain this information, we prepared living slices from cat visual cortex and injected cells intracellularly with Lucifer Yellow (J. Neurosci. 7: 1223, 1987). The slices were then fixed, sectioned, incubated in VVA and visualized using peroxidase immunohistochemistry. When sequentially viewed with epi-fluorescent and bright-field illumination, a subset of the intracellularly-filled neurons were labeled by VVA. Fifty to 70 cells were injected per experiment and of these, approximately 5% were double-labeled with the lectin. Of the double-labeled cells recovered so far, all were morphologically similar, with extensive and highly varicose dendrites. None of the other kinds of neurons intracellularly stained, which included spiny pyramids and sparsely spiny varieties, were labeled by the lectin.

These studies have identified a unique subset of GABAergic neurons with a distinct topography of glycosylated molecules across their surfaces. By this combined approach, we are beginning to link molecular composition to cellular architecture in specific subsets of cortical neurons.

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101.11 NICOTINIC CHOLINERGIC INPUT TO CAT PRIMARY VISUAL CORTEX. Nigel W. Daw, Kenneth E. Kratz and David Parkinson Dept. Cell Biology, Washington Univ. Med. School. St Louis, MO 63110.

Last year we reported that, in area 17 of the cat cerebral cortex, ^{3}H-Nicotine binds almost exclusively to layer IV (Parkinson and Daw, Soc. Neurosci. Abstr. 12: 162.1). This observation raised two questions: whether the binding was located on geniculocortical afferents, and whether the ^{3}H-nicotinic binding sites represent a physiologically relevant cholinergic receptor. We tackled the first question by making lesions in the lateral geniculate nucleus and the second by iontophoretic techniques.

To determine the location of the binding, we made lesions in the lateral geniculate nucleus. An electrode was angled at 20° to the vertical in the parasaggital plane, to pass through the same part of the field of view in all layers of the geniculate, and a lesion of approximately 1mm diameter was made in all layers. Two to four weeks later the animal was sacrificed, and the cortex frozen and sectioned for autoradiography. A gap was found in the nicotinic binding in layer IV of primary visual cortex. The location coincided with the loss of visual input to primary visual cortex determined elecrophysiologically. Staining with cytochrome oxidase revealed a gap in the same location. The pattern of labelling seen with ligands binding to the following receptors was not affected: muscarinic, GABA-A, benzodiazepine, and the GABA chloride channel.

To look for nicotinic effects on the activity of cells in the cortex, we recorded from single cells and iontophoresed acetylcholine, atropine, mecamylamine and hexamethonium. Results were obtained from 21 cells. In 17 of these cells the effect of acetylcholine was excitatory and/or the effect of an acetylcholine antagonist was inhibitory. In the other four acetylcholine had an inhibitory effect and/or an antagonist had an excitatory effect. Nicotinic antagonists were applied to 14 of these cells. In 7 cases activity was reduced by mecamylamine, usually both spontaneous and light-driven activity. In 6 of these cells atropine also reduced the activity: in the other case there was a slight increase in activity with atropine. Hexamethonium was applied to 6 cells, but a definite effect was not seen.

We conclude that there are nicotinic receptors in primary visual cortex; that these receptors appear to be located on the terminals of afferent fibers; and that the activity of cells in layer IV can be affected by nicotinic antagonists. It seems likely, therefore, that acetylcholine fibers have a nicotinic influence on primary afferent fibers at the level of their entry into the visual cortex.

101.12 THE NORADRENALINE, DOPAMINE AND SEROTONIN INNERVATION OF THE RAT VISUAL CORTEX. J.G. Parnavelas*[1], G.C. Papadopoulos*[2] and R.M. Buijs*[3] (SPON: T. Harrison). [1]Dept. of Anatomy, University College London, London WC1, U.K., [2]Veterinary School, University of Thessaloniki, Greece and [3]Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

The monoaminergic innervation of the rat visual cortex was examined by light and electron microscope immunocytochemistry using antibodies against noradrenaline (NA), dopamine (DA) and serotonin (5-HT).

Observations with the light microscope confirmed earlier reports concerning the density and distribution pattern of the noradrenergic fibers in the rat neocortex. Briefly, fibers running parallel to the pia predominate in layers I and VI, relatively straight radial fibers traverse layers II & III, and short tortuous or obliquely oriented axons prevail in layers IV and V. In addition, detailed examination revealed the striking presence of NA-immunoreactive fibers running in the mediolateral direction and "oscillating" regularly between layers II through IV. The DA innervation of the visual cortex was characterized by the differential density of labelled fibers within individual visual areas. Thus, area 18 contained a modest density of DA-labelled axons but areas 17 and 18a were sparsely innervated. All layers of area 18 appeared to be innervated by collateral branches emanating from fibers ascending from layer VI to layer I. These axons were thin and tortuous bearing irregularly spaced varicosities. The 5-HT innervation of the visual cortex appeared dense in all layers. Most axons, bearing numerous intensely-stained varicosities of various sizes, followed a tortuous path.

The ultrastructural features of the monoaminergic terminals in the cerebral cortex have been the subject of controversy in recent years. Systematic analysis of serial ultrathin sections, immunocytochemically stained with antibodies against NA, DA or 5-HT, has shown that nearly all stained terminals formed synapses characterized by specialized junctional appositions. These findings suggest that monoamines exert their influence in the cortex through conventional synaptic junctions.

CHEMICAL SENSORY SYSTEMS I

102.1 RAT ORAL TASTE SYSTEMS: COMPARISON WITH HUMAN. J.C. Boudreau. Sensory Sciences Center. Graduate School of Biomedical Sciences. University of Texas at Houston. Texas, 77030.

Almost all mammalian taste research is directed toward rodents and the results are usually interpreted in terms of an assumed human taste model. In our studies, oral taste systems of the rat were investigated by recording spikes from single sensory neurons in the rat geniculate ganglion (GG) and petrosal ganglion (PG). Utilizing stimulus-response measures, the neurons were parcelled into six different groups (GG salt, PG salt, GG acid, PG acid, Sugar-amino acid and X units--alkaloid and alkaloid plus). Only the latter two neural groups of units were found in both ganglia. On the basis of active stimuli, these neural groups were compared to humans where elicited sensations were utilized. Only the GG salt units (Na responsive) seem to clearly have a human counterpart. GG acid units of the rat were activated by a more restricted group of acids than those eliciting a sour sensation, and in addition, these rat units also fired to salts, which do not elicit a sour sensation. No clear human sensation exists as a counterpart for the rat PG salt and PG acid systems. No neural response was shown to glutamate (umami sensation) apart from GG salt units which fire to Na. No nucleotide responsive system has been found in the rat. Although some X units respond to a narrow range of alkaloids (atropine best), most bitter compounds are relatively inactive or inhibitory. No rat (or any other mammalian) excitatory neural analog exists for a general human bitter taste. The sugar-amino acid units do not discharge to some amino acids humans find sweet, nor do they innervate the front of the tongue. Little rat response has been shown to many of the intense sweeteners. Other human sensations (e.g., metallic) have been inadequately characterized to compare with rat units. Although the rat is frequently depicted in terms of a four basic taste model, this model and its accompanying simplistic chemistry improperly characterize the rat (and human). Since the rat's taste systems differ markedly from those of the human, it can be assumed that so also do its nutritional and metabolic systems.

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102.2 TASTE UNITS RESPONDING BEST TO NACL, SUCROSE OR KCL ARE FOUND IN SEPARATE LOCATIONS WITHIN THE SOLITARY NUCLEUS OF THE HAMSTER. M. McPheeters, S.C.Nuding*, T.P. Hettinger* and M.E. Frank. Dept. of BioStructure and Function, University of Connecticut Health Center, Farmington, CT 06032.

In mammals, the solitary nucleus is the first taste relay in the brain. Taste information from the tongue is carried via cranial nerves VII, IX, and X which synapse on second-order taste neurons in the solitary nucleus of the medulla. The information is then relayed to the parabrachial nucleus in the midbrain or to the thalamus. Knowledge of the physiological and anatomical properties of the solitary nucleus is necessary to understand the processing of taste information at this level. We are studying the response properties of single cells within the solitary nucleus in relation to their location.

We record from single taste responsive units in the solitary nucleus of of hamsters (Mesocricetus auratus) with glass microelectrodes filled with 0.5M KCl and 4% HRP. The rostral pole of the nucleus which receives afferent input from the anterior tongue via the chorda tympani is approximately 500 x 500 x 250um. Each unit is characterized by its response to NaCl (0.03M), sucrose (0.1M) and KCl (0.1M) presented to the anterior tongue. These chemicals identify three distinct populations of afferent inputs to the solitary nucleus. A unit is then classified as NaCl, sucrose or KCl best based on which stimulus evoked the maximal response measured by highest spike frequency. The location of the unit is marked with a small iontophoretic injection of HRP. Following sectioning of the brain and histological processing to visualize the location of the HRP reaction product (50 um in diameter), a three-dimensional reconstruction of the medulla is made either with clear plastic sheets or by computer simulation. We are then able to clearly see the location of each functionally identified unit within a model of the solitary nucleus. NaCl units tend to cluster in one location, sucrose units in another and KCl units in yet a third location. Thus we conclude that there exists a spatial organization of taste qualities at the second-order neuron level. This finding suggests that the organization of the gustatory system may be analogous to other lemniscal sensory systems.

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102.3 L-ARGININE-ACTIVATED CATION CHANNELS FROM THE CATFISH TASTE EPITHELIUM. J.H. Teeter and J.G. Brand* (SPON: R. Bruch). Monell Chemical Senses Center, University of Pennsylvania, and Veterans Administration Medical Center, Philadelphia, PA 19104.

L-Arginine is a highly effective stimulus for the taste system of the channel catfish (Ictalurus punctatus) in electrophysiological and behavioral experiments. In addition, binding studies using a sedimentable fraction from catfish taste epithelia (Fraction P2; Krueger and Cagan, J. Biol. Chem. 251:88, 1976) display specific binding of L-arginine with affinities of 10^{-7} and 10^{-5} M.

We have incorporated membrane fragments from a nucleotidase-enriched portion of Fraction P2 into artificial phospholipid bilayers (soybean phospholipids) formed over the tips of patch pipettes. Single-channel records revealed a variety of current fluctuations which resembled openings and closings of ion channels. Most of the channel activity was voltage-dependent and slope conductances ranging from 8 pS to 230 pS were observed. In 7 of 14 patches, which showed no channel activity at potentials between -100 mV and +100 mV, concentrations of L-arginine greater than 1 µM elicited single channel fluctuations. The amplitude of the open channel current varied approximately linearly with voltage between -80 mV and +80 mV, corresponding to a slope conductance of about 40 pS. Both individual openings and bursts of openings lasting several seconds were recorded. The frequency of openings increased with increasing concentrations of L-arginine (1-100 µM). The L-arginine-activated currents reversed at about 0 mV (-8 mV to +12 mV) with Ringer in the bath (mM: 110 NaCl, 2.5 KCl, 1.6 $MgCl_2$, 1 $CaCl_2$. 5 HEPES, pH 7.4) and a pseudo-intracellular solution in the pipette (85 KCl, 12.5 NaCl, 1.6 $MgCl_2$, 0.25 $CaCl_2$, 0.5 EGTA, 5 HEPES), indicating that the channel was cation selective with similar permeability to Na^+ and K^+. L-Arginine-activated channels were not observed in preparations derived from previously frozen samples of Fraction P2, which exhibit little specific binding of L-arginine. However, spontaneous channel activity was frequently observed in previously frozen preparations.

These results demonstrate the presence of L-arginine-activated cation channels in membranes from the catfish taste epithelium. Although modulation of these channels occurred in the absence of added second messengers or substrates, suggesting that L-arginine may directly gate channel openings, it is possible that sufficient second messenger substrate was present in the preparation to mediate the observed responses.

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102.4 **ADENYLATE CYCLASE AND GTP BINDING PROTEIN IN RAT SWEET TASTE TRANSDUCTION.** Doron Lancet§, Benjamin J. Striem*†, Umberto Pace*§, Uri Zehavi*† and Michael Naim*† (SPON: Sara Fuchs). †Dept. of Biochemistry and Human Nutrition, Faculty of Agriculture of the Hebrew University of Jerusalem, Rehovot, Israel, and §Dept. of Membrane Research, the Weizmann Institute, Rehovot, Israel.

The response of vertebrate olfactory epithelial neurons to many odorants has been shown to involve activation of adenylate cyclase via a stimulatory GTP-binding protein (olfactory G_s) (Lancet and Pace, Trends Biochem. Sci. 12:63, 1987). Taste transduction has long been proposed to be similarly mediated by cyclic nucleotides, but direct evidence has been lacking. Such evidence is provided here through the demonstration that rat lingual adenylate cyclase is activated by sweet tasting compounds. Membranes were prepared from the anterior dorsal rat tongue (tip), rich in fungiform papillae, by polytron homogenization in hypotonic buffer, followed by differential velocity centrifugation. Tip membranes, but not control ones from a non-sensory tongue region, tongue muscle or olfactory cilia, showed a significant (91±14%) elevation of adenylate cyclase activity by 1.0M sucrose. cAMP production was dose-dependent (8-142% enhancement for the physiological range of 0.2-1.2M sucrose) and required the presence of GTP or its non-hydrolysable analogue GTPγS. The latter observation suggests that a G_s-like protein couples sweet taste protein receptors and adenylate cyclase. Other disaccharides, all eliciting appealing taste in rats, were also activatory. Maltose was roughly a half as effective as sucrose, in agreement with with its potency relative to sucrose in eliciting chorda tympani nerve electrophysiological responses (Hagstrom and Pfaffmann, J. Comp. Physiol. Psychol. 52:259, 1959). Non-sugar sweeteners, aspartame and neohesperidine dihydrochalcone (NHD), which are essentially non-tasting for rats, did not significantly affect adenylate cyclase levels in tip membranes. Saccharin, a strongly appealing compound, gave about 100% enhancement at 20mM, but such activation was observed also in muscle membranes, and its specificity needs yet to be established. Adenylate cyclase measurements in tongue membranes could thus be used to assess the effectiveness and kinetics of sweet-tasting compounds in different species. Our results suggest that sweet disaccharides induce an elevation of intracellular cAMP in taste cells. This could give rise to the documented membrane depolarization by modulating ion channel conductance directly (as in olfactory cilia) or indirectly, via cAMP-dependent protein phosphorylation. The second possibility is supported by recent patch clamp recordings in isolated frog taste cells (Avenet, Hofmann and Lindemann, Pflugers Arch. 408:R35, 1987).

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102.5 MECHANISM OF TRANSDUCTION OF BITTER TASTE IN RAT TASTE BUD CELLS. M.H.Akabas*, J.Dodd & Q.Al-Awqati* (SPON: J.Ripellino). Dept. of Medicine & Physiol., Columbia Univ., New York, N.Y. 10032

Since most poisonous plant alkaloids are intensely bitter, taste permits animals to avoid potentially toxic foods. We have studied the mechanism of bitter taste transduction in rat taste cells. We developed a procedure to dissociate the rat lingual epithelium into single cells and to identify the taste cells using a fluorescently labelled antibody. Using fura 2, to measure single-cell intracellular calcium levels, and patch clamping, we have characterized the response of the taste cells to denatonium, the most bitter substance known. In a small subset of taste cells, denatonium induced a rise in intracellular calcium due to release from intracellular stores. Potassium induced depolarization did not alter cell calcium levels. This implies that voltage-dependent calcium channels are not involved in the transduction process.

Using patch clamp recording, delayed rectifier K^+ currents have been identified in the taste cells. Single channel studies have shown the presence of two types of K^+ channels, a voltage-dependent channel and a calcium-dependent channel. Using cell-attached patch clamping, we have shown that denatonium induces the closure of the voltage-dependent K^+ channels in a small subset of the cells. Since denatonium has no direct access to these channels, its effects must be mediated by a second messenger. The role of channel closure in the transduction process is unclear, however, since K^+ induced depolarization had no effect on cell calcium.

These results suggest that bitter taste transduction occurs via a biochemical pathway, not an electrophysiologic one. The initial event involves binding of a bitter substance to an apical cell surface receptor. This interaction leads to the generation of an intracellular second messenger which causes release of calcium from intracellular stores and the closure of a class of K^+ channels. Presumably, these events lead to neurotransmitter release.

102.6 ODORANT- AND SECRETAGOGUE-INDUCED CHANGES IN THE CATION CONCENTRATION OF OLFACTORY MUCUS. H. Joshi*, M.L. Getchell*, B. Zielienski* and T.V. Getchell. Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48236.

Atomic emission and absorption spectrophotometry was used to determine [Na], [K] and [Ca] in olfactory mucus of grassfrogs. Samples were obtained at 10-min intervals for 70 min using a modification of the "filter paper" technique. In untreated frogs, the mean (N=72) ion concentrations (mEq/L) were: Na, 52.7 ± 4.1; K, 10.6 ± 1.9 and Ca, 10.7 ± 1.7. These concentrations did not change appreciably during the 70 min period. This stability suggested that representative mucus samples were obtained without substantial cellular or extracellular fluid contamination. The odorant 1,8-cineole was delivered to the epithelial surface at 10, 20 and 30 min after an initial sample was obtained by moistening filter paper with 0.4 ul of 1 mM cineole in deionized water. [Na] increased transiently to 88.3 ± mEq/L at t-30 followed by a systematic decrease to within 15% of initial values. The changes in [Na] were statistically significant ($P<0.001$). Similar results were obtained for [Ca], but not [K], that were significantly different ($P<0.02$) from control values. The secretagogues methacholine (5 mg/ml, topical) and isoproterenol (30 mg/kg, i.p.) caused transient increases in [Na] at t-20 to 68.1 ± 26.4 mEq/L for the cholinergic agonist and 58.8 ± 28.4 mEq/L for the B-adrenergic agonist; due to the high variability in the data as evidenced by the standard deviations, mean values were not significantly different from control values. The [K] and [Ca] were not affected significantly. Histological examination of tissue from experimental animals showed signs of mucus and electrolyte/water secretion from sustentacular cells that included formation of apical domes and vacuoles and from acinar cells that included secretory granule depletion. Effects ranging from disaggregation of the mucociliary matrix to partial deciliation were also observed. The results show that the cation concentrations in frog olfactory mucus resemble those found in human nasal and tracheobronchial secretions, perilymph of the inner ear and gastrointestinal mucus. The transient increases in [Na] and [Ca] induced by the application of an odorant and secretagogues reflect secretory activity associated with perireceptor events. Supported by NSF-BNS-07949 (MLG) and NIH-NS-16340 (TVG).

102.7 PROPERTIES OF VOLTAGE DEPENDENT IONIC CURRENTS IN ISOLATED SALMON OLFACTORY RECEPTOR CELLS Gabrielle Nevitt, Department of Zoology, University of Washington, Seattle, Washington, 98195

Salmon offer a unique opportunity to study the biophysical basis of olfaction as it relates to behavior. Their keen ability to discriminate between specific chemicals by smell has been shown to play a key role in homing as well as predator avoidance. Here, I present a characterization of ionic currents present in olfactory receptor cells of Coho salmon (Oncorhynchus kisutch) using the whole-cell recording technique of Hamil, et al (1981).

Receptor cells were isolated from 2 year old fish by gently agitating rosette tissue in 0 Ca/0 Mg ringer, followed by an incubation in papain (.5 mg/ml / 15 min). Ciliated rod receptor cells retained their morphology and were easily recognizable. 10-20 G Ohm seals were routinely obtained, and in cell-attached configuration, spontanious action potentials were frequently observed.

Under voltage clamp recording conditions, both inward and outward currents were observed. Transient inward currents occurred in response to depolarizations from holding potentials of -60 to -120 mV. Amplitudes ranged from 200 to 700 pA and peak currents occurred at voltages between -5 and -30 mV. Peak currents activated within 1 ms and ½ inactivation times were of the order of 3 ms. This current is most likely carried by Na ions because it was blocked by TTX, and absent when choline replaced Na in the external ringer. Using CsCl-filled pipets and 10 mM Sr substitution for 3 mM Ca externally, a second inward current was observed during depolarizations from a holding potential of -40 mV. Peak current occurred at voltages between +5 and +15 mV. This current did not inactivate during an 800 ms pulse, and completely disappeared within 3-6 min following the establishment of whole-cell configuration. It is likely that this current is carried by Sr ions passing through Ca channels.

In addition to inward currents, an outward Ca-activated K current occurred in response to depolarizations from holding potentials of -120 to -40 mV. Amplitudes ranged from 200 to 1000 pA with peak currents occurring in a similar voltage range to the peak Ca current. This current was not present when Sr replaced Ca in the external ringer. Preliminary studies in which .15 mM cyclic GMP has been added to the control pipet solution suggest that cyclic GMP activates a second outward current in addition to an approximately 10% increase in steady state membrane conductance. Further investigations will focus on elucidating the details of these effects, and possible roles that these currents may play in the olfactory response to behaviorally relevant odorants.

102.8 **MOLECULAR CORRELATES OF OLFACTORY ADAPTATION: ADENYLATE CYCLASE AND PROTEIN PHOSPHORYLATION.** **Umberto Pace*, Judith Heldman*, Iris Shafir*, Galia Rimon* and Doron Lancet (SPON: Michael Y. Spiegelstein). Dept. of Membrane Research, the Weizmann Institute, Rehovot, Israel.**

Olfactory adaptation is manifested at two levels: 1) A slow (~ 1 min), often complete loss of odor sensation, most probably centrally mediated; 2) A faster (~ 1 sec) partial diminution of odorant-induced electrophysiological activity in the sensory epithelial neurons, possibly analogous to hormone and neurotransmitter receptors desensitization. We studied molecular correlates of olfactory adaptation using an in-vitro assay: odorant activation of adenylate cyclase in a rat olfactory cilia preparation. Olfactory epithelia (whole turbinates) were dissected after quick decapitation, and incubated in isotonic buffer, with or without odorant cocktail (1 mM each, n-amylacetate, l-carvone, 1,8-cineole and citral) for 60 min at 30°C. Subsequently, a cilia-enriched preparation was obtained by 5 sec sonication and differential centrifugation (Shirley et al., Biochem. J. 240:605 ,1986). This preparation displayed the same odorant activation of adenylate cyclase as olfactory cilia obtained by calcium shock, although the specific activity of the enzyme in it was ~ 5 times lower. We found that preparations from epithelia pre-exposed to odorants and washed, gave an appreciably diminished GTP-dependent odorant effect on cyclic AMP (cAMP) production: 41±38% enhancement above basal level, compared to 98±45% activation in untreated controls ($N=17$, $p<0.002$). Such decreased efficiency of stimulation was not due to constitutive, lingering activation, as odorant pre-exposure had no effect on basal adenylate cyclase levels, i.e. odorant preactivation was fully reversible. The desensitization of olfactory receptor (OR) coupling to adenylate cyclase may be of the homologous type, stemming from odorant-induced receptor phosphorylation by hypothetical olfactory receptor kinase (ORK), akin to β-adrenergic receptor kinase (βARK) (see Lancet and Pace, Trends Biochem. Sci. 12:63, 1987), or by cAMP-dependent protein kinase. cAMP-regulated transmembrane phosphoproteins, recently identified in olfactory cilia of rat and cow incubated with $\gamma[^{32}P]$-ATP may be substrates for such odorant-activated modifications. This possibility is currently examined by studying protein phosphorylation in epithelial explants incubated with $[^{32}P]$-phosphate under conditions that promote desensitization. Other sites at which phosphorylation related to heterologous desensitization could occur are the stimulatory GTP-binding protein (olfactory G_s) and the cAMP-gated ion channels. Cross adaptation studies with different odorants, studies of olfactory G_s modification and attempts to identify and examine the phosphorylation of the ciliary cation channel would be instrumental in determining the molecular sites of chemosensory adaptation.

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102.9 MOLECULAR ASPECTS OF OLFACTORY RECEPTION. P.B. Sklar, R.C.A. Pearson, J.C. Venable*, and S.H. Snyder. Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Recent advances in the study of the initial events which transduce odorant signals across the olfactory epithelium suggest 1) that a wide variety of odorants interact with an odorant-sensitive adenylate cyclase (Pace et al., Nature 316:255, 1985) and 2) the presence of a cyclic nucleotide gated channel in isolated ciliary membrane patches (Nakamura and Gold, Nature, 1987).

In the bullfrog Rana catesbeiana, many fruity, floral, minty, and herbaceous odorants stimulate adenylate cyclase activity in isolated olfactory cilia, while numerous putrid odorants and odorous chemical solvents do not (Sklar et al., J. Biol. Chem. 261:15538, 1986). We now report that mammalian cilia (rat) display a similar response profile to odorants. In rat olfactory cilia odorants such as citralva, 2-isobutyl-3-methoxypyrazine, carvone, menthone, and hexylpyridine all activate enzyme activity as in amphibian cilia. Odorants which had no effect in frog cilia similarly were ineffective when tested in our rat cilia preparation.

We wondered if the regeneration of olfactory neurons into adulthood might result from oncogene expression. To investigate this we examined mRNA expression in the olfactory epithelium by in situ hybridization. Preliminary experiments suggest that the message for the c-fms, c-cis, c-abl, H-ras, N-ras, and K-ras oncogenes are present in the olfactory epithelium. Currently, we are investigating the changes in oncogene expresion following unilateral bulbectomy.

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102.10 ODORANT-BINDING PROTEIN: cDNA CLONING AND HOMOLOGY TO RAT α2-MICROGLOBULIN. J. Pevsner, R.R. Reed* and S.H. Snyder. Depts. of Neuroscience, Pharmacology and Molecular Sciences, and *Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

An odorant-binding protein (OBP) has been purified from nasal epithelium of rat and cow. OBP has a subunit molecular weight of 19 kDa (bovine) or 20 kDa (rat). The protein binds several odorants with micromolar affinities, and has been immunohistochemically localized to bovine mucus-secreting glands in the nasal epithelium. We now report the cloning and sequence analysis of two cDNAs encoding rat OBP.

The sequence of the N-terminal 14 amino acids of rat OBP was determined. A set of oligonucleotide probes was synthesized corresponding to the first seven amino acids. These probes were used to screen a λgt10 cDNA library derived from rat olfactory epithelium (provided courtesy of Dr. E. Barbosa, Johns Hopkins). Two clones (800 and 850 base pairs) which were recognized by the probes were isolated and sequenced by the Sanger chain termination method. Initial results suggest that the cDNA clones encode OBP because amino acid sequences of the protein adjacent to the region used to generate the oligonucleotide probes are identical to those determined from DNA sequencing of that region.

Based on preliminary sequencing results, OBP is homologous to rat α2-microglobulin, an abundant 18 kDa protein secreted by the liver. Both of these proteins appear to be members of a supergene family that includes retinol-binding protein (which binds retinol), apolipoprotein D (which binds cholesterol), and protein BG, a 20 kDa soluble protein present in the Bowman's glands of frog olfactory epithelium. The homology between OBP and members of this gene family supports the hypothesis that OBP serves as a carrier for odorants within the nasal epithelium.

Supported by a grant from International Flavors and Fragrances, Incorporated.

102.11 CORRELATIONS BETWEEN 2DG ACTIVITY AND MONOCLONAL ANTIBODY STAINING IN THE OLFACTORY SYSTEM OF THE FETAL RAT. P.E. Pedersen, B. Friedman, R. Smith*, C.A. Greer, G.M. Shepherd and S. Hockfield, Secs. of Neuroanatomy and Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

The accessory olfactory bulb (AOB) is the site of intense 2-deoxyglucose (2DG) activity in E(embyronic day)22 fetal rats in utero. Together with behavioral and anatomical evidence, this finding has suggested that fetuses may use this pathway to sample and detect their chemical milieu. We now report the use of monoclonal antibody Rat 204 generated in this laboratory in combination with 2DG autoradiography to compare the biochemical and functional differentiation of components of the peripheral olfactory pathways.

In the adult rat, Rat 204 recognizes a subset of olfactory receptor neurons situated in the dorsal recess of the main olfactory epithelium and in the vomeronasal organ and their respective axonal projections. In the fetal rat, at E15, the immunoreactivity is similarly spatially restricted to the dorsal recess and to the vomeronasal organ. In contrast to this selective staining of a subset of receptor neurons, another monoclonal antibody, Rat 203 (Friedman et al., 1986), recognizes receptor neurons throughout the embryonic olfactory epithelium.

Fetuses that receive 2DG in utero according to previously reported methods (Pedersen et al., 1983) exhibit differentially dense areas of metabolic activity throughout the neuraxis. At the earliest age examined, E15, areas of dense 2DG uptake are evident within the olfactory mucosa and presumptive olfactory bulb. Within the olfactory mucosa, these areas appear in the dorsal recess. There is also dense uptake in the vomeronasal organ. Of interest is the finding that the areas of high metabolic activity in the mucosa overlap with the areas that are recognized by Rat 204.

The early expression of antigens in subcomponents of the olfactory system, correlated with metabolic activity, suggests that the neurons in the dorsal recess and in the vomeronasal organ may undergo early functional differentiation. This may be significant for the development of odor detection.

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102.12 **Olfactory marker protein (OMP)-like immunoreactivity in discrete nuclei of rodent brain.** H. Baker and F. L. Margolis*. Dept. Neurology, Cornell Univ. Med. Col., New York, NY 10021 and *Roche Inst. Molec. Biol., Nutley, NJ 07110

Olfactory marker protein is a major cytoplasmic protein present in mature olfactory receptor neurons. Antibodies prepared against the SDS-PAGE homogeneous protein have been reported to exhibit exquisite specificity for olfactory receptor neurons in several vertebrate species. Immunoprecipitates of *in vitro* translations driven by mRNA isolated from olfactory tissue manifest a single band of 35S-met labeled material which co-migrates with authentic isolated OMP on SDS-PAGE. Only a single band is observed on immunoblots of olfactory tissue extracts. OMP has not been detected by radioimmunoassay in several grossly dissected non-olfactory brain regions or in a variety of peripheral tissues. Similarly on Northern and slot blot hybridization, RNA isolated from 14 non-olfactory tissues gave no reaction when probed with an OMP cDNA clone. The amino acid sequence of isolated rat OMP has not evidenced any significant homology within the N.B.R.F. data base. Thus, it was with some surprise that we observed OMP-like immunoreactivity in very restricted, discrete groups of neurons in both mouse and rat CNS. Major areas of CNS such as neocortex, cerebellum and basal ganglia, among others, were unstained. However, staining was seen in several auditory related structures such as medial geniculate, inferior colliculus and lateral lemniscal nuclei. Several medullary nuclei contained labelled perikarya including area postrema, nucleus of the solitary tract-motor vagal complex and spinal trigeminal nucleus, pars zonale. The preoptic area and hypothalamus also manifested reactive neurons. In the latter, the most heavily stained neurons were located peri- and para-ventricularly. Similar staining patterns were observed with OMP antisera prepared in both rabbit and goat. In the hypothalamus the mouse exhibited more robust immunoreactivity and a somewhat wider distribution of fibers and terminals than in the rat. The staining in hypothalamus and preoptic region was abolished by preincubation of the antierum for one hour with 1μM purified OMP. Among the possible interpretations of these observations are: 1) OMP or a closely related protein is present in these neurons, 2) these antisera cross react with a hypothalamic peptide/protein, or 3) OMP antisera contain immunoglobulins directed against a previously unsuspected, highly immunogenic contaminant. The extensive use, by several laboratories, of these antisera as reagents has prompted this preliminary report. Evaluation of these diverse possibilities is under investigation as are the implications of these observations with regard to the function of OMP.

EPILEPSY I

103.1 **KINDLED SEIZURES EVOKED BY REPEATED EXPOSURE TO BICUCULLINE: CROSS-KINDLING TO LIDOCAINE AND A NON-CONVULSANT BETA-CARBOLINE, (FG7142).** M. Fabrazzo*, P. Noonan*, and K. Gale, Dept. of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington D.C. 20007

For the generation of convulsive seizures induced by GABA antagonism, an epileptogenic site within the deep prepiriform cortex, "area tempestas" (AT), has been shown to be crucial (1). This area is subject to electrical kindling and exhibits bi-directional transfer ('cross-kindling') with electrical stimulation of amygdala (2,3). As the latter, in turn, exhibits bi-directional transfer with kindling induced by repeated systemic treatment with lidocaine (LIDO) (4), we expected transfer of 'kindling' between LIDO and the GABA antagonist, bicuculline (BIC). To test this, rats were kindled with LIDO (**Group I**) (60 mg/kg/day i.p. for 21 days) or BIC (**Group II**) (14 daily s.c. injections starting at 2 mg/kg and reducing the dose until ≤1 mg/kg was given during the last 4 days). Approximately 30% of **Group I** became kindled as indicated by the appearance of clonic seizures in response to LIDO (60 mg/kg i.p.); no control rats (receiving injections of saline for 21 days) exhibited seizures with this challenge. LIDO-kindled rats also exhibited an enhanced seizure sensitivity to BIC in comparison either to LIDO-treated rats that did not become kindled or to saline treated controls. In **Group II**, over 70% became kindled as indicated by clonic seizures in response to a dose of BIC (≤ 1mg/kg) less than half the convulsive threshold dose in controls. 100% of BIC-kindled rats exhibited clonic convulsions with acute LIDO (60 mg/kg i.p.) challenge. BIC- and LIDO-kindled seizures typically included facial and forelimb clonus, rearing and falling (Stage 5, using the scoring system applied to amygdala-kindled seizures). These resemble seizures induced by application of BIC into AT as well as those induced by electrical kindling of amygdala ('limbic motor seizures'), but they are distinct from the seizure pattern observed in response to an acute challenge with BIC in non-kindled rats. BIC-kindled rats were also tested for their response to a beta-carboline (FG7142, 15 mg/kg i.p.) and 100% exhibited Stage 5 motor seizures; parallel controls did not show these seizures in response to FG7142 (15, 30, or 60 mg/kg i.p.). Days or weeks without convulsant exposure did not appear to diminish the kindled seizure responses.

In BIC-kindled rats, the threshold dose of BIC methiodide for eliciting motor seizures when placed directly into AT was decreased and the resulting seizures were more prolonged in comparison either to non-kindled rats or to pre-kindling baseline responses.

Thus, chemical kindling with LIDO and drugs which interfere with GABA transmission may share a common substrate. It is likely than an alteration in limbic circuitry with which amygdala and AT are interconnected is responsible for this kindling process.

(1) Piredda and Gale (1985) *Nature* 317:623, (1986) *Eur. J. Pharm.* 120:115, (2) Morimoto et al (1986) *Exp. Neurol.* 94:637, (3) Zhao and Moshe (1987) *Epil. Res.* 1:94, (4) Post (1981) *Kindling* vol. 2, 149

103.2 **CHOLINERGIC-GABAERGIC INTERACTIONS IN THE GENERATION OF LIMBIC MOTOR SEIZURES IN RATS.** M. Garnett*, M. Fabrazzo *, and K. Gale, (spon: J. McConnell) Department. of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington D.C. 20007

Blockade of GABA receptors, as well as stimulation of muscarinic receptors locally in area tempestas (AT, an epileptogenic site within the deep prepiriform cortex) elicits clonic convulsions that are prevented by the local application of a GABA agonist (1). Moreover, inhibition of excitatory drive within AT blocks limbic seizures induced by systemic treatment with the muscarinic agonist, pilocarpine (2). Based on this, we expected that the GABA antagonist, bicuculline (BIC) would potentiate the convulsant action of pilocarpine. To test this, rats were given a dose of pilocarpine (100 mg/kg i.p.) that was less than one-third the convulsant threshold. Ten min later, BIC was given in a dose (0.5 or 1.0 mg/kg s.c.) less than 25% of the convulsant threshold. At 20-30 min after BIC, 50% of the pilocarpine-treated rats exhibited clonic seizures. These seizures resembled the 'limbic motor seizures' induced by high doses of pilocarpine (400 mg/kg), consisting of facial and forelimb clonus, rearing and falling (Stage 5 according to the scoring system applied to amygdala-kindled seizures). Over 25% of the rats exhibiting convulsions also exhibited status epilepticus lasting over 3 hrs. These results are in contrast to those reported by Turski et al (3) who found that injection of BIC 10 min **prior** to pilocarpine did not potentiate convulsions. It is likely that the relatively brief duration of action of BIC and the 20-30 min latency for the convulsive action of pilocarpine does not allow for synergism when BIC is administered **prior** to pilocarpine.

To further explore a BIC-pilocarpine interaction, rats were 'kindled' by daily treatments (for 14 days) with BIC (starting at 2 mg/kg s.c. per day and reducing the dose by 0.5 mg/kg every 4-5 days). After this period, the threshold convulsive dose of BIC was <50% of that required in non-kindled (saline-treated) controls. After 10 days drug-free, these rats were challenged with 200 mg/kg pilocarpine, a subconvulsive dose in controls, and 50% of the BIC-kindled rats exhibited full Stage 5 convulsions.

Thus, the epileptogenic effects of BIC and pilocarpine probably share one or more common substrates. We have shown that not only does BIC potentiate the convulsant action of pilocarpine, but rats sensitized to BIC by repeated exposure, show cross-sensitization to pilocarpine. As the AT is a site at which both GABA antagonists and muscarinic agonists act to elicit limbic motor seizures, this site is likely to mediate BIC-pilocarpine synergism. In addition, pilocarpine convulsions can be potentiated by a reduction of GABA transmission within substantia nigra (4), so this is another site at which BIC may act to enhance pilocarpine seizures. Finally, as repeated application of muscarinic agonists (but not GABA antagonists) into amygdala can induce kindled seizures (5), a combined action of pilocarpine in amygdala and AT may contribute to the generation of seizures with pilocarpine in BIC-treated rats.

(1) Piredda and Gale (1985) *Nature* 317:623, (2) Millan et al (1986) *Neurosci. Lett.* 70:69, (3) Turski et al (1985) *Brain Res.* 361:309, (4) Turski et al (1986) *Brain Res.* 370:294, (5) Wasterlain et al (1981) *Kindling* vol. 2:315

103.3 DEPTH INJECTION OF INHIBITION-BLOCKING CONVULSANTS SUGGESTS THAT PROPAGATING EPILEPTIFORM RESPONSES REQUIRE THE POTENTIATION OF THALAMOCORTICAL CIRCUITRY IN NEOCORTICAL LAYER 4. A.B. Chatt* and J.S. Ebersole. Epilepsy Center, VA Medical Center, West Haven, CT 06516 and Department of Neurology, Yale University School of Medicine, New Haven, CT 06510.

Simultaneous recordings from three laminae within the cat visual cortex following differential intralaminar injections of strychnine (a putative blocker of glycine, taurine and beta-alanine) 1) confirmed that low strychnine concentrations (5 mM) induce intrinsically-mediated epileptiform abnormalities preferentially when injected into the superficial cortical layers, 2) reveal that these abnormalities are generated locally and 3) that they remain local phenomena by not spreading vertically into other cortical layers. Higher strychnine concentrations (20 mM), however, 4) obscure this differential laminar sensitivity by potentiating middle layer sensitivity to this agent, and further 5) reveal vertically propagating abnormalities following layer 4 injections which are associated with the potentiation of the thalamocortical circuitry that is prevalent within this layer.

In our previous experiments where the GABA-blocker, penicillin has been used as the convulsant agent, response changes induced in any layer were always preceded by a thalamocortically mediated response from layer 4 which generally recruited intrinsically-mediated activity there and in adjacent layers (Brain Res 1984: 290, 361-366; Brain Res 1984: 298, 253-272), a condition clearly unlike the 5 mM but similar to the 20 mM strychnine foci observed in this study.

These results suggest that convulsant action upon the thalamocortical circuitry of layer 4 is essential for the development of propagating as opposed to local epileptiform activity. While we do not know the mechanism of action of layer 4 thalamocortical activation many minutes following 20 mM strychnine injection, the absence of similar effects at 5 mM would suggest a non-specific mechanism perhaps on the axonal membranes of the multitude of thalamic terminals within this layer. What is clear is that only when this activation is present does strychnine induce propagating epileptiform abnormalities in the neocortical column and perhaps between columns as well. Further, the inability of strychnine to induce propagating abnormalities at concentrations where some specificity of action can be inferred makes this agent much less useful than the GABA-blockers in investigating epileptogenesis.

(This work was supported by USPHS Grant NS0608, the Veterans Administration, and the Swebilius Trust Fund.)

103.4 **POSSIBLE MECHANISM FOR THE TRANSITION FROM INTERICTAL TO ICTAL ACTIVITY IN HIPPOCAMPAL SLICES BATHED IN HIGH EXTERNAL POTASSIUM.** S.F. Traynelis* and R.J. Dingledine. Dept. of Pharmacology, Univ. North Carolina, Chapel Hill, NC 27514.

We have previously reported that rat hippocampal slices bathed in 8.5 mM $[K^+]_o$ display both interictal activity and spontaneous electrographic seizures. Interictal bursts arise in the CA3b or c region (frequency ~1 Hz) and propagate to the CA1 region, triggering bursts which gradually increase in intensity. At some threshold level an incoming CA3 burst triggers a tonic-like seizure component in CA1 which lasts 5-10 sec, consists intracellularly of a 8-12 mV depolarization capped by a train of action potentials, and appears extracellularly as a long train of population spikes superimposed on a 4 mV negative envelope. This is followed by a clonic-like phase lasting tens of sec in which CA3 interictal bursts trigger large amplitude CA1 afterdischarges. Seizures occur in 22% of bursting slices, last 52.0 ± 1.9 sec (mean ± SEM, n=192 slices), occur at intervals of 2.6 ± 0.1 min for up to three hours, and are dependent on CA3 interictal input, elevated $[K^+]_o$, and elevated temperature.

Throughout the duration of the seizure and the interseizure interval, the CA3 region generates interictal bursts apparently unaltered in intensity or frequency. The focal nature of the electrographic seizure suggests that development of hyperexcitability occurs within the CA1 region itself. In the 30-60 sec preceding a seizure, both CA1 pyramidal cells and glia depolarize, and the extracellular DC potential drifts negative. These results are all reflective of an increase in baseline $[K^+]_o$. Seizures could be blocked by the competitive NMDA antagonist D-APV (5-10 uM), but not its inactive isomer L-APV (25 uM).

We suggest that a positive feedback loop develops in high $[K^+]_o$, in which several initial consequences of elevated potassium bring about an increase in the number and/or synchrony of CA1 neurons firing during each interictal burst. This in turn increases the amount of potassium released into the CA1 interstitium following each interictal burst, and the entire cycle becomes regenerative. Consequences of increased $[K^+]_o$ include decreased IPSPs and AHPs, cellular swelling, neuronal depolarization, and decreased spike threshold. The actual initiation of a seizure may occur when the CA1 region fails to clear the potassium from its interstitium before the next interictal burst arrives; the resulting threshold-level of K^+ enables the next burst to synchronize or enlist a sufficient number of neurons to become the leading edge of the tonic phase of the seizure. This work supported by NS17771 and a predoctoral fellowship from the National Science Foundation.

103.5 TONIC ELECTROGRAPHIC SEIZURES IN THE CA3 REGION OF RAT HIPPOCAMPAL SLICES BATHED IN HIGH POTASSIUM. N.L. Chamberlin* and R. Dingledine. (Spon: J.L. Giacchino) Dept. of Pharmacology, Univ. North Carolina at Chapel Hill, Chapel Hill, N.C. 27514

Hippocampal slices bathed in 8.5 mM potassium exhibit spontaneous interictal burst discharges. Events that resemble tonic-clonic electrographic seizures often occur in CA1 but rarely in CA3. We have found that treatment of slices with 2 μM phorbol ester 12,13 diacetate can produce activity in CA3 that resembles the tonic phase of a CA1 electrographic seizure. Treatment with phorbol ester significantly increased the rate of spontaneous interictal bursts from 47±2 to 69±3 per minute prior to the onset of tonic electrographic seizures in CA3 (paired "t" test, p<.01, n=26). Since burst intensity was slightly decreased (CA3) or unchanged (CA1), we suggest that an increase in interictal burst frequency was responsible for the interictal to ictal transition in CA3. Under control conditions, interictal bursts usually began in CA3b or c and spread to other pyramidal cell regions. In phorbol esters the interictal pacemaker could shift, but usually remained in CA3b or c. Tonic seizures in phorbol esters were initiated in any subfield of the hippocampus proper, as judged by onset latency in simultaneous recordings from multiple sites. Often they appeared in all regions of the slice simultaneously. These events were associated with a negative DC shift of -2.4±0.4 mV (n=10) in CA3 and -3.6±0.7 mV (n=7) in CA1. Tonic seizure components lasted 10-53 seconds and occurred every 15-150 seconds. The frequency and duration of tonic seizures was fairly constant within a given slice.

Phorbol esters have been shown to have a number of effects on hippocampal neurons, including reduction of burst afterhyperpolarizations (AHPs) in CA3 neurons (Williamson and Alger, Soc. Neurosci. Abst. 12(1):728,1986). Preliminary results suggest that the AHP was reduced in CA3 neurons prior to the onset of ictal events with no change in resting potential or input resistance. These findings are consistent with the idea that AHPs limit the rate of spontaneous interictal bursting and thereby prevent the appearance of electrographic seizures in CA3. Supported by NS-17771.

103.6 GRAFTS OF FETAL NORADRENERGIC NEURONS FROM LOCUS COERULEUS SUPPRESS DEVELOPMENT OF KINDLING EPILEPSY EVOKED BY ELECTRICAL STIMULATION IN THE HIPPOCAMPUS OF THE RAT. O. Lindvall*, D. Barry*, I. Kikvadze*, P. Brundin*, T. Bolwig* and A. Björklund. Dept. Histology, University of Lund, S-223 62 Lund, Sweden and Neurobiology Research Group, Dept. Psychiatry, Rigshospitalet, DK-2100 Copenhagen, Denmark.

Lesions of the ascending noradrenergic projections from the locus coeruleus facilitate the development of kindling (i.e. the number of stimulations needed to reach generalized seizures) caused by electrical stimulation in the amygdala, hippocampus or neocortex. The objective of the present study was to investigate if grafts of fetal locus coeruleus neurons could reverse this lesion-induced facilitatory effect on hippocampal kindling. Three groups of rats were prepared: (1) Normal control group; (2) Lesion group, which received 6-hydroxydopamine (6-OHDA) in the lateral ventricle; (3) Transplant group, which first received 6-OHDA in the lateral ventricle and then bilateral injections of cell suspension grafts of locus coeruleus from fetuses (crown-rump length 10-12.5 mm) into the hippocampus. All animals were subjected to electrical kindling daily in the CA1-CA3 region of the dorso-caudal hippocampus.

The development of seizures was considerably faster in the noradrenaline (NA)-depleted rats than in the control rats. Both the onset (number of stimulations to first grade 1 seizure) and the progression (number of stimulations from first grade 1 to first grade 5 seizure) of kindling was affected. In the grafted rats the onset and progression of kindling was markedly retarded compared to the NA-depleted animals. There was a clear graft-derived NA fiber ingrowth with a normal terminal pattern in all subfields of the dorsal two-thirds of the hippocampal formation. The extent and density of the graft-derived NA innervation varied, however, markedly between the individual animals. The number of stimulations to first grade 5 seizure was significantly correlated with the degree of reinnervation of the stimulated hippocampus.

Our data show that grafted noradrenergic locus coeruleus neurons can retard the development of kindling induced seizures. This study is the first demonstration that intracerebrally grafted neurons can modulate the excitability of epileptic brain regions. Intracerebral implantation of inhibitory neurons may, therefore, provide a new strategy to control the generation or spread of seizure activity.

103.7 THE SPREAD OF EPILEPTIFORM DISCHARGES IN RAT SOMATOSENSORY CORTEX MEASURED WITH A VOLTAGE SENSITIVE DYE. J.A. London, J.-Y. Wu,* M. Rioult,* M. Cattarelli,* and L. B. Cohen. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

Optical recordings were used to study the propagation and spatial distribution of epileptiform discharges in somatosensory cortex. Adult male rats were anesthetized with equithesine, paralyzed, and artificially ventilated. The cortex was stained with the fluorescent styryl dye, RH795 (kindly supplied by R. Hildesheim and A. Grinvald) at a concentration of 1 mg/ml for 1 hr, followed by a 30 min saline wash. The stimulus consisted of brushing a thin nylon filament across mystacial whiskers. Optical signals were recorded via a 12 X 12 element photodiode array. A ball electrode was placed on the somatosensory cortex adjacent to the region of optical recording.

To measure the optical signal in response to whisker movement in the control preparations, 32 trials had to be averaged. Epileptiform discharges were then induced by application of 1 mM bicuculline to the cortex. After bicuculline, optical signals could be recorded in single trials. Two general classes of responses were recorded, those evoked by whisker stimulation and spontaneous interictal discharges. The evoked response originated from the same site as the control signal, but propagated over a much larger area. It was relatively stereotypic from trial to trial in one animal. In addition to the increase in size of the evoked response after the addition of bicuculline, the signal after bicuculline was also both longer lasting and it occurred with a larger latency after the whisker movement. The spontaneous response was much more variable in signal size, origin, propagation direction, and spatial location than the evoked response.

The electrical activity recorded with the ball electrode was sometimes not well correlated with the optical signals. On several occasions the optical signals disappeared even though there was little change in the ball electrode signal. The optical signals returned following the readdition of bicuculline to the cortex. Optical recording appears to provide detailed information about the location and propagation of epileptiform discharges.

Supported by NIH grant number NS08437.

103.8 RAPID CESSATION OF FOCALLY-INDUCED GENERALIZED SEIZURES IN THE RAT VIA DIRECT ANESTHETIZATION OF THE FOCUS. Douglas C. Smith and Ronald A. Browning, Department of Psychology and School of Medicine, Southern Illinois University-Carbondale, Carbondale, Illinois 62901-6502

In this report we will demonstrate the rapid cessation of behavioral and electroencephalographic (EEG) seizures induced by application of bicuculline into the deep prepyriform cortex (DPC) or hippocampus (HC) of the rat. Such rapid cessation is accomplished via infusion of 2% lidocaine hydrochloride into the focal discharge area. While lidocaine delivered intraveneously is well-known to produce seizures, direct application of lidocaine into neural tissue produces a complete, and reversible, cessation of all action potentials in neurons to which it is applied. This is due to the blocking of sodium channels caused by this local anesthetic.

In eight Long-Evans hooded rats, we delivered 1.0 µl of 100 ng/µl bicuculline (BIC) unilaterally into the DPC through an implanted cannula. As has been reported by Piredda and Gale (1986), such infusion initiates seizures which begin unilaterally and increase in intensity to bilateral clonus with rearing and falling (Stage 5 on Racine's [1972] scale). As soon as the seizure became generalized, 1.0 µl of 2% lidocaine was infused unilaterally at a maximum rate of 1 µl/min. In all cases, behavioral seizures were stopped (Racine Stage 0) within 30 seconds following infusion of lidocaine. Saline infusion had no effect on these BIC induced seizures. EEG recordings indicate a return to pre-BIC baselines following lidocaine, but not saline.

In four additional rats, wet dog shakes were produced via unilateral BIC infusion into the HC. During BIC the mean number of wet dog shakes/min was 5.9. For the minute following administration of lidocaine, $\bar{X} = 0$.

In summary, we have shown that infusion of the local anesthetic, lidocaine, into a focal discharge area rapidly terminates behavioral and EEG generalized seizures which are induced by focal application of bicuculline. We are currently exploring whether lidocaine will arrest focally-induced generalized seizures when other methods of inducing such seizures are employed. If this proves to be the case, the delivery of lidocaine into a focal discharge area, once we perfect the technology, may hold promise for the control of seizures in human epileptics which are unresponsive to conventional anticonvulsant drug therapy.

103.9 DECREASED NEUROTRANSMISSION IN HIPPOCAMPAL-SUBICULAR PATHWAYS OF HUMAN TEMPORAL LOBE EPILEPSY. M. Isokawa-Akesson, C.L. Wilson and T.L. Babb. Brain Research Institute and Department of Neurology, UCLA, Los Angeles, CA 90024-1761.

Although studies in both animal models (Wong & Traub, J. Neurophysiol., 49:442,1983) and humans (Prince & Wong, Brain Research, 210:323, 1981; Schwartzkroin & Haglund, Epilepsia, 27:523, 1986) have characterized the way hippocampal bursts were altered under conditions of epilepsy, how epileptic properties expressed by individual neurons propagate into seizures remains unknown. The present study was designed, in chronic temporal lobe epilepsy patients, to investigate interictal physiological characteristics of an existing limbic pathway, i.e. the hippocampal-subicular path, using electrical stimulation to test for response-differences in normal and epileptic connections. The hypothesis tested was that the presubicular (PRESUB) neurons activated by stimulation of epileptic hippocampus (HC) would exhibit "hyperexcitability" compared to PRESUB neurons activated from non-epileptic HC.

In vivo extracellular single units were recorded bilaterally from 56 HC and 41 PRESUB neurons in 17 patients with a unilateral HC epileptic foci. Neurons located in the hemisphere contralateral to epileptic foci were considered as normal and used as a comparison control. In epileptic HC, burst firings were observed with high probability, and the pattern of those bursts were found to be stereotyped using auto-correlation analysis. Synchronized firings were also characteristic of HC bursting neurons as previously reported in human epileptic amygdalae (Isokawa-Akesson, et al. Epilepsy research, 1:17, 1987). In contrast, PRESUB neurons neither fired as a burst nor showed synchronized firings even though they were located near the HC epileptic focus. Single electrical stimulation (0.1Hz) to non-epileptic HC evoked responses of initial excitation followed by inhibition in 40% of the PRESUB neurons, which agrees with the intracellular responses in rat subiculum following HC stimulation (Finch et al., Brain Research, 197:11, 1980). However, electrical stimulation of epileptic HC failed to evoke any response in PRESUB neurons (0 of 20). Within the epileptic HC, however, 25 of 54 neurons responded with excitation-inhibition to local epileptic HC stimulation, indicating the stimulus strength was effective and that the cell-poor HC is not electrically inexcitable. Furthermore, the PRESUB neurons near the epileptic HC were equally activated by stimulating locally or stimulating parahippocampal gyrus.

The present results demonstrate that, in the interictal period, the epileptic hippocampal outputs to cytologically-normal PRESUB (Babb et al., Epilepsia, 25:729, 1984) appear to be poorly transmitted, possibly due to HC sclerosis and its own restructured (Davenport & Babb, NS Abstr. 343, 1986) epileptic circuitry.

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103.10 DIFFERENT THALAMOCORTICAL EXCITABILITY PATTERNS UNDERLYING STATE DEPENDENT SEIZURES IN FELINE MODELS OF PETIT MAL AND TEMPORAL LOBE EPILEPSY. M.N. Shouse.* (SPON: M.B. Sterman). Lab. of Sleep Disturbance Res., VA Med. Ctr., Sepulveda, CA 91343 and Dept. of Anatomy, UCLA Sch. of Med., Los Angeles, CA 90024.

Little is known about neural mechanisms underlying the important relation between biorhythms and epilepsy. This report summarizes three studies in cats suggesting thalamic mediation of sleep-activated seizures in the amygdala kindling model of secondary generalized temporal lobe epilepsy; in contrast, cortical hyperexcitability was implicated in the timing of "absences" and generalized tonic-clonic convulsions in the systemic penicillin model of primary generalized petit mal epilepsy. Amplitudes of centrally evoked neural responses in ventral lateral thalamus and motor cortex provided indices of thalamic and motor cortex excitability, respectively, in 18 cats. Six cats were kindled, six received 300,000 or 400,000 IU/kg sodium penicillin G intramuscularly, and six cats were nonepileptic.

Major results were: 1) During kindling, thalamic excitability increased with seizure generalization from the limbic system, especially during slow-wave-sleep (SWS) and transitions from SWS to rapid-eye-movement sleep when seizure susceptibility is highest in this model; cortical excitability accompanied penicillin epilepsy, particularly during SWS and drowsiness after awakening when vulnerability to these seizures peaks. 2) Sleep deprivation (24h) exacerbated seizures and increased thalamocortical excitability nonspecifically. 3) A temporal lobe anticonvulsant (carbamazepine) suppressed thalamic excitability and generalized seizures in kindled cats, whereas a petit mal anticonvulsant (ethosuximide) suppressed only cortical excitability and penicillin epilepsy.

The data suggest that thalamocortical excitability provides a final common pathway for the expression of sleep-waking state dependent generalized seizures. The results may also explain consistent sleep deprivation and differential anticonvulsant drug actions in these two quite dissimilar seizure models.

Supported by the Veterans Administration.

103.11 MORPHOLOGICAL ANALYSIS OF THE TEMPORAL LOBE OF AN EPILEPTIC PATIENT WITH A TUMOR IN THE ENTORHINAL CORTEX: NEUROTRANSMITTERS AND ELECTRON MICROSCOPY. C.E.Ribak[1], S.H.C.Hendry[1], E.G. Jones[1], B.H. Choi[2], J.W. Geddes[3], C.W. Cotman[3] and L.D. Cahan*[4]. Depts. of Anatomy and Neurobiology[1], Pathology[2], Psychobiology[3], and Surgery[4]. Univ. of California, Irvine, CA 92717.

A four year old girl with intractable epilepsy had a tumor removed from her left temporal lobe to stop her seizures. The brain tissue was blocked at the time of removal into small pieces that contained the hippocampal formation and associated parahippocampal structures, and it was immersed into fixative for light and electron microscopic preparations. Frozen sections (40 um thickness) were processed for Nissl staining and immunocytochemical localization of GABA, cholecystokinin, substance P and neuropeptide Y. In addition, Vibratome sections (100 um thickness) were dissected so that identified regions of the temporal lobe were embedded in plastic for electron microscopy. Light microscopy revealed a ganglioglioma in the entorhinal cortex where pyramidal cell bodies were apposed by tumor cells. The dentate gyrus displayed a decrease in the number of granule cells whereas the CA1 region (Sommer's sector) was devoid of virtually all pyramidal cells. In contrast, CA3 appeared normal. Cortical interneurons labeled in the immunocytochemical preparations were decreased in the entorhinal cortex and dentate gyrus. However, the basket plexus of axon terminals was labeled in CA3 but not in CA1 where the pyramidal cells were lost.

Electron microscopic observations have been limited to the dentate gyrus and hippocampus. The granule cells of the dentate gyrus were enveloped by profiles of reactive astrocytes. The terminal axons of granule cells, the mossy fibers, were rarely observed in the hilus and stratum lucidum of CA3. Very few axosomatic symmetric synapses were found whereas asymmetric axodendritic synapses were more numerous in the adjacent neuropil. Pyramidal cells in CA3 displayed numerous axodendritic asymmetric synapses. Many spines were compressed into dendritic shafts by the radially-oriented glial processes. Expanded, watery dendrites were also found. These dendritic observations are consistent with previous Golgi results that showed reduced numbers of spines and the presence of dendritic nodulations. Terminals of the basket plexus were packed with synaptic vesicles and were also compressed against the somata of pyramidal cells by glial processes. Finally, CA1 displayed only a few pyramidal cells and they contained many large dense-core (autophagic) vesicles in their perikaryal and dendritic cytoplasm. These remaining neurons in CA1 were encapsulated by glia and may be involved in the early stages of degeneration. These findings suggest that the most severe degeneration of neurons in the hippocampal formation occurs in CA1 and the dentate gyrus, the regions that receive the most excitatory afferents. The presence of some interneurons in these regions may indicate that they are more resistant to the excitotoxic effects of these afferents, including those from entorhinal cortex. This work was supported by NIH grants NS 15669, ES 02928 and NS 22317.

103.12 HIPPOCAMPAL SOMATOSTATIN, CCK, AND VIP LEVELS IN PATIENTS WITH TEMPORAL LOBE EPILEPSY. R.J. ROBBINS, T. ADRIAN*, N. de LANEROLLE, J. KIM*, S. HALVONIK* and D. SPENCER (SPON: S.Spencer). Sections of Neuroendocrinology and Neurologic Surgery, and The Yale Epilepsy Center, Yale Univ. School of Medicine, New Haven, CT 06510

The presence of peptide containing interneurons in various limbic structures has been well established in many species. Receptors for these peptides, and specific electrophysiologic effects have been demonstrated. Since immunohistochemical methods have localized such interneurons in hippocampal fields, where they may modify ongoing electrical activity we hypothesized that levels of specific peptides would be altered in states of abnormal electrical activity. We measured three neuropeptides, known to be present in the human hippocampus, in patients with medically refractory complex partial seizures (CPS) who were undergoing medial temporal lobectomy. As soon as the hippocampus was removed intact from the surgical field it was cut coronally into 2-3mm slices which were rapidly frozen and kept for up to 2 weeks at -80C. Slices were then allowed to warm to -10C and punch biopsies of various hippocampal regions were made using a dissecting microscope. Tissue was weighed, boiled in water, acidified, sonicated, centrifuged and the supernatants were stored at -80C until radioimmunoassay. Controls included tissue removed from patients dying of non-neurologic causes (NC), and tissue removed from patients with epilepsy due to glial tumors of the temporal lobe (TC). In the dentate granule cell layer, and in the CA4 field we found a significant reduction of immunoreactive somatostatin (IRS) in the CPS patients compared to NC or TC. No such changes in CCK or VIP were present. In the CA1 region IRS in TC was increased compared to CPS patients or NC. CCK levels in CA1 were much higher in both CPS patients and TC than in NC. VIP levels in CA1 were similar in all groups. No significant differences in peptide levels have been detected in the subiculum or entorhinal cortices to date. We conclude that specific alterations of somatostatinergic and CCK-ergic interneurons are occuring in the hippocampal regions of patients with epilepsy. Whether these changes are important in the pathophysiology of the seizures or simply reactions to the abnormal electrical signals remains to be determined.

PROCESS OUTGROWTH: GROWTH CONE BEHAVIOR

104.1 TURNING OF *APLYSIA* GROWTH CONES AT SUBSTRATE BORDERS *IN VITRO*. D.W. Burmeister and D.J. Goldberg, Dept. of Pharmacology, Columbia Univ. College of Physicians & Surgeons, N.Y., NY 10032.

Cultured *Aplysia californica* neurons can extend axons on either poly-lysine treated or untreated glass coverslips. When they are confronted with a border between the two, however, growth cones approaching from the treated side will turn and continue to grow only on the poly-lysine side. Recently we have em - ployed video enhanced contrast - differential interference contrast microscopy to examine, in detail, the behavior of growth cones on homogeneous substrates (Goldberg, D.J. & Burmeister, D.W., J. Cell Biol. 103:1921, 1986). We ob - served that growth always took place through the same sequence: vesicle-free veils formed between filopodia, these were invaded by vesicles from the growth cone center, and the veil was progressively transformed into the growth cone center. The growth cone center, in turn, rounded into the axon cylinder. Because substrate cues are thought to play an important role in directing neurite growth *in vivo* we were curious to observe how this developmental sequence was modified as growth cones approached and turned at a defined substrate boundary.

Turning neurites advanced through the same sequence observed previously. Turning occurred because the growth cones upon reaching the border generally started to split, developing two separate active growth areas, areas where new filopodia and veils formed. This behavior was similar to that seen when neurites branched at substrate obstructions. At the borderline, if one of the active areas extended onto treated and the other onto untreated glass, both areas usually continued to advance for a time, but the regions growing on the untreated glass would eventually, without initially losing adhesive contact with the substrate, wither, dying back to the point where the growth cone had originally split. Growth cones that approached the border at angles where both active regions formed on glass, stopped growing and eventually withered entirely, retracting back to a previous branch point, even if this was well back from the substrate boundary. Turning was not the result of differential filopodial adhesion. Because new filopodia tended to be at the front of approaching growth cones, and because growth cones tended to initially grow beyond the substrate boundary, often more filopodia were attached to the untreated substrate than the treated substrate during the early stages of turning.

Neurite turning was largely the result of asymmetric regression of developed areas of the growth cone, rather than asymmetric elongation. The selective re - gression of branches was competitive in that growth cones, or active areas, could survive and advance on untreated glass, but only if nearby areas or branches were not growing on the treated side. Both because of its competitive nature, and because withering occurred back to the branch point, growth cone turning may be analogous to, and may share mechanisms with, the pruning of exuberent branches seen during many stages of neural development.

104.2 LOCAL EFFECTS OF CALCIUM IN VEIL FORMATION AND MAINTENANCE IN APLYSIA GROWTH CONES IN VITRO. D.J. Goldberg. Dept. of Pharmacology, Columbia U. Coll. of P. & S., N.Y., NY 10032.

Our recent observations of the growth cones of Aplysia neurons in vitro using high resolution video microscopy have defined stages in the growth of an axon (Goldberg, D.J. and D.W. Burmeister, J. Cell Biol. 103:1921, 1986). Initially, there is protrusion on a filopodial framework of an organelle-free veil of membrane, followed by the morphological transformation in place of this veil eventually into the neurite. The filopodia clearly direct the growth of the neurite by defining the pathway along which veil protrudes, but it is unclear what causes veil protrusion or what determines along which of many filopodia the protrusion will occur.

The possibility of the involvement of Ca^{++} in the formation or maintenance of veils in Aplysia growth cones in vitro was investigated using video microscopy. Reduction of $[Ca^{++}]_o$ from the normal concentration of 11 mM to 0.3 - 20 μM through the use of Ca^{++} - EGTA buffers caused retraction of veils within minutes without visibly affecting filopodia or the bidirectional transport of membranous organelles in the neurite. Reduction of $[Ca^{++}]_o$ to 0.2 - 1.3 mM (without EGTA) usually, though not always, caused veil retraction. Addition of 20mM Co^{++}, which blocks Ca^{++} channels, to a solution containing the normal $[Ca^{++}]_o$ also caused rapid veil retraction. Perfusion with normal medium after any of the aforementioned treatments usually caused reformation of veils. Ba^{++}, which is known to readily permeate Ca^{++} channels, could substitute for Ca^{++} in causing veil reformation. Concentrations of trifluoperazine which should block calmodulin- and protein kinase C-dependent protein phosphorylation and concentrations of calmidazolium which should block only the former both caused veil retraction. Taken together, these data indicate that Ca^{++} plays a role in veil formation and maintenance, perhaps via calmodulin, and that, in these Aplysia neurons in vitro, substantial influx through the Ca^{++} channel is needed to maintain the requisite $[Ca^{++}]_i$ for this role. Whether changes in $[Ca^{++}]_i$ normally act as the trigger for veil formation is unclear.

Evidence was also obtained that Ca^{++} acts quite close to the site of veil formation. Microelectrodes containing either 1M $CaCl_2$ (for diffusion or iontophoresis) or 20 mM Ca^{++} in medium (for puffing) were moved to within a few μm of large (>10 μm) growth cones in low Ca^{++} medium. When veils formed in response, they were almost always restricted to the filopodia close to the orifice of the microelectrode (which was usually no larger than the space between neighboring filopodia). This suggests that, if veil protrusion is triggered by Ca^{++}-dependent exocytotic membrane addition, this occurs at or near the site of protrusion and determines where on the growth cone protrusion occurs.

104.3 PROPERTIES OF THE RETROGRADE MICROTUBULE-BASED TRANSLOCATOR FROM SQUID AXOPLASM. B.J. Schnapp, T. Schroer, M.P.Sheetz, and T.S. Reese. Laboratory of Neurobiology, NINCDS, NIH, at the Marine Biological Laboratory, Woods Hole, MA. 02543.

Two microtubule-based translocators have been identified in squid axoplasm and implicated in rapid axonal transport. Kinesin is a 600 kd, soluble protein thought to promote vesicle movement in the anterograde direction by associating with appropriate organelles and promoting their translocation along microtubules; the retrograde translocator is a different protein first identified in Triton extracts of low speed axoplasmic supernatants by examining bead movement on an oriented population of microtubules in vitro (Vale et al, Cell 43: 623, 1985). We now show that the retrograde activity is present in high speed, soluble supernatants from axoplasm in the absence of Triton, indicating that some of the retrograde protein, like kinesin, is in a soluble form in the axoplasm. The soluble retrograde activity appears to be part of a large complex because it can be separated from kinesin activity by sedimentation for 45 min in an airfuge; the resuspended pellets show retrograde activity. SDS-PAG electrophoresis of the soluble axoplasmic supernatant before and after the airfuge spin indicates that most proteins are not sedimented under these conditions. When supernatants containing retrograde activity are exposed to AMP-PNP in the presence of microtubules (conditions which promote tight binding of kinesin to microtubules and which provided the means for first purifying kinesin), no retrograde activity is recovered after the supernatant is desalted into ATP-containing buffer. Thus, a number of high molecular weight proteins, which we have suspected to participate in retrograde movement because of their AMP-PNP dependent binding to microtubules, are ruled out as retrograde factors. The role of the retrograde activity in organelle transport still remains unclear, as suggested by the following experiments. Soluble supernatants from squid axoplasm support both anterograde and retrograde movement of salt-stripped organelles as well as bidirectional bead movement. When these supernatants are depleted of kinesin using a monoclonal affinity resin specific for the 110 kd subunit, retrograde but not anterograde bead movement remains; movement of salt-stripped organelles in both directions, however, is lost. This suggests that the retrograde translocator by itself can not support organelle movement and that kinesin (which mediates the movement of beads in the anterograde direction only), or some protein binding to kinesin, is required for retrograde organelle movement.

104.4 PRUNING OF HIPPOCAMPAL PYRAMIDAL NEURON DENDRITIC ARCHITECTURE IN VITRO BY GLUTAMATE AND A PROTECTIVE EFFECT OF GABA PLUS DIAZEPAM. M. P. Mattson, P. Dou* and S. B. Kater. Program in Neuronal Growth & Development, and Dept. of Anatomy, Colorado State Univ., Ft. Collins, CO 80523

Glutamate is believed to be the major excitatory amino acid (EAA) neurotransmitter, and GABA the major inhibitory transmitter impinging upon hippocampal pyramidal neurons (HPN). In the hippocampus, glutamate, acting at synapses on HPN dendrites, is believed to be involved in a variety of normal and pathological processes including: learning and memory, epilepsy, Alzheimer's disease, and stroke. In each of these cases neuroarchitectural changes have been documented. In the present study we tested the possibility that interactions of **excitatory and inhibitory neurotransmitters can regulate the outgrowth status of pyramidal neurons**; these experiments were based upon preceding studies in Helisoma neurons which revealed roles for neurotransmitters in the regulation of neurite outgrowth (Science 226:561). Isolated, cultured HPN (obtained from 18 day rat fetuses) in which the axon and dendrites are morphologically distinct were used to demonstrate a new role for EAAs and GABA in the regulation of hippocampal architecture.

Glutamate selectively pruned, in a dose-dependent manner, the dendritic arbors of these neurons. With increasing concentrations of the EEAs glutamate, kainic acid (KA), and quisqualic acid (QA) we observed the following graded sequence of morphological changes in HPN: 1) Dendritic elongation was suppressed; axonal elongation was unaffected (10 uM glutamate). 2) Dendritic retraction; axons unaffected (50 uM glutamate). 3) Dendritic retraction; suppression of axonal elongation (100 uM glutamate); under these conditions cell death was often induced which occured over a period of 6 to 24 hr. 4) Rapid cell death ($\leq$ 1 hr) with somal swelling and process fragmentation ($\geq$ 1 mM glutamate). Outgrowth was insensitive to mM concentrations of N-methyl-D-aspartic acid (NMDA). The effects of the EAAs were prevented with the general glutamate antagonist d-gamma-glutamylglycine but not with the NMDA receptor-specific antagonist DL-2-amino-5-phosphonovaleric acid suggesting mediation by receptors of the KA/QA types. Co^{2+} (100 uM) prevented both the dendritic pruning and cell death induced by glutamate, KA, and QA. Furthermore, the calcium ionophore A23187 caused dose-dependent graded process retraction in HPN (100 nM - 1 uM) and was toxic at high levels ($\geq$ 10 uM). Calcium influx thus appears to play an important role in mediating the EAA effects on neuroarchitecture. Given these negative effects of EAAs on dendritic outgrowth, we next examined the effects of the inhibitory neurotransmitter GABA alone or in combination with glutamate on the outgrowth of HPN. GABA alone ($\leq$ 100 uM) had no significant effect on dendritic or axonal elongation. Neither did GABA alter the ability of glutamate to selectively prune dendrites. However, the combination of GABA (10 uM) and the benzodiazepine Diazepam (10 uM) was able to prevent the dendritic pruning and cell death caused by glutamate.

Taken together, these findings suggest roles for excitatory and inhibitory neurotransmitters in the regulating the growth status of neurons in the mammalian brain. The graded pruning of dendrites of isoalted HPN in response to increasing levels of EAAs is strikingly similar to the graded reductions in HPN dendrites seen in the brains of patients with increasing severities of epilepsy and Alzheimer's disease. Our finding that Diazepam is effective in countering the neurodegenerative effects of EAAs in HPN suggests that such inhibitory agents might be effective in preventing the kind of dendritic pruning of HPN associated with various neurodegenerative disorders. (Supported by NIH grants NS 08054 [MPM]; NS 24683, NS 2456, NS 15350 [SBK]).

104.5 GROWTH CONE-TARGET CELL INTERACTIONS IN DEVELOPING CEREBELLUM IN VITRO. C.A. Mason, J. Sanchez*, and M.E. Hatten, Dept. of Pharmacology, New York University School of Medicine, New York, NY 10016.

One of the tasks of the axonal growth cone is to select and make synapses with specific target cells. We have developed a model culture system, using mouse cerebellum, to characterize growth cone behavior, membrane appositions, and synaptic morphology during axon-target cell interactions.

Two afferent systems (mossy and climbing fibers) derive from separate brainstem sources (pontine and inferior olivary nuclei, respectively) and in mature brain contact granule cells or Purkinje cells, respectively. Granule neurons and Purkinje cells were purified into highly homogeneous cell populations by gradient separation and preplating, and plated as microcultures (30 µl) on polylysine-coated coverslips, in medium containing horse serum and FGF (Hatten, '85, JCB 100:384; Hatten et al., '87, SfNA). Olivary and pontine nuclei were excised from brain slices and subdivided into small chunks, which were placed as explants onto a sparse monolayer of their appropriate target cells. Within 24-36 hrs, neurite outgrowth from the explant is profuse, and target cells extend neurites. During this period, we focused on growth cones about to approach target cells. Neurites were identified as being afferent by either following them back to the explant, or by injecting the explant with HRP or rhodamine. Target cells were identified by specific antigen markers and by characteristic morphology.

The behavior of growth cones as they associate with target cells was monitored in real time with video-enhanced differential interference contrast optics, coupled with a time-lapse optical memory disc recorder. When a growth cone meets a target cell, within minutes, filopodia and ruffles on both the growth cone and target cell actively extend and withdraw. Filopodia from each cell often appear to be continuous. Such interactions precede either growth cone binding to, or withdrawal from, the target cell. After a growth cone forms a stable attachment to a cell via filopodia and veils, other projections extend and further probe the cell. We are currently determining the time course from first interactions to stabilization of contacts, by performing a periodic time-lapse study. The cytology, membrane interactions and synaptic morphology of identified growth cones will be analyzed in the EM and with antisera to synaptic vesicle antigens.

This model system forms an assay for future studies on the molecular basis of target cell selection and synapse formation. (Supported by NIH grants NS-16951 and NS-21457).

104.6 AXON INGROWTH AND TARGET CELL INTERACTIONS IN DEVELOPING CEREBELLUM. S. Catalano* and C.A. Mason, (SPON:L.A. Greene), Dept. of Pharmacology, New York University School of Medicine, New York, NY 10016.

During the assembly of neural circuitry, different groups of axons enter target regions and interact with specific populations of target cells. Demonstration of the temporal and spatial features of axon-target cell interactions has been impaired by the difficulty of labeling identifed immature afferents and localizing target cells, particularly before cell migration is completed. To address this issue, we have a) labeled cerebellar afferents (mossy and climbing fibers) from their brainstem sources (pontine and inferior olivary nuclei respectively), with rhodamine, a dye that densely labels axons and their growth cones over a long distance, and b) localized Purkinje cells, the target cell of climbing fibers, with antisera to the 28,000 M_r vitamin D-dependent calcium binding protein (gift of S. Christakos, NJ Med. Sch., Newark), which selectively stains Purkinje cells, including dendrites and growth cones.

At birth, climbing fibers extend well into the cerebellar anlage, reaching to the external granule cell layer as relatively unbranched fibers tipped by small growth cones. Purkinje cells are either still migrating into position, or have grouped in a multi-layered tier, and bear radiating somatic processes and growth cones. The majority of pontine mossy fibers at this age are just entering the cerebellum, and also have a simple shape, but have larger growth cones. There is considerable overlap in the projection of both axon groups at P2-3. By P3-4, climbing fibers form profuse finely branched arbors that extend laterally over several Purkinje cells, which now form a more condensed multilayered tier. Mossy synaptic boutons within the granule layer become enlarged, and some mossy fibers send fine branches into the Purkinje cell zone, that are tipped with climbing fiber-like endings. This latter finding confirms our original prediction that such HRP-labeled fibers derive from a mossy source (Mason and Gregory, '84, J. Neurosci. 4:1717). At P5 climbing fibers that have focused onto individual Purkinje neurons with a 'nid' formation, do so on Purkinje cells that have settled into a monolayer.

These first observations raise several issues: the placement and role of afferent axons during migration of their target cells; the relationship of axonal exuberance to the multiple layering and somatic protrusions of target cells; the rationale for mossy fiber projections to the Purkinje cell layer. Experiments are in progress to label cerebellar afferents and to localize Purkinje cells at embryonic ages, to further understand the whereabouts and morphology of immature axons and target cells, and their early interactions. (Supported by NIH grant NS 16951).

104.7 INFLUENCE OF SUBSTRATE ON THE DISTRIBUTION OF CALCIUM CHANNELS IN SPROUTS OF IDENTIFIED LEECH NEURONS IN CULTURE. H. Arechiga, J.G. Nicholls, and W.N. Ross, CINVESTAV-IPN, Mexico 14, D.F. Mexico, Biocenter, Basel 4056, Switzerland, and Dept. of Physiology, New York Medical College, Valhalla, N.Y. 10595.

When leech neurons are plated on two different substrates, the plant lectin Con A and an extract of extracellular matrix (ECM), they grow very differently (Chiquet and Acklin, PNAS 83: 6188-6192, 1986): on Con A the sprouts are thick with broad flattened growth cones; on extract the sprouts are long, straight, extremely fine, and branched. The aim of the present experiments has been to determine whether these two substrates have different effects on membrane properties, particularly the distribution of calcium channels.

A mixture of arsenazo III (a sensitive indicator of calcium concentration changes) and Lucifer Yellow (to indicate membrane area) was injected into the soma of an identified neuron and allowed to diffuse into the fine processes. The cell was imaged onto a 10x10 array of photodiodes and each element detected changes in light intensity at 660nm corresponding to changes in intracellular calcium. Each array element detected calcium changes in an area 40x40 um^2. Earlier work with cells on Con A (Ross, Arechiga, and Nicholls, J. Neurosci., in press) had shown that calcium entry following impulses was pronounced in the soma and initial segment but rarely detectable in thick processes or broad flat growth cones. These results have now been confirmed. By contrast, the same types of cells (Retzius, P, or AP) grown on ECM extract showed clear calcium signals, not only over the cell bodies and initial segments but also over the slender processes. The membrane area was far smaller than that for processes on Con A. The amplitudes of the calcium signals are proportional to the number of calcium channels in each pixel. Hence, the larger calcium signals and smaller membrane area on ECM extract imply a greater density of calcium channels. These results indicate that the laminin-like molecules of the ECM extract which neurons of the CNS contact in situ can have pronounced effects on the distribution of calcium channels.

We thank Ms. H. Niederer for excellent technical assistance. Supported by USPHS grant NS16295 and the Whitaker Foundation (WNR), and the Swiss National Fund and the U.S. Navy (JGN).

104.8 ANALYSIS OF GROWTH CONE MORPHOLOGY AND AXONAL BRANCHING IN INITIAL LIMB INNERVATION. M. Hollyday, Bryn Mawr College, Bryn Mawr, PA, 19010.

Previous studies have demonstrated that motor axons grow to the periphery with a high degree of precision. It is not known how axons make these accurate pathway choices. One possibility is that axons are guided by specific chemotrophic cues. An alternative mechanism of axon guidance might involve branching at the growing tip and maintenance of only those portions of the growth cone in contact with a suitably adhesive substrate. In order to gain insight into this issue in vivo, anterograde transport of WGA-HRP was used to selectively label individual axons and their associated growth cones during the stages of initial innervation of the chick embryo wing (stages 17-28). Labeled axons were studied with the light microscope in 40-60 µm thick sections reacted with DAB and intensified with cobalt. Axon identity was determined either directly by tracing into the ventral root or the dorsal root ganglion, or indirectly by inferring from the HRP injection site. More than 300 axons and their associated growth cones were reconstructed and drawn with a camera lucida.

Both motor and sensory axons terminated in growth cones which could be classified as either filopodial, lamellopodial, lamellopodial with associated filopodia, or club shaped. No blunt or rounded growth cones were observed at the ends of axons except in the "waiting zone" at the base of the dermomyotome, and even here they did not predominate. Many lamellopdial growth cones had complex shapes and were divided in ways which suggested the incipient formation of branches. Branched axons, terminating in two or more separate growth cones separated by axon segments as long 50-70 µm, were observed at all stages examined. These were found distributed all along the outgrowth pathway, from the ventral root or dorsal root ganglion, to the spinal nerve, in the plexus region, and further distally in the limb bud. Identification of "branched" axons (as opposed to divergence of two tightly fasciculated axons) was based on the presence of a distinctive triangular or "tricorne" shaped structure. The frequency of axon and growth cone branching was higher in decision regions than along spinal nerve pathways or in nerve trunks in the limb bud. The decision regions examined most thoroughly were in the limb plexus region and proximally in the ventral nerve pathways where n. pectoralis diverges from n. inferior brachialis, and where n. inferior brachialis splits into median and ulnar nerves. Similar patterns of growth cone and axon branching were observed among both identified sensory and motor axons in the same decision regions.

Examination of the labeled material revealed beaded and even fragmented, apparently degenerating, axons and growth cones among both motor and sensory populations in decision and non-decision regions alike. It is unlikely that these structures resulted from HRP cytotoxicity since other axons and growth cones in the same or adjacent sections appeared perfectly healthy. Rather they may be the remnants of inappropriately directed exploratory growth cones and axonal branches. Taken together, these observations suggest that one important determinant of accurate pathfinding is the formation of exploratory growth cones which not infrequently produce axonal branches. The mechanisms which act to select and maintain an appropriately directed branch and to withdraw inappropriately directed branches are presently unknown.

104.9 INTRINSIC POSITIONAL INFORMATION GUIDES THE EARLY FORMATION OF THE RETINOTECTAL PROJECTION OF XENOPUS. S. E. Fraser. Dept of Physiology & Biophysics, University of California, Irvine, CA 92717.

The lower vertebrate visual system has served as an important testing ground for the cellular processes that underlie patterned neural connections. A wide range of evidence is consistent with a role for cell-autonomous positional cues in the shaping of the retinotectal projection, but definitive tests have not been possible. The work presented here provides a test of this proposal by demonstrating that single retinal cells can project appropriately to the tectum, even when grafted to inappropriate regions of the eyebud.

The experiments use the axonal transport of fluorescent dextrans to follow the patterning of retinotectal connections in living, intact Xenopus embryos and larvae (O'Rourke & Fraser, Dev Biol 114:265). Lysinated fluorescein dextran (LFD) or lysinated rhodamine dextran (LRD) was injected into a fertilized egg, the animals were allowed to develop to eyebud stages, and were then used as donors for embryonic grafts of eyebud tissue to unlabeled hosts. The labeled optic nerve fibers arising from the grafted eyebud cells were then followed in the intact animal using epifluorescence microscopy. A refinement of this technique permits grafts of as few as one cell from each of the labeled donor animals. In animals with small grafts, individual optic axons and their growth cones were clearly visible, and the terminal arbors were easily distinguished from the axons leading to the terminal arbors.

In this set of experiments, LRD and LFD labeled cells were grafted to a single unlabeled host in either an equivalent location to which they were removed (homotopic grafts) or to non-equivalent locations (heterotopic grafts). The grafting of both LFD and LRD labeled cells to a single host permitted unambiguous evaluation of the topography of the projection. The animals were then raised either in the dark or in light filtered to remove wavelengths that could bleach the fluorescent dyes.

The results from homotopic grafts were in agreement with our previous work on labeled half-eye grafts. Homotopic dorsal and ventral grafts projected to the ventral and dorsal tectum at all stages examined. Homotopic anterior and posterior grafts initially projected indistinguishably, and then slowly sorted into a topographic order by stage 48.

Axons from heterotopic grafts always behaved in a fashion appropriate for their position of origin in the donor, independent of their final position in the host. Donor-specific behavior persisted under all conditions examined, including: 1. dark rearing, 2. light rearing, 3. grafts performed in "full-strength" embryological medium, 4. grafts performed in dilute artificial pond water, 5. grafts performed in artificial pond water intentionally titrated to an inappropriate pH (from pH6.5 to pH8). The results from grafts performed on animals ranging from stage 26 to stage 34 were indistinguishable, and the topography of the projection remained stable until at least stage 52.

These observations indicate that small groups of eyebud cells (as small as a single cell) possess positional information that helps to guide the optic nerve fibers to the correct site in the tectum. This positional information can be autonomously expressed and acted upon even when the cells are confronted with inappropriate neighbors. (Supported by NSF and the McKnight Found.)

104.10 CHANGES IN OPTIC FIBER MORPHOLOGY DURING DEVELOPMENT. N. A. O'Rourke , B. E. S. Fox and S. E. Fraser. Dept. of Physiology & Biophysics, Univ. of CA, Irvine, CA 92717.

In the lower vertebrate visual system, the axons of retinal ganglion cells form a topographically ordered projection in the tectum. The optic fibers respond to a number of different cues in the tectal environment during both development and regeneration of this ordered projection. A common link in these interactions is the ability of fibers to respond to these cues by extending and retracting branches and changing their position within the tectum. Neuronal activity appears to be involved in dynamic refinement of the projection during regeneration. In this study, we have investigated the role of neuronal activity in a similar refinement process which occurs during the initial formation of the topographic projection in *Xenopus* tadpoles. In addition, we have evaluated the role of dynamic changes in the morphology of individual retinal ganglion cell axons in the tectum during early fiber ingrowth as well as later stages of development.

We have utilized the fluorescent vital-dyes, fluorescent dextrans, to directly visualize dynamic changes in the retinotectal projection in live *Xenopus* larvae over a period of weeks. In a recent study, we described the technique and used it to follow the initial anteroposterior patterning of the projection. Fibers from nasal and temporal poles of the eye first overlapped in the tectum and then sorted out into the normal topographic pattern over a period of days (O'Rourke & Fraser, Dev. Bio., 1986). We have used this early refinement paradigm to test the role of neuronal activity in the initial development of topographic order. From other studies, we have results suggesting that glutamate is the primary neurotransmitter in the *Xenopus* retinotectal system (Fox & Fraser, Neur. Sci. Abst., 1987). Tadpoles which had recieved grafts of fluorescently labeled nasal and temporal retinal ganglion cells were reared in solutions of agonists and antagonists of NMDA-type glutamate receptors. These treatments block visually-evoked activity in the tectum. Preliminary results show that perturbing the NMDA class of glutamate receptors in this way delays the onset of the initial segregation of optic fibers.

To further investigate dynamic behavior in the retinotectal projection, we have refined the fluorescent dextran technique to follow changes in the morphology of single optic fibers in the tectum. In a parallel approach, the fluorescent carbocyanine dye, diI, was also used. In both cases, the dyes label the axons completely and diffusely, permitting visualization of terminal branches and growth cones of single fibers. An image intensifying camera and image processing system have allowed us to observe the fibers several times over a period of a week or more without phototoxic effects on the fibers. Morphological changes in the optic nerve terminal arbors were observed not only during initial ingrowth of the younger fibers into the tectum but in older fibers as well. These observations support the idea that axons display dynamic behavior during early stages and then maintain this behavior during later development.(NSF Grant BNS 8608356).

104.11 DISTRIBUTION OF IPSILATERALLY-PROJECTING AXONS IN *XENOPUS* OPTIC NERVE. S.G. Hoskins. Dept. of Biol. Sci., Columbia Univ., NY, NY 10027.

The ipsilateral retinothalamic projection in *Xenopus* is formed during metamorphosis by axons of a subset of ganglion cells which lie interspersed with contralaterally-projecting cells in the retina. The development of the ipsilateral projection is thyroxine-dependent (Hoskins and Grobstein, *Nature 307*: 730, 1984), and contralateral projections to the thalamus and tectum are present well before ipsilaterally-projecting fibers begin to develop. Developing ipsilaterally-targeted axons thus initiate growth toward the brain in a contralaterally-projecting nerve. The mechanism(s) which controls the accurate routing of ipsilaterally-projecting fibers is unknown. One possibility is that such axons might fasciculate selectively in the optic nerve, and be routed as a group at the optic chiasm.

To examine the routes taken by ipsilaterally-projecting fibers, I injected HRP into the thalamus of juvenile frogs, and examined the distribution of retrogradely-labelled fibers in the ipsilateral optic nerve and tract. Pressure injections of concentrated HRP were made into the thalamus on one side of the brain. Two to three days later, the animals were anaesthetized and killed, the optic nerves severed behind the eye, and the brains and attatched nerves fixed, processed, and sectioned serially. The positions of HRP-labelled fibers in the optic nerve and tract were reconstructed from frozen or paraffin sections using a camera lucida. 1. At the level of the eye, the nerve is cylindrical in cross section, with glial cell bodies concentrated centrally and the periphery essentially cell-free. All HRP-labelled fibers lie in the periphery, the majority at the extreme posterior or postero-ventral periphery, interspersed with unlabelled, presumably contralaterally-projecting axons. No labelled fibers are seen in the anterodorsal periphery. 2. Near the brain the nerve widens along the antero-posterior axis and glial cells are distributed heterogenously. Labelled axons occupy more of the peripheral circumference, appearing in ventral and dorsoposterior regions as well as posteriorly. As in distal nerve, labelled axons are interspersed with unlabelled axons. 3. In the optic tract, ipsilaterally-projecting fibers form a closely associated bundle lying anteriorly and do not appear to be interspersed with unlabelled fibers, suggesting that substantial reorganization of fibers occurs in the optic chiasm. 4. In contralateral nerves, in contrast, labelled fibers are found centrally as well as throughout the entire periphery, at all levels. The central vs peripheral distribution of labelled retinothalamic fibers thus correlates with the birthdates of their cells of origin, as is to be expected based on studies of fiber organization involving central or peripheral retinal lesions (Taylor, *Development 99*: 393, 1987).

In summary, ipsilaterally-projecting axons do not form a closely associated group in the optic nerve, and instead are scattered throughout much of the periphery. It is thus unlikely that routing of ipsilaterally-projecting axons at the optic chiasm can be achieved by deflection of a particular fascicle. The mechanism controlling pathway selection at the chiasm appears to be one which affects axons individually, perhaps on the basis of cell-surface differences, rather than routing them as a group. Since the development of ipsilaterally-projecting axons requires thyroxine, and the early establishment of the contralateral projections does not, it is possible that hormone-associated molecular differences between the two groups of axons might underly their divergent routing at the chiasm. As a first step in pursuing this possibility, I have constructed a cDNA library from metamorphosing retina, and am in the process of screening it for thyroxine-regulated genes.

104.12 TARGET RECOGNITION AND PATHWAY CUES IN THE PRIMARY DEVELOPMENT OF THE RETINOTECTAL PROJECTION. J.S.H. Taylor,* (Spon. G. Fink), MRC Neural Development and Regeneration Group, Dept. of Zoology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT.

It has been shown that in the normal Xenopus embryo, retinal ganglion cell axons grow directly toward their target cells in the optic tectum. Harris (Nature, 320, 1986) has shown that axons from displaced eyes, which enter the caudal part of the ipsilateral midbrain still grow directly to the tectum.

By translocating the midbrain rudiment of stage 24 Xenopus embryos, the eventual midbrain target cells of the developing retinotectal projection can be moved to novel sites. In these experiments the tectal anlage, contained within the developing midbrain, has been placed rostral or caudal to its normal position, or separated into two spatially distant parts, or removed altogether. By using the axonal tracers Horseradish peroxidase and Di-i, the pathways of the first growing optic axons in such preparations have been examined. It is shown that retinal ganglion cell axons follow well defined pathways, in spite of the abnormal structure of the brain. In cases where the target has been removed, the normal optic tract is followed to a point just ventral to the tecto-diencephalic border; here the axons turn caudally, and extend into the hindbrain where they enter spinal tracts. Where the target has been displaced caudally a similar pathway is initially followed, however, in these cases the axons turn dorsal at the level of target and terminate. In cases where the targets have been displaced rostrally, the axons follow the normal optic pathway to the level of the target and then turn to innervate the tectal anlage. In these cases no axons are found growing caudally away from the tectum. Where dual targets have been made, the optic tract bifurcastes, such that both structures are subsequently innervated.

These experiments clearly show that optic axons can seek out and form terminal arbors in ectopically placed target structures. It is suggested that retinal ganglion cell axons preferentially grow along stereotyped pathways which, in the normal animal lead them towards the optic tectum. In these experimental preparations and most probably in normal animals, a target derived trophism acts as an important short range guidance cue, directing the axons from the pathway towards the tectal anlage and their target cells.

104.13 REGULATION OF THE PHOSPHORYLATION AND STEADY-STATE LEVELS OF MAP1.2 BY NERVE GROWTH FACTOR. J.M. Aletta, N.J. Cowan*, S.A. Lewis* and L.A. Greene. Depts. of Pharmacology and Biochemistry, NYU Medical Center, New York, NY 10016.

MAP1.2 is a phosphorylated microtubule-associated protein whose relative level is increased during NGF-mediated neurite outgrowth in cultured PC12 cells (Greene et al. JCB:96; 76-83, 1983). Although the NGF-induced accumulation of MAP1.2 protein in these cells has been purported to be 10-20 fold (Drubin et al. JCB:101; 1799, 1985), quantification of MAP1 mRNA levels during NGF treatment revealed a much more modest increase (Lewis et al. JCB:102; 2106, 1986). To resolve this apparent discrepancy, we have now further characterized the NGF-dependent regulation of MAP1.2 in PC12 cells during process outgrowth.

MAP1.2 was identified in lysates of whole cells by its comigration with immunoprecipitated material on low percentage SDS-polyacrylamide gels. The gels were either silver-stained or dried for autoradiographic detection of proteins previously labeled metabolically with ^{35}S-methionine (3 days) or ^{32}P-orthophosphate (2 hrs). Using quantitative densitometry of gel autoradiograms generated from sister cultures, we found that over a time course of 15 min to 2 weeks or more of NGF-treatment, the steady-state levels of MAP1.2 protein, relative to total cell protein, were increased only 3.5x (±0.4 SEM; n=5). This was corroborated by the silver-stained material. Phosphorylation of MAP1.2 in cells treated with NGF for at least 2 weeks, when corrected for these increases in protein levels, was elevated an average of 4.1-fold per molecule (±1.0) above that in untreated cells. The increase in phosphorylation commenced rapidly (2.6x ±0.3 within 15 min.) followed by a slower increase over days to weeks; in contrast, the increase in protein level commenced only after 2-3 d of treatment, rising slowly thereafter. NGF also evoked a progressive decrease in the mobility of MAP1.2 on gels. This appeared due to enhanced phosphorylation since MAP1.2 from NGF-treated cells could be converted to the more mobile form characteristic of untreated cells by exposure to alkaline phosphatase.

Our results demonstrate that under the influence of NGF both the steady-state levels and the phosphorylation of MAP1.2 are increased. The level of phosphorylation is elevated prior to the induction of protein levels and continues to increase afterwards as well. Activators of kinases A or C were much less effective than NGF with regard to MAP1.2 phosphorylation, although with respect to a Triton-insoluble cytoskeletal substrate, they were equally or more effective mediators of phosphorylation.
(Supported by NIH grant NS16036, a basic research grant from the March of Dimes Birth Defects Fdn. and NRSA fellowship NS07754.)

105.1 STOCHASTIC ANALYSIS OF THE CENTER OF PRESSURE MOVEMENT IN QUIET STANDING Z. Ladin*, M.H. Hanno*, S.H. Roy and C.J. De Luca (SPON: H. Voigt) NeuroMuscular Research Center, Boston University, Boston, MA 02215

The momentary movements of the center of pressure (COP) of a standing subject, as measured by a force platform, produce a trace called a stabilogram. This trace represents the output of a complex sensorimotor system aimed at maintaining an erect posture of a human subject standing still. Some global aspects of this trace (e.g. total distance traveled, minimum bounding radius, etc.) have been studied, but only recently was it attempted to extract some quantitative information from the momentary movements of the COP. This study presents another step in that direction and describes the stochastic nature of the direction changes in the COP trajectory.

A series of stabilogram studies were conducted on human subjects of a young age-group (20's to early 30's) and an old age-group (70's). The subjects were asked to stand still on a force-platform in a comfortable posture while looking at a picture on the wall opposite them. Data were collected for a period of 15 seconds at a sampling-rate of 200Hz. Experiments were repeated for a total of eight times interchanging between "eyes-closed" and "eyes-open" states. An algorithm to calculate the angle change occuring between two samples was developed. The histogram of the angle changes in the range of $\pm 180^{\circ}$ was calculated and displayed, excluding stationary points, i.e. the sample points which represented movements smaller than the resolution of the measurement system.

Preliminary results suggest the existence of qualitative differences between the histograms of the two age-groups. The histogram for the young age-group had a U-shape with peak values at $\pm 180^{\circ}$ and a uniform distribution in-between. such a distribution is indicative of a movement which occurs mostly along a straight line. The histograms of the older age group showed significant deviations from the above. The main feature we observed included two peaks at angles close to $\pm 130^{\circ}$. Such a distribution suggests that the minute sway motions which are along a straight line for the young age-group, are replaced by deviations from a straight line in preferred directions for the older age-group.

An interesting finding involved the percentage of time spent in a stationary manner (i.e. - sample points which moved a total distance smaller than the resolution of the system). Stationary samples were in the range of 0.2-6% of the total time. No correlation with age was found for the specific group of subjects in our study. Hence, the results suggest that age affects the nature of the CP movement, rather than its ability to be stationary. The study was supported by Liberty Mutual Ins. Co.

105.2 IDENTIFICATION OF AN M2 LATENCY RESPONSE TO POSITIONAL STRETCH. J. S. Thomas, Department of Physiology, Meharry Medical College, Nashville, TN 37208

During behavioral tasks requiring maintenance of a defined posture (of elbow, wrist, or finger) against an expected load change, a step increase in load evokes a characteristic pattern of EMG response peaks usually labeled "M1" (25-50 msec.), "M2" (50-100 msec.), and "M3" (100+ msec). Flutter frequency (8 - 50 Hz) square wave modulation of torque load produces similar M1/M2 response peaks (as timed from the onset of the loading phase of the forcing), but this evolving pattern is truncated by the inhibition resulting from the subsequent unloading phase of the load modulation. If the periodic load modulation is terminated by the omission of the unloading half of the last cycle, the result is a step increase in net load equal to one half the periodic amplitude whose onset is timed from the point where the missing unloading torque step would have occurred. As tested in six human volunteer subjects, such "Missing Unload" termination of periodic forcing produces an M2 latency peak of EMG response as timed from the point of the missing unload. Using various frequencies of terminated forcing it can be demonstrated that this "Missing Unload Response" (MUR) bears no constant latency relationship to the M1/M2 latency events evoked by the loading transient of the periodic forcing and thus must derive from a different reflex mechanism.. Responses to other waveforms of terminated periodic forcing (i.e., 'sawtooth' vs 'inverse sawtooth' waveforms which shift the onset of the periodic load termination by 180 deg. with respect to the phase of the kinetic parameter variations induced by the preceding periodic torque) show that the MUR is NOT a response to the detection of the onset of deviation from an accommodated forcing pattern, but seems instead to be timed from achievement of a critical level of positional stretch away from the minimal length associated with the preceding periodic fluctuations in joint angle. This conclusion is reinforced by tests which vary the waveform of the terminal cycle so as to retard or accelerate the resulting positional excursion and which produce corresponding changes in the latency of the MUR burst. Missing Unload termination of periodic forcing thus demonstrates an M2 latency response component to load change which is independent of the afferent source of the M1/M2 latency responses to acceleration and which can be conveniently used to assess the static components of behavioral reflex gain in human and animal subjects. Supported by NIH grant RCMI 03032

105.3 ABSENCE OF DISTINCT MUSCLE SYNERGIES DURING POSTURAL RESPONSES IN CATS TO TRANSLATION OF THE SUPPORT SURFACE. J.M. Macpherson. Dept. of Anatomy, Queen's University, Kingston, Ont. Canada K7L 3N6

It has been suggested that the computational problems faced by the nervous system in producing controlled movements could be simplified by controlling several muscles as a unit, a muscle synergy, thereby reducing the number of degrees of freedom (Bernstein, 1967). This study was designed to explore the synergic organization of muscle activation patterns in limb muscles during postural reactions to movements of the support surface in the horizontal plane.

Cats were trained to stand on a moveable support surface, with each paw on a miniature, triaxial force plate. The animals were required to keep their weight about equally distributed between the left and right sides. Trials consisted of ramp-and-hold movements of the platform in one of sixteen different directions in the horizontal plane, with 0° being tailward, 90° leftward, 180° headward and 270° rightward. Recordings consisted of 50 ms of quiet stance followed by the platform movement and postural response, for a total of 500 ms. The data included the three components of force exerted by each paw, vertical, longitudinal and lateral, the position of the platform, and electromyographic (EMG) activity. Eight EMGs were recorded from each of 6 cats using implanted pairs of wire electrodes, and included 6 muscles of the left forelimb and 9 of the left hindlimb.

During quiet stance, all cats exerted tonic horizontal forces directed backward and outward for the hindlimbs and forward and outward for the forelimbs. The maximum corrective forces exerted by any limb were observed for translations that were directly opposite to the horizontal force vector of quiet stance. Thus, the left hindlimb was maximally loaded, and exerted the largest horizontal forces for translations of the platform that were forward and to the right (225°).

Each muscle was active over a broad range of angles of platform movement, showing a gradual increase in activity to a peak and then a decrease, as angle of translation increased, the 'tuning curve'. While the tuning curves of many muscles overlapped, they were not identical. For example, gluteus medius was active between 157° and 315°, with maximum activity at 225°. Gracilis was active from 135° to 247°, with a maximum at 180°. The tuning curve for each muscle appeared to be related to its biomechanical action on the limb. The postural response in any one direction consisted of a unique blending of activity of many muscles such that the limb exerted the appropriate force vector to correct trunk position. Therefore, for postural responses, muscles appear to be controlled independently rather than in distinct synergic groupings.
Supported by MRC of Canada and Queen's University.

105.4 INDEPENDENT ACTIVATION OF COMPARTMENTS OF FELINE BICEPS FEMORIS DURING POSTURAL RESPONSES TO TRANSLATIONS OF THE SUPPORT SURFACE. C.M. Chanaud and J.M. Macpherson. Lab of Neural Control, NINCDS, NIH, Bethesda, MD 20892 and Dept. of Anatomy, Queen's Univ., Kingston, Ontario K7L 3N6.

A recent glycogen depletion study of biceps femoris (BF) in the cat has revealed three separately innervated compartments: anterior (BFa), middle (BFm) and posterior (BFp) (English & Weeks, J. Morph. 19:161-175, 1987). Recordings of EMG during various natural behaviors have demonstrated differential activation of these three regions (Chanaud et al., Neurosci. Abstr. 12:686, 1986). The current study explores the differential functions of BF during postural responses to translations of the supporting surface in the horizontal plane.

Each of two cats were implanted with a chronically indwelling Silastic patch containing 8 pairs of electrodes that were evenly spaced across the width of the BF muscle of the left hindlimb. The cats were trained to stand quietly on a hydraulically-driven, moveable platform with each of the four paws placed on separate, triaxial force plates (Macpherson et al., J. Neurosci. Meth., in press). The platform was moved in a linear trajectory in any of 16 directions in the horizontal plane (presented in varying orders), with 0° defined as a tailward translation, 90° a leftward translation, 180° a headward translation and 270° a rightward translation. The EMGs, the forces exerted by each paw, and the position of the platform were recorded on-line for 100 ms before and 900 ms after the onset of platform movement. At each new direction, several practice trials were presented before data collection.

During those perturbations that loaded the left hindlimb (203°-270°), BFa was activated along with other hip extensors and abductors (Macpherson, Neurosci. Abstr. 13, 1987) while BFp was inactive. In contrast, perturbations that unloaded the left hindlimb (45°-135°) resulted in inhibition of tonic BFa activity and activation of BFp, the latter presumably for knee flexion. BFm was activated at angles in between those for which BFa and BFp were involved, but was most similar to BFa.

Activation of the various compartments of biceps femoris depended on the direction of translation of the support surface in the horizontal plane, and could be related directly to the differential biomechanical actions of BFa and BFp at the hip and knee joints, respectively, as required for the postural correction.

105.5 KINESTHETIC COORDINATION OF A MULTI-JOINT ARM MOVEMENT. P.J. Cordo. Neurological Sciences Institute Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209

To control targeted movements of the whole arm, the nervous system must coordinate the motions of different joints. Coordination might be learned and then implemented via open-loop control or it might be produced by reafferent sensory input. Sensory control of coordination might be preferable to open-loop control, because sensory input could compensate for variability in motor output and changes in the mechanical environment. This experiment examined whether the nervous system can use kinesthetic input from the arm to coordinate a throwing-like movement of the elbow and hand.

In a movement task resembling frisbee throwing, subjects extended the elbow horizontally at 16 deg/s through an arc of 40-50 deg and opened the hand as the elbow passed through a 2 deg wide target zone. The target zone was located approximately half way between the starting and ending angles. The forearm was placed in a cuff that resisted elbow extension with a standard stiffness of 0.3 Nm/deg. In randomly presented trials, the stiffness was either increased to 0.5 Nm/deg or decreased to 0.1 Nm/deg just before the subject began to extend the elbow. The function of stiffness changes was to speed up or slow down the elbow rotations.

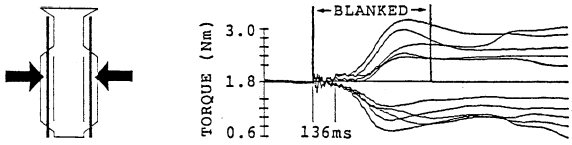
The experiment was designed to determine whether subjects were able to open the hand in the target zone independent of the changes in elbow velocity. To do this, subjects would have to employ kinesthetic input from the moving limb since no visual feedback of elbow angle was provided, and the changes in stiffness and velocity were unpredictable.

Subjects were able to open the hand in the target zone with a high degree of accuracy, independent of the imposed changes in elbow velocity. Subjects compensated for the changes in elbow velocity with two distinct mechanisms. Approximately 250 ms after subjects initiated their elbow rotations, the first mechanism corrected for errors in elbow velocity by adjusting the level of agonist muscle activity and elbow torque. Nevertheless, the elbow passed through the target zone at distinctly different times due to early leads and lags produced by stiffness changes. Subjects compensated for these timing differences with a second mechanism that adjusted the timing of the hand movement.

105.6 KINESTHETIC CONTRIBUTIONS TO HUMAN BIMANUAL COORDINATION. M. Flanders and P.J. Cordo. N.S.I., Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209

Although our everday experience suggests that kinesthetic sensory input helps to coordinate voluntary movement, experimental evidence for this is incomplete. For example, Cole et al. (Exp. Brain Res.,56:582,1984) showed that during pinching, perturbation of one finger increases the agonist muscle activity of both fingers. Our research (Flanders et al., J. Motor Behav.,18:427, 1986) suggested that this type of increase in activity may be similar to a startle response. A kinesthetically driven system that coordinates movement should be capable of evoking accurate decreases as well as increases in muscle activity. The present study was designed to demonstrate that kinesthetic input from a perturbed elbow can evoke graded increases or graded decreases in the muscle activity of the other elbow.

Five subjects were trained to respond to an imposed rotation of the right elbow by quickly changing the torque of the isometric left elbow. All rotations and torques were in the horizontal plane. Subjects began each of 50 trials by producing flexion torque at both isometric elbows. As shown below, this torque moved left and right, visually displayed, tracking lines toward each other and into their respective target zones. After 1-3s, the display was blanked and the right elbow was randomly extended or flexed 4,6,8,10, or 12 deg. The correct response was a corresponding 0.4,0.6,0.8, 1.0, or 1.2 Nm increase (for extension) or decrease (for flexion) in the torque of the left elbow. Knowledge of results was provided by the reappearance of the display, 700ms after its disappearance.



The averaged (N=5 trials) torque responses of one subject are shown above. For all subjects, the average latency for responding in the correct direction was 123ms (SEM=8ms). Kinesthetically evoked responses were graded with stimulus amplitude before the visual display reappeared. Further data analysis was focused on the latency of accurately graded muscle activity and on improvement of this activity during motor learning.

105.7 EMG MEASUREMENTS SIGNIFICANCE IN THE EVALUATION OF FATIGUE. A.B. Arsenault, D. Gagnon*, S. Nagata* and G. Smyth*. Ecole de réadaptation, Université de Montréal and Centre de recherche, Institut de réadaptation de Montréal, Montréal, Qc, Canada H3S 2J4.

Ten normal subjects participated in a study designed to contrast results obtained pre and post-fatigue. The measures contrasted were; the slope of the EMG/Torque relationship, the mean and the median frequencies of the EMG power spectrum and the IEMG ratios of agonist/antagonist pairs of muscles. The experimental task was a 8 second ramp isometric elbow flexion ranging from 0 to 100% maximal voluntary contraction (MVC) (elbow angle = 90°). IEMG ratios and power spectra statistics were obtained over 1024 points of data at levels of 20, 40, 60 and 80% MVC. The following muscles were recorded from with miniature Beckman surface electrodes and TECA MarkIII pre-amps and amplifiers; Biceps-brachii (BB), Brachio-radialis (BR), Triceps-brachii (TB) and Anconeus (AN). The torque was measured at the wrist using a CybexII dynamometer. Fatigue was induced using a 60% MVC of elbow flexion maintained during at least 5 minutes. The data were collected on-line using a PDP 11/23+ computer and a sampling rate of 1kHz. The results were as follows. The expected increase, post-fatigue, of the EMG/Torque relationship for both BB and BR was found in only half of the subjects. In the other half, a drastic loss of the linearity of this relationship was observed with a levelling off of the EMG at 40% MVC. The statistics of the power spectra showed significant shifts (ANOVA for repeated measures, $p<.05$) towards the low frequencies at the levels of 40, 60 and 80% MVC. Finally, the IEMG ratios (BB/TB and BR/AN) presented a marked decrease at the levels of 40, 60 and 80% MVC at the post relative to the pre-fatigue state. The BB/BR ratios remained stable. It appears that these changes in the post-fatigue ratios disclose an increased level of contraction of the antagonists which may exert an inhibitory effect on fatigued agonist. Furthermore, while the torque and the statistics of the power spectra are sensitive, as measures, to the fatigue state, it appears that the EMG/Torque relationship cannot be considered a reliable measure for this physiological state, as has been suggested previously.

105.8 MOVEMENT MODULATION OF A HUMAN Ib INITIATED HETERONYMOUS REFLEX. J.D. Brooke, W.E. McIlroy, Human Biology/Biophysics, College of Biological Sci., University of Guelph, Guelph, Ont. Can. N1G 2W1.

Modulation of the electromyographic (EMG) response to stimulation of flexor reflex afferents occurs over the locomotor cycle in cat and human. There is also modulation of responses to group I stimulation during fictive locomotion in the mesencephalic cat. The present study reports that, following transcutaneous stimulation of Ib fibres in humans, a heteronymous reflex from ankle afferents to knee extensors shows substantial modulation over the cycle of leg movement. Subjects pedalled an ergometer instrumented for angular position of the pedal crank and for pedal reaction force. EMG responses were evoked in vastus medialis (VM) muscle, following low threshold, 1 ms square wave, stimulation of the common peroneal nerve serving the dorsiflexors of the ankle. The stimulus was applied at different points in the 360° rotation of the pedal crank, with 20 to 50 samples taken per point, for each subject. The direct activation (M wave) of the tibialis anterior muscle (TA) was also recorded and compared against M max obtained separately for each movement point, to control for variations in the intensity of stimulation. During normal pedalling, VM was active from approx. 290° to 110° of pedal crank rotation (0° being top dead centre for the crank).The burst in rectus femoris started earlier, closer to 220° of rotation. TA ranged approx. 163° to 7°, soleus 25° to 155° and lateral gastrocnemius 25° to 250°. In response to stimulation, reflexes were observed in VM at short latency (22-30 ms), with maximum peak to peak amplitudes up to 35% of those seen in maximum voluntary contractions. Around the movement cycle, the substantial modulation of this VM reflex was uncorrelated with changes in the magnitude of the M wave. The modulation was strongly associated, in timing and magnitude, with the locomotor burst normally occurring in VM, reducing from its peak to the extent that, between approx. 110° and 245°of pedal crank rotation, no VM reflex occurred. The threshold for the reflex was approx 10% ongoing contraction in VM. If bursts of VM activity were voluntarily intruded into the movement phase when VM normally was quiet, the reflex reappeared. In contrast, no effect of the group I stimulation on contralateral VM or TA was observed, with or without contraction of the target muscles. This supports the view that the contralateral path involves higher threshold interneurones. It is concluded that this ipsilateral reflex depends on the contraction of the target muscle. This is probably through facilitation of the motoneuron pool, similar to the locomotor drive potential described for the mesencephalic cat. However, the reflex modulation is not specific to the patterning of normal locomotion. Supported by NSERC (Canada) Grant #A0025.

105.9 NEURONAL CONTROL OF MOTOR BEHAVIOR IN THE ELECTRIC FISH Eigenmannia

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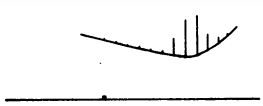
The segmental motor activity during so-called "probing" (Behrend, K., Neurosci., 13:171, 1984) of the electric fish Eigenmannia was investigated. When probing the fish bends its tapering caudal end (see fig.) presumably to fully exploit the dynamic properties of its sensory brain centers which in turn leads to an improved perception of objects of interest. The fish swam in a 10 x 20 cm channel and was stimulated at various positions in the channel by a short between a point electrode and a distant ground. The motor activity was monitored by a specially developed optical method with a time resolution of 20 msec. As a measure of motor activity the curvature of the fish was calculated every 8 mm along its longitudinal axis and interpreted as the activity of the motoneuron pool of the particular segmental muscles. The data show that the bending pattern is fairly uniform: the distribution of activity always has one peak and its amplitude decreases smoothly to the right and left (see fig.). Activity always starts simultaneously - within the limits of temporal resolution - in the segments involved in the movement. The center of activity may be shifted along the body axis to meet the needs of the fish. In the brainstem each body segment is represented in eight different nuclei by only one neuron in most cases (Behrend, K., Donicht, M. in prep.). Electrical stimulation in two of these nuclei elicits bending albeit in different contexts (Behrend, K., Neurosci. Abstr., 1985). The pattern may then be explained by assuming an excitatory coupling of each neuron to its neighbours weighted decreasingly as the distance between the neurons increases.



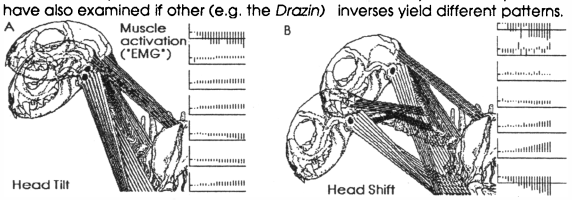
Fish (continuous line) seen from below; head is to the left. The amplitude of curvature is represented by the bars. The small square marks position of stimulation.

(Supported by DFG Be 658/4-1)

105.10 MULTIDIMENSIONAL SENSORIMOTOR "PATTERNS" ARISING FROM A GRAPHICS-BASED TENSORIAL MODEL OF THE NECK-MOTOR SYSTEM. **J. Laczkó***, KFKI/MSZKI Budapest, Hungary, 1525, **A. J. Pellionisz,** Dept. Physiol. Biophys., New York Univ. Med. Ctr. New York, NY, 10016, **B.W. Peterson,** & **T.S. Buchanan***, Dept. Physiol., Northwestern Univ. Med. School, Chicago IL, 60611. (SPON: G. Ostriker)

The general problem of how sensory reception is transformed, via neuronal networks, into motor execution led us to build a multidimensional tensorial model of the neuro-musculo-skeletal head control system of the cat *(Pellionisz & Peterson, 1987, in: "Control of Head Movement", Oxford U.P.).* The structuro-functional basis of the model is the availability of general (non-orthogonal, overcomplete) coordinates that are intrinsic to neural, muscular and skeletal expressions of sensory and motor events. With the numerical values of muscle orgin and insertion points and a single center of head-rotation revealed *(cf. Baker & Wickland, Ibid)* the tensorial model can provide experimentally verifiable predictions *(cf. Peterson et al., 1987, Proc. Symp. Biomech. & Neural Contr.).* When further developing this model, one of the considerations is that the center of rotation of the head/neck system is not fixed. This necessitates accurately modeling the skeleton. A further phenomenon to be accounted for by the model is that animals may use different muscle patterns when making a head movement in different paradigms *(Keshner et al., Soc Neurosci. Abstr, 1986).*

Thus, the tensor approach was extended with a graphics-based computer model *(Pellionisz,1987, In: "Comp. in Brain Sci.", Cambridge U.P.).* 2D diagrams of the skeletomuscular system were inputed. By specifying joints and muscle origin and insertion-points, the overcomplete non-orthogonal systems of coordinates were calculated. Predictions were made by the *Moore-Penrose* inverse, since the model requires the solution of an overdetermined system of equations. We have also examined if other (e.g. the *Drazin)* inverses yield different patterns.



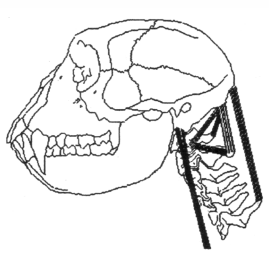
A surprising result gained by this generation of tensor models is that with only slightly different movement-intentions the emerging movement-patterns can be drastically altered (see *tilt* around a *single* center in **A**, versus *shift* around *two* centers in **B**). The emergence of different CNS patterns is of great interest because it provides insight into how a single neuronal mechanism might yield different "synergies", "schemas" or "strategies" to coordinate the multitude of degrees-of-freedom of multi-joint movements given a desired trajectory.

Such tensor models are research tools for the interpretation of sensorimotor experimental data in several species. They can also be used to predict muscle activity which can be used both for direct comparison with EMG data and for studies related to functional neuromuscular stimulation. — Supported by NS 22999

105.11 ROLE OF THE SUPERFICIAL & DEEP NECK MUSCLES IN THE CONTROL OF MONKEY HEAD MOVEMENT: APPLICATION OF THE TENSOR ANALYSIS APPROACH. F. Lestienne*, Ph. Liverneaux* CNRS Lab. Neurosensorielle, Paris, France 75270, A. Pellionisz, Dept. of Pysiol. & Biophys. New York Univ. Medical Center, New York, NY, 10016, USA. (SPON: A. Berthoz)

The understanding of structuro-functional principles of sensorimotor systems hinges on a proper quantitative knowledge of the complex anatomical features of the system and on the power of mathematical concepts and formalisms applied to the explanation of its functioning. On the basis of a recent multidimensional geometrical approach applied to model cat's head control system *(Pellionisz and Peterson 1987, In: "Head Control", Oxford U.P.)* our aim was to interpret, by this kind of a tensorial model, the complex neck muscle synergies acting on a multisegmental skeletal-cervical column of the monkey *Macaca Mulatta.*

The sub-occipital muscles (rectus capitis posteror major and minor, obliquus capitis superior and inferior) and the medial superficial neck muscles (semispinalis capitis, splenius capitis and trapezius) were implanted with bipolar electromyographic electrodes in female rhesus monkeys. X-ray opaque markers were placed in the two rectus capitis posterior major and in the two splenius capitis muscles to allow visualization, using successive X-ray exposures made in synchronization with a dedicated digital hardware system, for movement-analysis via real-time video signal processing. Head movements and the electromyographic activities were recorded during free voluntary head movements. A computer modeling technique was adapted that is applicable even if anatomical data are available only in graphical form: the computer was utilized to extract the quantitative information *(cf. Pellionisz, 1987, In: "Computers in Brain Sci.", Cambridge U.P.).* As the first step of such modeling, 2D diagrams of the cervical column and the neck muscles of *Macaca Mulatta* were inputed to a widely available graphical processor. By selecting joint-points and muscle origins and insertion points, the overcomplete system of coordinates of individual muscle contractions were automatically calculated. Since tensor network theory postulates a unique distribution of muscle activities even in such overdetermined systems, activations of mucles (EMG) and movements, according to any movement-intention in the 2D, could be calculated and displayed.



From EMG data it has been found that sub-occipital muscles and medial superficial muscles are simultaneously activated during pitch movent, whereas during yaw movements these two groups of muscles are activated in a sequential order. The tensorial modeling approach is able to predict the pitch-pattern of motor activation of the 7 pairs of deep and superficial neck muscles in its present form, and work is in progress to model full 3D rotations also including yaw and roll.— Support: CNES /87 /1228 and NS 22999 &NS13742

106.1 LATERAL MOBILITY OF CHICK ACETYLCHOLINE RECEPTORS IS UNAFFECTED BY FACTOR THAT INCREASES THEIR DENSITY. J.M. Dubinsky, D.J. Loftus*, G.D. Fischbach, E.L. Elson.* Washington University School of Medicine, St. Louis, Mo. 63110.

The mobility of acetylcholine receptors (ACHR) in rat myotubes and Xenopus myocytes has been measured by fluorescence photobleaching. Similar data are not available in chick muscle cells. In rat and Xenopus, a high fraction of the diffusely distributed receptors are mobile (rat=75% Axelrod et al 1976, Xenopus=68% Kidokoro et al 1985). The estimated diffusion coefficients (D, at 22°C) are $5*10^{-11}$ cm^2/s in rat and $2.5*10^{-10}$ cm^2/s in Xenopus. In contrast, few if any ACHRs are mobile within high density receptor clusters.

We have assayed the mobility of diffuse and clustered receptors in control chick myotubes and in myotubes treated with a partially purified factor known to increase ACHR density (Usdin & Fischbach 1986). Chick cells were grown on glass coverslips and the ACHRs were labeled with rhodamine-alpha-Bungarotoxin (RdBTX). Receptor mobility was assayed by monitoring the recovery of fluorescence within a 4um circle after bleaching the same area with an intense laser flash. 38% (±19% s.d., n=15) of the diffusly distributed ACHRs were mobile, with calculated D of $7.3*10^{-10}$ cm^2/s ($\pm 1.2 * 10^{-10}$). Less than 10% of the ACHRs within clusters were mobile. Similarly, in ciliary ganglion-muscle cocultures, few if any receptors within neurite-associated receptor patches (NARPs) were mobile, even if the NARPs were located at growth cones. The fact that the mobile fraction of chick ACHRs is significantly less than that estimated in Xenopus and rat is consistent with the observation that receptor migration and trapping does not make a significant contribution to the initial accumulation of ACHRs at developing NARPs in chick myotubes. The role of local insertion of new ACHRs was evident when cell surface ACHRs were blocked with unlabeled BTX and new ACHRs were visualized with RdBTX between 1-6 hr later. Digitized images revealed new receptors distributed throughout NARPs rather than in a rim around their perimeter.

As expected, the RdBTX fluorescence intensity of myotubes treated with a partially purified factor from chick brain extract, was greater than that of controls. However, the mobile fraction of diffuse ACHRs in treated cells (44% ± 15%, n=8) and diffusion coefficient ($5.7*10^{-10}$ cm^2/s ± $1.8*10^{-10}$) were comparable to control values. Significantly, the intensity of RdBTX fluorescence increased more at hotspots (5.7x) than at non-hotspot regions (2.1x). This is consistent with the notion that brain factor influences ACHR insertion in the vicinity of clusters more than eleswhere.

JMD was an MDA postdoctoral fellow. DJL was supported by NIH GM 27160.

106.2 SPONTANEOUS FORMATION OF ACETYLCHOLINE RECEPTOR CLUSTERS TRIGGERED BY BRIEF EXPOSURE TO EXTRACELLULAR ELECTRIC FIELD. B. Podbilewicz* and M-m. Poo (SPON: J. Dani). Department of Cell Biology and Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, CT 06510

Application of an extracellular electric field to culture cells may result in a redistribution of membrane-bound molecules in the plane of plasma membrane, a phenomenon termed in situ electrophoresis (Poo, M-m. 1981. Ann. Rev. Biophys. Bioeng., 10:245). It was found previously that prolonged (>3 hrs) exposure of embryonic Xenopus myocytes in 2-d old primary culture to a field of 10 V/cm caused formation of large immobile acetylcholine (ACh) receptor clusters on the cathode-facing side of the cell, as shown by the clustered distribution of binding sites for fluorescently labelled α-bungarotoxin. Brief exposure to the same field for durations less than 1 hr produced low levels of ACh receptor accumulation and formation of small receptor clusters on the cathodal side. Here we report a surprising finding that when the cells briefly treated with the field were incubated at culture conditions in the absence of the field for a duration of 6 hrs prior to the labelling of fluorescent α-bungarotoxin, the percentage of cells showing asymmetry of receptor distribution became higher and the average size of cathodal-facing receptor clusters became larger than those of similar field-treated cultures that were not incubated. Apparently small clusters of ACh receptors induced by brief treatment of the field served as a nucleus for the spontaneous formation of larger clusters at the same site, a process analogus to crystal formation in aqueous solution. For a field of 10 V/cm, the minimum duration of field exposure that can induce detectable post-field spontaneous clustering was found to be about 10 minutes. The resolution of the present technique does not allow us to determine whether this minimum duration corresponds to the time required for the formation of a small cluster of a certain critical diameter.

A field of 10 V/cm corresponds to a potential drop of 20 mV across a Xenopus myocyte 20 μm in width. Fields of similar strength could be generated at an active synapse by synaptic currents that occur at high-frequency. It seems possible that in the early stages of development brief bursts of synaptic current could produce a relatively long-term effect in the distribution or the density of membrane receptors and ion channels by a physicochemical mechanism similar to that described here. Work supported by NIH grant NS-22764.

106.3 DEVELOPMENT OF THE ACHR CLUSTERS: COMPETITION BETWEEN TORPEDO ELECTRIC ORGAN BASEMENT MEMBRANE EXTRACT AND POLYCATION-COATED LATEX BEADS. Ding-Liang Zhu*. H. Benjamin Peng and L.L. Rubin. Dept. of Anatomy, Univ. of North Carolina, Chapel Hill, NC 27514 and Rockefeller Univ., New York, NY 10021.

Molecules in the basement membrane of Torpedo electric organ have been shown to induce the formation of acetylcholine receptor (AChR) clusters in cultured chick myotubes. In our laboratory, we have found that polycation-coated latex beads induce the AChR clustering in cultured Xenopus muscle cells. In order to further understand the nature of the stimulation which leads to this postsynaptic differentiation, we examined the effect of the Torpedo electric organ basement membrane extract on Xenopus muscle cells and its competition with the latex beads. A high salt-soluble extract was prepared from the Torpedo electric organ basement membrane. At a protein concentration of 50 μg/ml, this extract caused a two-fold increase in the number of AChR clusters in cultured Xenopus muscle cells within 24 hr. Although individual clusters in extract-treated cells were somewhat smaller in size than those in the untreated cells, the total area occupied by the clusters in each cell was clearly larger in the extract-treated cells. When the cells were treated with polyornithine-coated latex beads, AChR clusters formed at the bead-muscle contacts. However, when the beads were applied in the presence of the extract, the bead-induced AChR clustering was suppressed. If the extract was applied first and followed by the addition of the beads, the latter failed to induce the cluster formation. Furthermore, if the extract was applied after the clusters were first induced by the beads, it caused a reduction in the number of bead-associated clusters and an increase in the number of extra-bead clusters. However, extract covalently linked to latex beads failed to exert any AChR clustering activity. These results indicate that the extract reversed the cluster-inducing effect of polycation-coated latex beads in Xenopus muscle cells. This may be due to an interference by molecules in the extract on the bead-induced local specializations responsible for the formation and the maintenance of the clusters. An understanding of this phenomenon should help elucidate the mechanisms of the postsynaptic induction by the basement membrane and by the latex beads. (Supported by NIH grants NS 23583 and NS 21767 and by the Muscular Dystrophy Association)

106.4 MOLECULES SIMILAR TO AGRIN ARE CONCENTRATED IN MOTOR NEURONS. C. Magill, B.G. Wallace & U.J. McMahan. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Monoclonal antibodies against agrin, which is extracted from the synapse-rich electric organ of Torpedo, selectively stain cell bodies of motor neurons in embryonic and adult spinal cord. Agrin causes the formation of several components of the postsynaptic apparatus on cultured myotubes, including aggregates of acetylcholine receptors (AChRs). These findings lead to the hypothesis that molecules similar to agrin are released by motor axon terminals to cause the formation of postsynaptic apparatus in embryonic muscle and the maintenance of the apparatus in adult muscle. It also suggests that motor neurons provide the molecules stably bound to basal lamina at the adult neuromuscular junction, that direct the formation of postsynaptic apparatus on regenerating muscle fibers in the absence of axon terminals. The experiments described here were aimed at determining if motor neurons contain and release agrin-like molecules with AChR-aggregating activity.

First we made extracts of the electric lobe of the brain of Torpedo, the region containing the cell bodies of the motor neurons that innervate the electric organ, and of spinal cords from adult Torpedo and frog and embryonic chick. Each of the extracts caused the formation of AChR aggregates on cultured myotubes. Monoclonal antibodies against agrin immunoprecipitated the AChR-aggregating activity indicating that the active molecules were antigenically similar to agrin. Extracts of regions of CNS that contain relatively few motor neurons had relatively little activity. Since anti-agrin antibodies selectively stain motor neuron cell bodies and since regions of the CNS in which cell bodies of motor neurons are concentrated are enriched for agrin activity, we conclude that motor neurons contain molecules similar to agrin.

Second, we made extracts from cultures containing dissociated chick spinal cord cells. The spinal cords were taken from a developmental stage when motor neurons are numerically predominant. As expected, such extracts contained AChR-aggregating activity. Moreover similar activity was detected in medium conditioned by the spinal cord cells, consistent with the hypothesis that motor neurons release molecules similar to agrin.

106.5 PURIFICATION AND CHARACTERIZATION OF AGRIN. M.A. Smith, B.G. Wallace, Y.-M.Yao, J. W. Schilling, P. Snow and U.J. McMahan. Depts. of Neurobiology and Biology, Stanford Univ., Stanford, CA 94305. Calif. Biotech. Inc., Mountain View, CA 94043.

Extracellular matrix-rich extracts of Torpedo electric organ contain agrin, which causes the formation of patches on cultured chick myotubes that resemble the postsynaptic apparatus at the neuromuscular junction; they contain high concentrations of acetylcholine receptors (AChRs), acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE). We now have found that other components of the postsynaptic apparatus are concentrated at agrin-induced patches and have purified to homogeneity four structurally related polypeptides, two of which have agrin activity.

Incubation of cultured chick myotubes with agrin-containing extracts induces the formation of patches at which six components of the postsynaptic apparatus are concentrated: two extracellular matrix molecules, a heparan sulfate proteoglycan and an asymmetric form of AChE; three membrane components, AChRs and globular forms of AChE and BuChE; and a cytoplasmic molecule, a 43kDa receptor-associated protein. All of the aggregating activities were immunoprecipitated by each of four different anti-agrin monoclonal antibodies. All four of the monoclonal antibodies immunoprecipitated polypeptides of 150, 135, 95, and 70 kDa. To determine which of the activities was associated with each of the polypeptide species, extracts were chromatographed on a gel filtration column and the fractions analyzed for each aggregating activity and for agrin-like polypeptides. Our results indicate that both the 150 and 95 kDa polypeptides cause aggregation of all of the postsynaptic components, while the 135 and 70 kDa polypeptides have no detectable aggregating activity.

To characterize agrin further, we have purified each of the antigenically related polypeptides to homogeneity by preparative polyacrylamide gel electrophoresis. N-terminal amino acid sequencing indicates that the 95 (active) and 70 kD (inactive) polypeptides are identical at their N-terminus, as too are the 150 (active) and the 135 kD (inactive) polypeptides.

Thus, we have used anti-agrin monoclonal antibodies to purify four structurally related polypeptides from electric organ extracts. Two of these, agrin-150 and agrin-95, cause the formation of patches on cultured myotubes at which at least six components of the postsynaptic apparatus at the neuromuscular junction are concentrated.

106.6 AGRIN-INDUCED ACETYLCHOLINE RECEPTOR AGGREGATION: Ca^{++} DEPENDENCE, INHIBITION BY PHORBOL ESTER, AND EFFECTS ON RECEPTOR DEGRADATION. B.G. Wallace. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Agrin, a protein extracted from Torpedo electric organ, is similar to molecules that direct the formation of the postsynaptic apparatus at the neuromuscular junction. For example, agrin causes the formation of patches on cultured chick myotubes at which several components of the postsynaptic apparatus are concentrated. The studies reported here were aimed at characterizing further agrin's effects on acetylcholine receptor (AChR) distribution and metabolism as a step toward determining its mechanism of action.

When agrin was added to the medium bathing chick myotubes it initially induced the formation of small ($< 4\ \mu m^2$) aggregates of AChRs. Aggregates began to appear within 2 hrs and increased rapidly in number until 4 hrs. Over the next 12-20 hrs the number of aggregates per myotube decreased as the mean size of each aggregate increased to approximately $15\ \mu m^2$. The large aggregates remained as long as agrin was present in the medium; if agrin was removed the aggregates disappeared slowly. For at least the first 6 hours of agrin-induced receptor aggregation all AChRs detected in aggregates accumulated there by lateral migration of AChRs present in the myotube plasma membrane at the time agrin was added. Agrin did not alter the rate of appearance of new AChRs.

Agrin-induced AChR aggregation required Ca^{++}; a half-maximal response occurred at 0.2 mM Ca^{++}. Co^{++}, Mn^{++}, and Ni^{++} inhibited agrin-induced AChR aggregation. Mg^{++} and Sr^{++} could not substitute for Ca^{++}, but did not block receptor aggregation in the presence of Ca^{++}. Agrin-induced receptor aggregation also was inhibited by the phorbol ester TPA, an activator of protein kinase C. Brief exposure (30 min-2 hrs) to 20 nM TPA completely prevented AChR aggregation. Two dimensional SDS-PAGE of extracts of cultures identified several polypeptides that were phosphorylated in response to TPA treatment. Agrin itself did not change the pattern of phosphoproteins.

Agrin-containing extracts of electric organ did cause a change in the rate of receptor degradation. In untreated cultures, two populations of AChRs were detected on the surface of cultured myotubes; most AChRs were rapidly turning over ($t_{1/2}$~1d), ~15% turned over more slowly ($t_{1/2}$~10d). Agrin-containing extracts increased the proportion of slowly turning over receptors to ~20%.

Thus agrin induces AChRs in the myotube plasma membrane to accumulate into aggregates by a rapid, Ca^{++} dependent process that is inhibited by protein kinase C-mediated protein phosphorylation and is accompanied by a decrease in the rate of degradation of a fraction of AChRs.

106.7 LOCALIZATION OF A SYNAPTIC ORGANIZING MOLECULE IN DEVELOPING MUSCLE. J. R. Fallon. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Agrin is a molecule derived from the extracellular matrix of Torpedo electric organ that organizes AchR and AchE on cultured muscle cells. Immunocytochemical studies with monoclonal anti-agrin antibodies have established that in normal and damaged muscle molecules closely related if not identical to agrin ('agrin-related molecules') are localized in the synaptic basal lamina (Fallon et al., Nature 315; 571-574, 1985; Reist et al.; Magill et al., Soc. Neurosci. Abstr.12(1): 189;190, 1986). These results provide strong evidence that agrin or related molecules play a role in directing the regeneration of the neuromuscular junction.

The present studies are aimed at determining the role of agrin in the development of the synapse. As a first step towards this goal the localization and time of appearance of agrin-related molecules has been compared to that of AchR clusters in the developing chick hindlimb. The dorsal muscle mass and two muscles derived from that structure, the fast posterior iliotibialis (PITB) and the predominantly slow iliofibularis (IFIB), were examined in detail. Frozen sections were double-labelled with monoclonal antibodies directed against Torpedo agrin and rhodamine-coupled α-bungarotoxin. Examination of serial sections of the entire thigh from embryos of stage 24-30 (embryonic day 4.5-7) revealed that anti-agrin staining was present throughout the dorsal muscle mass from as early as stage 24. AchR clusters were first detected at stage 25. At this stage and at all subsequent stages examined greater than 95% of the AchR clusters co-localized with agrin-related molecules. This co-localization was also observed in unpermeabilized whole mount preparations of stage 32-34 muscles, indicating that the agrin-related molecules as well as the AchR clusters were on the external surface of the cells. At all stages of development the distribution of agrin-related molecules in muscle was wider than that of AchR clusters. The extent of anti-agrin labelling on the muscle cell surface increased until at stage 42-44 (embryonic day 16-18) the entire surface of all myofibers was stained. At these later stages the level of extrajunctional staining was greater in the IFIB than in the PITB. However, in the mature chicken, agrin-related molecules in both muscles were restricted to the region at and immediately adjacent to the endplates.

These experiments demonstrate that in developing muscle agrin or a closely related molecule is 1) expressed before AchR clusters are detected and 2) co-localized with the earliest AchR clusters formed. These results suggest that agrin may play a role in directing the initial events of synapse formation during development.

Supported by NIH grant BRSG S07 RR05528

106.8 DISTRIBUTION OF PRE- AND POSTSYNAPTIC SPECIALIZATIONS DURING THE NORMAL DEVELOPMENT OF AN AMPHIBIAN MUSCLE. M.W. Cohen. Dept. of Physiology, McGill University, Montreal, Quebec H3G 1Y6.

The development of spatial alignment between pre- and postsynaptic specializations was examined in Xenopus myotomal muscle. Sites of clustered acetylcholine receptors (AChRs) were visualized after staining with fluorescent α-bungarotoxin. To view sites of presynaptic specialization in the same muscles indirect immunofluorescent staining was employed using a monoclonal antibody (generously provided by J. Bixby and L. Reichardt) directed against a synaptic vesicle antigen.

In mature myotomes the immunofluorescence was restricted to the ends of the muscle cells and aligned precisely with the sites of high AChR density. This immunofluorescence was not observed a) after denervation, b) in the absence of cell permeabilization, or c) when the monoclonal antibody was omitted from the staining protocol. These results, considered with those of previous studies, suggest that the monoclonal antibody is effective in revealing clusters of synaptic vesicles in Xenopus neurons.

At the onset of innervation of embryonic myotomes the presynaptic stain revealed nerve fibres coursing across the developing muscle cells. The nerve fibres were usually stained more intensely distally than proximally and their immunofluorescence terminated in broad expansions presumed to be growth cones. Co-localized with a few of these presumptive growth cones were small sites of postsynaptic (AChR) stain. More often sites of postsynaptic stain were situated more proximally along the path of nerve growth or were not present at all in the same myotome. More rostral myotomes of the same embryos exhibited larger numbers of stained sites but co-localization of both stains was still relatively low. With further development the incidence of co-localization increased. In further contrast to the pattern in mature myotomes, co-localized as well as individual sites of pre- and postsynaptic stain were not restricted to the ends of the muscle cells.

These observations suggest that growing axons contain clusters of synaptic vesicles in their growth cones. Clusters of AChRs form shortly after axon-muscle contact but synaptic vesicles do not initially remain at the majority of these postsynaptic sites. As development progresses the incidence of spatial alignment between clustered AChRs and clustered synaptic vesicles increases, presumably by the formation of new clusters at previously non-aligned sites as well as by unclustering of AChRs or synaptic vesicles at non-aligned sites. In addition, aligned as well as non-aligned sites eventually disappear from the central portions of the myotomal muscle cells, leaving neuromuscular junctions only at their ends. (Supported by MRC).

106.9 THE RELATIONSHIP BETWEEN PRE- AND POSTSYNAPTIC ELEMENTS DURING SYNAPTIC COMPETITION AT DEVELOPING NEUROMUSCULAR JUNCTIONS. R.J. Balice-Gordon and J.W. Lichtman, Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

During the first few weeks after birth, polyneuronal innervation of mammalian muscle fiber endplates is eliminated by retraction of motoneuron axon branches. The postsynaptic regions of acetylcholine receptors change shape during this period, from simple plaques containing a uniform distribution of receptors to a more complex distribution containing receptor rich and receptor poor regions. To determine how the alterations in axonal convergence and in postsynaptic receptor distribution are related, techniques were developed to study the pre- and postsynaptic elements of the same endplates in living mouse sternomastoid muscle during the first month of postnatal life, using a flourescent marker for motoneuron nerve terminals, 4-Di-2-Asp (J. Neurosci. 7: 1215), and flourescently tagged alpha-bungarotoxin to label acetylcholine receptors. Both nerve terminals and the postsynaptic distribution of receptors were observed to undergo rapid changes during the time synapse elimination occurs. In 1 to 2 day old mouse pups, the distribution of acetylcholine receptors on most muscle fibers is simple and doughnut shaped and has a relatively uniform staining intensity. Nerve terminal staining with 4-Di-2-Asp is diffuse at this age and is observed over most, if not all of the receptors. To verify that 4-Di-2-Asp exclusively stains nerve terminals and not other components of the endplate, the sternomastoid muscle was permanently denervated by nerve section in 2-4 day old mice. No endplate staining was seen, indicating that even in neonatal animals this marker is specific for components of nerve terminals. By 3-6 days, many of the receptor plaques have broken up into branched regions which contain receptors and regions which do not. Similarly, 4-Di-2-Asp staining indicates that nerve terminals become progressively more well-defined; the diffuse staining observed at earlier ages is replaced gradually by a pattern of distinct terminal branches. Interestingly, at many endplates at these ages it appears that some receptor regions no longer have nerve terminals overlying them. By 2 weeks of age, however, endplates have receptors distributed in a highly branched pattern which the nerve terminal staining matches completely. To investigate more precisely whether receptor-rich regions of endplate become vacated as axons are eliminated and to determine the relationship between the remaining axons and receptors, we have begun first, to examine the same endplate at multiple time points and second, to differentially label axons innervating the same endplate with flourescent markers that can be internalized by active terminals. Our results argue for rapid changes in the distribution of motoneuron terminals and postsynaptic receptors as competitive synaptic reorganization occurs.

106.10 AN ANTIGEN CONCENTRATED IN THE BASAL LAMINA OF THE NEUROMUSCULAR JUNCTION. D.D. Hunter*, J.R. Sanes, and A.Y. Chiu[1]. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110, and [1]Division of Neurosciences, City of Hope Medical Center, Duarte, CA 91010

Axons preferentially reinnervate original synaptic sites in denervated muscle, a selectivity mediated in part by cues associated with synaptic portions of the muscle fiber's basal lamina (BL) sheath. Molecular correlates of this functional specialization are provided by antibodies that selectively stain synaptic BL (Sanes and Chiu, Cold Spring Harbor Symp. Quant. Biol. 48: 667, 1983). To learn whether the synaptic antigens thereby defined are recognized by axons, it is necessary to purify and characterize them.

Four monoclonal antibodies, C1, C4, D5, and D7, stain synaptic BL far more intensely than extrasynaptic BL. All 4 also stain BL of arteries but not veins in muscle, and BL of glomeruli but not tubules in kidney, suggesting that they recognize the same antigen(s). Biochemical results support this suggestion. On immunoblots of reduced kidney glomerular extracts, C4 and D5 intensely stain a protein of ~185 kD and faintly stain a protein of >400 kD. D7 stains the >400 kD band more intensely than the ~185 kD band. C1 recognizes no proteins on blots, but immunoprecipitates a ~185 kD, C4-reactive protein. Thus, these antibodies recognize at least 3 different epitopes on a common set of polypeptides. The relationship of the >400 kD to the ~185 kD band is unknown: we have no evidence for conversion, and C4, D5, and D7 recognize only material of $>10^6$ kD when gels are run without reducing agent.

C4-reactive material of ~185 kD is present in rat muscle and cultured muscle cells, as well as in kidney. However, kidney was chosen as a source for purification because of its higher apparent content of antigen. Kidney cortices or glomeruli were pulverized, and depleted of cellular components by extraction with a series of detergent- and high salt-containing buffers; antigen was then solubilized in guanidinium with dithiothreitol, and detected by dot blot or Western blot. This selective extraction procedure results in a 50-100-fold purification over the starting material, but the extract still contains many proteins detectable by gel electropheresis. For further purification, C4-agarose was used. Over 75% of the antigen, but less than 2% of the protein, bound to the column; antigen was then eluted with chaotropic agents. We are now maximizing the yield and assessing the purity of eluted antigen; preliminary results suggest that sufficient material can be obtained for sequence determination. (Supported by NIH grant NS19195.)

106.11 FIBROBLASTS FROM DENERVATED MUSCLE SYNTHESIZE NCAM, J1 AND FIBRONECTIN. C.L. Gatchalian* and J.R. Sanes (Spon: C.J. Forehand). Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

We have previously studied the distribution of several adhesive macromolecules, including NCAM, J1, and fibronectin (FN), in rat skeletal muscle. NCAM is present on denervated but not innervated muscle fiber surfaces, FN on both, and J1 on neither. However, all 3 molecules accumulate in interstitial spaces near synaptic sites following denervation (Sanes et al., J. Cell Biol. 102: 420, 1986). Because regenerating axons traverse these spaces, and may therefore be influenced by these molecules, as they reinnervate original synaptic sites (see accompanying abstracts), we have sought the source of these perisynaptic, extramuscular deposits of NCAM, J1 and FN.

Immunoelectron microscopy revealed that J1 is associated with collagen fibers in interstitial areas near denervated endplates. FN is also matrix-associated, at least in part. NCAM, however, is associated with the surface of a major class of cells in interstitial spaces. These cells are likely to be fibroblasts, in that they are rich in rough ER, free of basal lamina, and bear long processes. The processes frequently abut J1- and FN-rich matrix. Mast cells, macrophages, capillary endothelial cells, perineurial cells, and endplate-associated Schwann cells bear little or no NCAM. Autoradiography following administration of ^{3}H-thymidine showed that at least some NCAM-bearing fibroblasts arise by proliferation following denervation. Thus, fibroblasts that proliferate and accumulate near denervated endplates are a leading candidate for the source of interstitial NCAM, FN, and J1.

To study these cells further, we cultured small pieces (~2mm^2) of endplate-rich regions of denervated muscle on collagen substrata. Cells that migrated from the explants were stained after 2-5 days in vitro. More than 90% of these cells were typically fibroblastic in morphology; <2% were myoblastic (spindle-shaped or myosin-positive). More than 80% of the fibroblastic cells were FN-positive; of these, >50% were NCAM-positive and >70% were J1-positive. When monensin, a drug that inhibits glycoprotein secretion, was added to the cultures, NCAM, J1, and FN accumulated inside the cells. This result demonstrates that fibroblasts from denervated muscle synthesize multiple cell adhesion molecules. These perisynaptic cells will be compared with fibroblasts from other sources to determine whether they comprise a unique cell type, or whether they are conventional fibroblasts responding to a localized, nerve-dependent signal. (Supported by NIH grant NS19195.)

106.12 NEURITE OUTGROWTH ON CRYOSTAT SECTIONS OF INNERVATED AND DENERVATED MUSCLE. J. Covault and J.R. Sanes, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Axons implanted in innervated skeletal muscle grow little and form no neuromuscular junctions; however, if the muscle is denervated, the axons readily sprout and form synapses. Regenerating axons preferentially reinnervate original synaptic sites, although "ectopic" synapses sometimes form. Several molecules have been proposed as mediators of these interactions; some are distributed throughout denervated muscle, some are concentrated perisynaptically, and some are localized precisely at synaptic sites (Sanes et al., J. Cell Biol. 102: 420, 1986; and preceding 2 abstracts). However, tests of these candidates in vivo have not been feasible. To bridge the gap between studies of axon guidance in animals and in culture we have studied neurite outgrowth on cryostat sections of muscle.

Chick ciliary ganglion neurons (known to form cholinergic skeletal neuromuscular junctions) were plated on cryostat sections of innervated and denervated rat diaphragm; 1-3 d later, cultures were fixed and neurons stained with anti-chicken NCAM. Our main results are as follows: 1) On both innervated and denervated muscle, neurons extended neurites that grew preferentially along cell surfaces. 2) On average, neurites regenerating on denervated muscle were longer, broader and more highly branched than those on innervated muscle. The ratio of neurite lengths on endplate-rich areas of denervated and innervated diaphragms was 1.7±0.1 in 29 experiments. 3) Average neurite length was greater on sections taken from endplate-rich areas than on sections from endplate-free areas of denervated diaphragm (1.4±0.1; n=20), although both were greater than that on endplate-rich areas of innervated muscle. 4) In 80% (24/30) of the cases in which neurites contacted original synaptic sites (marked with rhodamine-α-bungarotoxin), neurites terminated within 1 μm of the site. These results support the idea that denervated muscles use cell surface and extracellular matrix molecules to modulate axon growth.

Our aim is to study effects of antibodies to putative recognition molecules on neurite behavior in this "cryoculture" system. In preliminary experiments, we have found that anti-NCAM inhibits neurite outgrowth on sections of brain but not on sections of peripheral nerve, while antibody to a laminin-proteoglycan complex (INO; Chiu et al., J. Cell Biol. 102: 1383, 1986) inhibits outgrowth on nerve but not on brain. These results encourage us to test these and other antibodies, singly and in combinations, on muscle. (Supported by NIH and MDA).

107.1 ANTIBODIES TO GABA RECEPTOR RECOGNIZE FUSION PROTEINS ENCODED BY CLONED BRAIN cDNAs. M. Khrestchatisky*[1], R.W. Ransom*[2], R.W. Olsen[2,3], and A.J. Tobin[1,3,4]. Departments of [1]Biology and [2]Pharmacology, [3]Brain Research Institute, and [4]Molecular Biology Institute, University of California, Los Angeles, CA 90024.

We have screened three bacterial expression libraries with a polyclonal antiserum raised against purified rat GABA receptor. The antibody used to screen these libraries reacts principally with a single 52 kD polypeptide of affinity purified GABA receptors, with 52 and 66 kD polypeptides in extracts of rat and bovine cortex and whole rat brain, and with a 52 kD polypeptide in extracts of rat or bovine cerebella.

The libraries screened, all in lambda gt-11, contained cDNAs copied from poly (A) RNA from fetal human brain (obtained from R. Neve), from mouse cerebellum (prepared in this laboratory by T. Wood), and from rat brain (obtained from D. Chikaraishi). We isolated nine immunoreactive clones from the human library, three from the mouse library, and 16 from the rat library. We used fusion proteins from lysogens of these recombinants to select antibodies ("epitope selection"). These affinity purified antibodies were then tested for their reactivity to purified GABA receptor. The fusion proteins encoded by several rat clones appear to share epitopes with the 52 kD, benzodiazepine binding, polypeptide of purified GABA receptor.

One of the cloned human cDNAs, 4.3 kb long, encodes a polypeptide that shares epitopes with a 31 kD polypeptide that copurifies with the 52 kD and 56 kD components of GABA receptor. Partial nucleotide sequence of this cDNA shows no significant homology with other reported sequences in the NIH Gene Bank Database. Southern blot analysis indicates that the genomic sequence corresponding to this cDNA is 10-15 kb long.

This cDNA hybridizes to several rare RNAs in adult rat and human brain, as well as in two central neuroblastoma lines (B103 and B65) known to have GABA binding activity. No hybridizable RNAs are detectable in the rat pheochromocytoma cell line PC12. After two days exposure to purified nerve growth factor or dexamethasone, however, hybridizable RNAs appear. Their concentration increases after further treatment with NGF.

This work was supported by a grant to AJT from NINCDS (#NS 22256), a program project grant to Dr. A.V. Delgado-Escueta (#NS 21908), and a fellowship to MK from the Fondation de l'Industrie Pharmaceutique pour la Recherche.

107.2 DISTRIBUTION OF ACETYLCHOLINE RECEPTOR mRNA AT THE NEUROMUSCULAR JUNCTION OF SKELETAL MUSCLE LOCALIZED BY IN SITU HYBRIDIZATION. J.H. Caldwell and D.M. Chikaraishi. Dept. of Molecular and Cellular Biology, National Jewish Center, Denver, CO 80206 and Neuroscience Program, Tufts Univ. School of Medicine, Boston, MA 02111.

Skeletal muscle fibers are long, cylindrical, multinucleated cells. Some membrane proteins, such as the acetylcholine receptor (AChR), acetylcholinesterase, NCAM and the sodium channel, are highly concentrated at the neuromuscular junction. This distribution could be a consequence of differential insertion or accumulation of these proteins at endplates or different transcriptional programs in junctional versus nonjunctional nuclei. In this regard Merlie and Sanes (Nature, 317:66, 1985) demonstrated by RNA blot analysis more AChR RNA near endplates. We have tested this with in situ hybridization by using anti-sense RNA probes to the BC_3H1 muscle α subunit of the AChR.

Frozen sections of adult rat muscle were labeled with alpha-bungarotoxin to identify the neuromuscular junction and with Hoechst dye 33258 to identify nuclei. These same sections were then used for in situ hybridization performed with ^{35}S labeled riboprobe.

Preliminary results indicate that (1) denervation produces a large increase in the amount of αAChR mRNA, in agreement with Merlie et al., J. Cell Biol., 99:332, 1984 and Goldman et al., J. Neurosci., 5:2553, 1985). (2) At exposure times at which denervated muscle shows heavy labeling, innervated muscle is unlabeled even at the endplate. This implies that the endplate nuclei are not making 10-100 fold more message than extrajunctional nuclei. (3) At longer exposure times both endplate and non-endplate nuclei appear to synthesize αAChR mRNA. In all cases this hybridization seems to be perinuclear.

The results suggest that AChR mRNA is associated with endplate and non-endplate nuclei and implies that intracellular trafficking may be an important factor in the localization of AChRs.

107.3 Glucose Dependent Regulation of the Glucose Transporter mRNA in Rat Glial and Neuronal Cells in Primary Culture. P.S. Walker*, B. VanNess*, R.E. Fellows and J.E. Pessin*. Dept. of Biochemistry and Physiology & Biophysics, The Univ. of Iowa, Iowa City, IA 52242.

Although the brain depends on glucose as an extrinsic metabolic energy source, the regulation of glucose transport activity in the brain is not completely understood. To directly assess the regulation of glucose transport in neuronal and glial cells, in the absence of complications due to the endothelial cell blood brain barrier, we have developed methods to isolate and culture these cells from fetal and newborn rat brains. Neuronal cultures were prepared by enzymatic dissociation and plated onto polylysine coated dishes at $8x10^5$ cells/cm^2 and maintained in a serum free defined medium. Glial cultures were prepared in an analogous manner, plated at $1x10^5$ cells/cm^2, and maintained in DMEM plus 10% fetal calf serum. These cultures were determined to be at least 90% homogeneous based upon immunofluorescent staining by tetanus toxin (neuronal) or glial acidic fribrillary protein (glial), respectively. Total cellular mRNA was isolated by homogenizing the cells in 4M guanidine isothiocyanate, cesium chloride ultracentrifugation and ethanol precipitation. Northern blot analysis was performed by RNA glyoxylation, agarose gel electrophoresis, transfer to ABM paper and hybridization against the ^{32}P labelled rat brain glucose transporter cDNA.

Northern blot analysis revealed the presence of a single band at 2.9Kb corresponding to the expected rat glucose transporter mRNA from both neuronal and glial cultures. However, glial cells incubated for 24h with 25 mM fructose instead of 25 mM glucose (starved cells) in the culture medium displayed a 4-6 fold induction of the steady-state glucose transporter mRNA content. In contrast, starvation of the neuronal cultures under the same conditions only demonstrated a 1.5-2 fold induction of the glucose transporter mRNA. This effect was found to be concentration dependent, with half-maximal induction occurring at 5 mM glucose, maximal induction at 0.5 mM and maximal inhibition occurring at 25 mM glucose. These results demonstrate that the regulation of the glucose transporter by hypo and hyperglycemia in the rat brain is cell type specific. These results further suggest a role for glucose transport regulation in glial cells by altered metabolic states.

107.4 DIFFERENTIAL EXPRESSION WITHIN NEURONS AND GLIA OF mRNA ENCODING A PUTATIVE THYROID HORMONE RECEPTOR (cerbA1). S.R. Fox and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Thyroid hormone (T3) affects many functions of the adult central nervous system. However, the cells containing T3 receptors remain poorly described due to the high nonspecific binding of T3 analogs and the absence of T3 receptor antibodies. Therefore, we sought to identify cells containing T3 receptors by using in situ hybridization to measure T3 receptor mRNA within individual cells with a probe thought to be specific for this mRNA (cErbA1).

In situ hybridization was performed on frozen 10um tissue sections postfixed in paraformaldehyde and taken from forebrain areas of 6 female rats. Sections were prehybridized for 24 h. Putative T3 receptor mRNA was identified by incubating sections at 42°C for 72 h in 30ul of buffer containing 50,000 cpm of a tritiated cRNA complimentary to 3Kb of the human cerbA1 mRNA and hydrolyzed to 0.2Kb. Sections were then RNAse treated, rinsed in 0.1 x SSC for 24 h at R.T., dehydrated and dipped in emulsion. Autoradiograms were developed after 20-60 days and stained with cresyl violet. Cells were identified as glia or neurons on the basis of their size, shape and staining intensity.

Neurons in all parts of the brain examined appeared to contain many more copies of putative T3 receptor mRNA than did glia. To see if this difference was due to the smaller size of glia compared to neurons, the relative concentration of putative T3 receptor mRNA within individual cells was determined. We assumed that the number of reduced silver grains was a measure of the number of copies of the putative T3 receptor mRNA contained within a cell. Grain counting was performed as follows: a grid was positioned over cells magnified 100x in coronal sections of forebrain, and the number of reduced silver grains over each cell was counted. The cell areas were then measured and the relative concentration of silver grains calculated for each cell. Cells in three brain regions were compared: primary olfactory cortex, anterior cingulate cortex deep to the molecular layer, and corpus callosum. The mean concentration of grains found over neurons in different brain regions did not differ among themselves; neither did the grain concentrations over different glial populations. However, the mean concentration of grains over glia was about five-times less than that found over neurons in all brain areas studied. These observations suggest that neurons and not glia contain the majority of T3 receptors in the forebrain. Moreover, most neurons may constitutively express putative T3 receptor mRNA, since this mRNA was found at similar concentrations within neurons varying widely in their location and function.

107.5 THYROID HORMONE CONTROLS THE EXPRESSION OF SPECIFIC mRNAs IN THE FETAL AND NEONATAL MOUSE BRAIN. S. A. Stein*(Sponsor: P.M. Adams), Dept. of Neurol., Univ. of Tx. Hlth. Sci. Cent. at Dallas, Dallas, Tx. 75235.

Thyroid hormone(s)(TH), thyroxine(T4) and triiodothyronine(T3), play critical but only partially understood roles in the development of normal peripheral and central nervous system function. In the rodent, reduction in thyroid hormone levels (hypothyroidism) during the fetal and early neonatal period leads to significant behavioral abnormalities and anatomical abnormalities in the developing cerebral cortex and cerebellum. We have characterized a useful model of fetal and neonatal hypothyroidism, the hyt/hyt mouse on a BALB/cBY background, which has profound primary hypothyroidism, starting after 15 days(d) post conception (pc)(Stein, S.A. et al, Ann. Neurol., 20:402,1986). This model demonstrates many of the TH related anatomical and behavioral abnormalities seen in other rodents. The onset of rodent TH function after 15 d pc corresponds with cerebral cortex neuronal differentiation and new brain mRNA synthesis. Given the function of TH in regulating specific gene expression in adult rodent brain(Stein, S.A. et al, Ann. Neurol.,18:385,1985) and in a variety of tissues and developing systems, it is possible that some of the behavioral and anatomical abnormalities that are observed in the hypothyroid rodent might be due to alteration of fetal brain gene expression by hypothyroidism. We have isolated and characterized a group of rat and mouse cDNAs which are reflective of specific thyroid hormone regulated mRNAs by sequential colony hybridization, and northern gel hybridization(NGH). These cDNAs represent abundant but unknown mRNAs that are present in adult, fetal, and neonatal mouse and rat liver, brain, and cerebral cortex. Along with other cDNAs reflective of known and common brain mRNAs, including actin and tubulin, we have used our unknown TH regulated cDNAs to construct developmental mRNA abundance profiles using mixed cDNA probes from developing fetal(12 d pc to 19 d pc) and neonatal[1 day of age(Day of birth)] total brain and cerebral cortex in the BALB/cBY mouse and the hyt/hyt hypothyroid mouse. In comparing mRNA abundance in the normal and hyt/hyt animals for these 40 mRNAs at 1 day of age(DB), mRNAs that are potentially altered by hypothyroidism in the hyt/hyt animal have been identified. Of these potential fetal brain TH regulated mRNAs, cDNA MNB1 is present in 10-20 fold greater abundance in the normal total brain compared to the hyt/hyt total brain on slot blot(SBH) and NGH. MNB1, a 2300 b.p. mRNA, is detected at 12 d pc, rises in abundance at 18 d pc in total brain, and is also found in cerebral cortex(DB). The fact that fetal and neonatal mouse brain MNB1 might be regulated by thyroid hormone is suggested by: 1)The combined results of the SBH and NGH; and 2)The rise in MNB1 abundance corresponding with the endogenous fetal mouse rise in thyroid hormone after 15 d pc. mRNAs such as MNB1 may have relevance for understanding how thyroid hormone works in the normal and hypothyroid developing brain.

107.6 EXPRESSION OF THE THY-1 GENE IN TRANSFECTED NEUROBLASTOMA CELLS AND IN THE NERVOUS SYSTEM OF TRANSGENIC MICE. G. L. Andreason*, H. A. Ingraham*, S. Chen*, Florence Botteri*, Herman van der Putten* and G. A. Evans. Gene Expression and Cancer Biology Laboratories, The Salk Institute for Biological Studies, San Diego, CA 92138

The Thy-1 glycoprotein is a developmentally regulated cell surface antigen structurally related to the immunoglobulin superfamily of cell surface proteins. Thy-1 is expressed at high levels on the surfaces of most neurons; in mice, it comprises 2.5 to 7.5% of the neural cell surface protein and is induced in the central nervous system at 2 to 3 weeks after birth. In addition, Thy-1 gene expression is induced in PC12 pheochromocytoma cells upon treatment with nerve growth factor (NGF). Previous studies from our laboratory demonstrated that the mouse Thy-1 gene has two promoters which initiate transcription from one of two alternate first exons (Ingraham, H. A. and Evans, G. A., *Mol.Cell. Biol. 6*:2923-2931, 1986). S-1 nuclease protection assays and RNAase protection studies of RNA isolated from murine tissues and cell lines, as well as construction of hybrid genes using Thy-1 promoter and the CAT (chloramphenicol acetyl transferase) indicator gene, have demonstrated that these promoters lack tissue specificity and are equivalently induced when PC12 cells are grown in the presence of NGF. To evaluate the possibility that additional *cis*-acting regulatory sequences might be important for the expression of the Thy-1 gene in the brain, hybrid genes were constructed in which fragments of the Thy-1 gene were attached to the CAT or Luc (firefly luciferase) indicator genes. These constructions were introduced into neural cell lines by calcium phosphate-mediated transfection or electroporation and activities of the indicator genes determined as CAT activity or light production. These studies suggest that sequences downstream of the Thy-1 promoters have the properties of transcriptional enhancers and may be important for developmental control of gene expression in the developing mouse nervous system.

To extend these studies, we have begun studying expression of the native Thy 1.2 gene or a modified Thy 1.2 gene in transgenic mice. Results of these experiments should corroborate *in vitro* studies and allow us to localize sequences involved in brain-specific regulation of the Thy-1 gene expression.

107.7 L7 IS A MARKER FOR THE TERMINAL DIFFERENTIATION OF PURKINJE CELLS IN MOUSE CEREBELLUM. J. Oberdick*, F. Levinthal* & C. Levinthal (SPON: S. Schuetze). Dept. of Biological Sciences, Columbia Univ., NYC, NY 10027.

Three cDNA clones from a λgt10 library were isolated on the basis of reduced expression in Lurcher (Lc) cerebellum vs. wild-type. Lc is a mutation affecting post-natal viability of Purkinje cells (PC). Hence these sequences are likely to be PC specific or regulated by the presence of PC's.

By Northern analysis the three clones correspond to RNA's of about 500 bases in length. Clone L7 shows expression only in the cerebellum, L19 is brain specific but not cerebellum specific, and L14 is expressed in every tissue examined.

By primer extension the 5' end of the L7 RNA has been mapped relative to the end of the clone and the tissue distribution has been verified by repeating the experiment with RNA's from multiple tissues. In addition L7 has been completely sequenced. L7 could code for either a 131 amino acid protein or one of about 80 aa's, depending upon the start Met. This protein has no significant homology to other proteins; however its amino acid content and distribution is similar to several known peptide hormones. We are in the process of using both fusion proteins and synthetic peptides to raise antisera against L7 for immunocytochemical experiments.

By in situ hybridization L7 has been shown to be PC specific as predicted on the basis of its method of selection. By in situ hybridization as well as by primer extension L7 RNA first appears between post-natal days 4 and 8 (P4 and P8) and remains into adulthood. Perhaps most striking is the observation that not all PC's on P8 express L7 whereas all PC's at later stages do (see poster by F. Levinthal, et al.). Hence L7 is a marker for the terminal stages of PC differentiation. Similar experiments are in progress with L14 and L19.

Isolation of the corresponding genomic clones is in progress. We are searching for shared elements in the control regions of these three genes which might impart PC specificity. Differences between the three are also of obvious interest in that they all have different tissue distributions.

107.8 THE USE OF THE SELECTIVE NEUROTOXICANT, TRIMETHYLTIN AND A NOVEL AVIDIN-BIOTIN SUBSTRATE HYBRIDIZATION TECHNIQUE TO ISOLATE mRNAS UNIQUE TO SUBCLASSES OF RAT BRAIN NEURONS. J.K. Krady* and M.L. Billingsley. Department of Pharmacology and Center for Cell and Molecular Biology, Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

Trimethyltin (TMT) is a neurotoxicant which selectively destroys subclasses of neurons in the rodent limbic system and neocortex. We have used a sensitive silver degeneration stain to map specific neurons which were destroyed by intoxication with TMT (8 mg/kg, i.p.). After 7 days, the greatest neuronal damage was seen in the CA3-4 fields of the hippocampus, the pyriform and entorhinal cortices. In order to determine whether there were mRNAs or gene products common to cells destroyed by TMT, we used the following selection and subtractive hybridization protocol. On day 7 post TMT, poly(A) mRNA was isolated from vehicle and TMT-treated Long-Evans rats using guanidine isothiocyanate extraction and enrichment via oligo d(T)-cellulose chromatography. Poly(A) mRNA from control rats was made into single-stranded ^{32}P-cDNA using reverse transcriptase; poly(A) mRNA from TMT-treated rats was biotinylated using photobiotin. Solution hybridization using control ^{32}P-cDNA and 20-fold excess of biotinylated mRNA from TMT-treated rats proceeded at 50°C for 24 hr. Avidin was added, and complexes of avidin-biotin-nucleic acid were retained on an imidoacetic acid chelating Sepharose column; unique, single-stranded ^{32}P-cDNA was not retained on this matrix. This procedure resulted in a 1000-fold enrichment of mRNA/cDNAs unique to neurons destroyed by TMT. The unique ^{32}P-cDNAs were made double stranded, annealed into the Pst I site of pBR322, and used to transform either E.Coli HB101 or the recA positive strain RR1. Positive transformants were tetracycline resistant and ampicillin sensitive (Pst I inserts). A total of 15 clones were isolated; slot blot hybridization analysis with total mRNA probes from both TMT-treated and control rats revealed that 5 of the 15 clones reacted only with mRNA from control rat brain but not with mRNA probes from TMT-treated brain. Studies are currently underway to sequence these 5 clones, and to examine the pattern of in situ hybridization in brain tissue with the unique cDNA probes. These results indicate that specific populations of neuronal mRNA can be selectively enriched by using a combination of selective neuronal elimination and avidin/biotin-based subtractive hybridization. We are currently using other selective neurotoxicants to enrich for brain region-specific mRNAs. Supported by a research grant from the International Life Sciences Institute Research Foundation and by EPA grant CR-200576 to MLB

107.9 mRNA EXPRESSION OF CYTOSKELETAL PROTEINS FOLLOWING β, β'-IMINODIPROPIONITRILE INTOXICATION. I.M. Parhad, E.A. Swedberg*, D.I. Hoar*, C.A Krekoski*, A.W. Clark. Depts. of Pathology and Medical Biochemistry, University of Calgary, Calgary. Alta, Canada, T2N 4N1.

β,β'-iminodipropionitrile (IDPN) when given to experimental animals produces a disorganization of the cytoskeleton, followed by a selective impairment in the transport rate of neurofilament (Nf) proteins. This impairment in transport produces in turn an accumulation of Nfs in the proximal axon, and a depletion of Nfs distally. The redistribution of Nfs along the axon results in marked changes in axonal caliber. Even with chronic IDPN intoxication, there is no neuronal death, and the lesions are reversible when the agent is discontinued. In this study we asked whether IDPN produces an alteration in mRNA expression of cytoskeletal components following acute or chronic intoxication. Female rats (Sprague-Dawley, 6 week old, 130-170 gms) were treated with IDPN (1.5gm/Kg of body weight intra-peritoneally, followed by 0.02% in drinking water) and killed 2, 7 or 30 days later. Total RNA was isolated from the spinal cord of each rat and 20μg resolved by electrophoresis on a 1.2% agarose/ 2.2M formaldehyde gel, transferred to nylon membranes and hybridized with ^{32}[P]-dCTP labeled cDNA probes. The following probes were used: Nf-M for the 145Kd Nf protein (Julien et al., Mol Brain Res: 1, 243, 1986), Nf-L for the 68Kd Nf protein (Lewis & Cowan, J Cell Biol: 100, 843, 1985), bαl tubulin (Cowan et al., Mol Cell Biol: 3, 1738, 1983), and GFAP (Lewis et al., Proc Nat Acad Sci: 81, 2743, 1984). Our results show that the total RNA remains unchanged in the spinal cords of the IDPN treated group as compared to the controls at each time point (2-ANOVA, p>0.05, n=4/group). Northern blots revealed bands at approximately 3.5 Kb for the Nf-M, 2 bands at 2.5 and 4.0 Kb for Nf-L, 1.8 Kb for b-αltubulin, and 3.0 Kb for GFAP in control and IDPN treated rats. Quantitation of mRNA bands by densitometry showed no alteration in Nf-M, Nf-L, bαl tubulin or GFAP in the IDPN groups as compared to their controls. Cytoplasmic dot analysis of IDPN and control groups at 7 days following intoxication confirmed these observations and showed no difference in the levels of the mRNAs evaluated. These results indicate that abnormalities at various stages of cytoskeletal processing such as 1) the early disorganization of the cytoskeleton, 2) the impairment of Nf transport, or 3) the long-term redistribution of Nfs along the axon are not directly due to, nor do they affect the mRNA expression of Nf or tubulin components in this model.

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107.10 OPIOID PEPTIDE mRNA IN STRIATUM: DIFFERENTIAL EXPRESSION AND EFFECTS OF CORTICAL LESIONS. G.R. Uhl, B. Navia* and J. Douglas. Neurology Dept. and Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 and IABS, Portland, OR 97201.

Preproenkephalin and preprodynorphin are the principle opiate peptide genes expressed in the brain. Since expression of one or the other gene could exert substantially different influences on brain function, we have developed an approach to quantitative assessment of the levels of expression of each of these genes and applied it in the striatum, where both are expressed at high levels.

Hybridization to several ^{35}S-labeled oligonucleotide cDNAs complementary to specific regions of each mRNA but displaying similar length, guanine/cytosine content, and specific activity characteristics reveals densities of hybridization corresponding to each probe in serial sections of the rat striatum. The hybridization of each probe fulfills several anatomic and biochemical criteria for specificity. Testing in a model system in which known amounts of mRNA sense RNA are immobilized to a filter and subject to the same hybridization and wash conditions confirms that hybridization densities increase as added mRNA increases.

Studies of serial striatal sections hybridized with cDNAs directed against each of the mRNAs reveal that preproenkephalin-expressing neurons are more numerous than cells expressing preprodynorphin. Hybridization densities above enkephalin-positive neurons are also more than twice those noted above preprodynorphin expressing cells. Northern analyses of mRNA extracted from the striatum are consistent with these relationships.

In order to examine the regulation of the expression of these striatal enkephalin-expressing neurons, we assessed striatal cellular enkephalin mRNA hybridization densities at various times following unilateral cerebral cortical aspiration lesions. The mid-striatal preproenkephalin hybridization densities are decreased ipsilateral to these lesions. This change evolves largely between one and five days following the insult. Cellular hybridization densities in positive neurons of the adjacent central nucleus of the amygdala, on the other hand, are unaffected. Striatal preproenkephalin mRNA is thus more prominent than preprodynorphin mRNA, and depends on cerebral cortical inputs for its full expression.

107.11 CHRONIC LESIONS DIFFERENTIALLY DECREASE MESSENGER RNA IN DOPAMINERGIC NEURONS OF RAT SUBSTANTIA NIGRA. G.M. Pasinetti, S.P. Lerner*, S.A. Johnson, D.G. Morgan, N. Telford, M.M Myers* and C.E. Finch. Gerontology Ctr. USC, Los Angeles, CA 90089

Chronic lesions of rat substantia nigra pars compacta were produced by a single 6-hydroxydopamine injection (6-OHDA) that caused striatal depletion of dopamine (DA) by 65% . Morphological changes and effects on messenger RNA (mRNA) of tyrosine hydroxylase (TH) and β-tubulin by in situ hybridization in the remaining dopaminergic neurons were evaluated 9 months after lesion. Adult Fisher 344 male rats (250-275 g) were injected stereotaxically with 6-OHDA into the right substantia nigra pars compacta (8 μg in 3 μl volume of 0.1 % ascorbic acid). Three weeks later rats were tested for rotational behavior (amphetamine 5 mg/kg i.p.). Nine months after 6-OHDA lesions, rats were sacrificed by decapitation. Striata were dissected for estimation of DA and dihydroxyphenylacetic acid (DOPAC) by HPLC and midbrain fixed and embedded in paraffin. Immunocytochemistry (ICC) was performed using an ABC-PAP kit (Vector) with a primary antibody to TH (Eugene Tech). TH (PSTI/KPNI) cDNA (D. Chikaraishi) and B-tubulin (PSTI/HINDIII) cDNA (D. Cleveland) fragments, were recloned in PGEM I vector allowing single strand cRNA transcripts. In situ hybridization was performed on 10 um paraffin sections and the sections were treated first for TH-ICC. Neuronal content of mRNA for TH and β-tubulin was assayed by in situ hybridization in TH-positive neurons. A computerized grain counting system was used to quantify the grain density over neurons. Both mRNA types were reduced per TH-positive neuron, but TH mRNA showed a relatively greater loss (-60% ; P<.001), either per cell or by density per neuron area, compared with the contralateral nigra. TH mRNA was correlated with the loss of the ipsilateral striatal DA levels (P<.05). β-tubulin mRNA in TH positive cells did not correlate with the loss of ipsilateral striatal DA and did not differ with lesioned induced reduction in cell size. Among the remaining TH-positive neurons, cytoplasm, nuclear and nucleolar area were reduced by 30-40% (P<.001). The nigra contralateral to the lesion contained fewer TH neurons <20 um in diameter than the ipsilateral side. This apparent increase in number of small neurons after lesioning, must have resulted from the shrinkage of large neurons. These results confirm the findings that the remaining nigral neurons at the death of Parkinson's victims have less RNA and smaller nucleoli (Mann D. et al., J. Neuropathol. Exp. Neurol. 36:379-383, 1977). We hypothesize that genes for neuron specific proteins, such as enzyme for neurotransmitters, are more susceptible to neuronal damage in contrast to generally expressed genes like β-tubulin. (Supported by AG-00443 and the MacArthur Foundation Research Program on Successful Aging to CEF)

107.12 EXPRESSION OF THE GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) GENE IN THE MOUSE RETINA. P.V. Sarthy and M. Fu*. Department of Ophthalmology, Univ. of Washington, Seattle, WA 98195.

In response to photoreceptor degeneration, Müller cells, the predominant glial cell type in the retina, undergo 'reactive gliosis'. In normal mouse retinas, immunocytochemical studies show that polyclonal anti-GFAP sera stain the astrocytes while the Müller cells remain unstained. Photoreceptor degeneration resulting from rd, nr, and pcd mutations or constant light exposure (cld), however, lead to the appearance of GFAP-immunostaining in Müller cells. Since Müller cells in normal retina contain large numbers of intermediate filaments, what is the mechanism of GFAP expression in Müller cells from retinas with photoreceptor degeneration? Is the appearance of immunoreactivity due to depolymerization, proteolysis, or chemical modification of existing GFAP filaments, or is it due to formation of new GFAP molecules? We have used Western and Northern blotting and in situ hybridization techniques to address these questions. Our immunoblotting data show that there is an increase in the GFAP content in the rd, nr, and light-damaged retinas. Northern blot analysis with a GFAP cDNA probe shows that there is a 10 to 25-fold increase in the GFAP mRNA level in the rd, nr and cld retinas. Hence the increase in GFAP content probably results from de novo transcription of the GFAP gene and not from enhanced translation of pre-existing mRNA. In order to locate the sites of mRNA synthesis, in situ hybridizations were carried out with ^{35}S-labeled DNA and RNA probes. Both aldehyde-fixed and frozen sections were used for localization and DNA probes were 80-150 bases or 1.5Kb long. In normal retinas, silver grains were concentrated on cell bodies located just below the ganglion cell layer, the area where astrocytes reside. Few grains were found in other parts of the retina. In retinas with photoreceptor degeneration, silver grains were found on cells in the inner nuclear layer and on astrocytes. Furthermore, silver grains were also seen in the inner plexiform layer, and the density of silver grains was higher in astrocytes. These studies establish that reactive gliosis leads to transcriptional activation of the GFAP gene in Müller cells and astrocytes of the mouse retina. A similar mechanism is probably responsible for the increased GFAP-staining in Müller cells observed following photoreceptor degeneration in other mammalian retinas.

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108.1 SYNAPTIC CONNECTIONS OF THE AXON TERMINAL OF ROD BIPOLAR CELLS IN THE RABBIT RETINA. E. Strettoi*, E. Raviola and R.F. Dacheux*. Dept. of Anatomy, Harvard Medical School, Boston, MA 02115.

The synaptic connections of the axonal arborization of rabbit rod bipolar cells were reconstructed at the electron microscope from continuous series of thin, radial sections using computer graphics. The axonal arborizations of four adjacent rod bipolars were reconstructed from the mid-periphery of the ventral retina.

Rod bipolar axons crossed the scleral portion of the inner plexiform layer (IPL) without branching. In the vitreal region of the IPL they gave rise to a small number of large endings arranged in a row at the boundary between IPL and ganglion cell layer. All synaptic contacts were confined to the vitreal portion of the axon and to the endings. Rod bipolar terminals were presynaptic exclusively at ribbon synapses and usually contacted a dyad of postsynaptic processes; monads were very infrequent. As a rule, the postsynaptic processes were dendrites of amacrine cells (99.4%, 159 processes); only one instance was observed of a ganglion cell dendrite postsynaptic to a rod bipolar (0.6%, 1 process). In about three quarters of the dyads, one of the postsynaptic dendrites had dark matrix and few vesicles; it contained a heterogeneous collection of cytoplasmic organelles. When these processes were followed through the IPL, they conformed in course, size, pattern of branching and fine structure with the dendrites of the narrow-field, bistratified (NFB) rod amacrine cell. There was a high degree of overlapping in the connections between rod bipolars and NFB rod amacrine cells: a large proportion of the ribbon synapses of an individual rod bipolar cell converged onto a single NFB rod amacrine; often, each amacrine cell dendrite received multiple synapses from the same rod bipolar ending. In two thirds of the dyads, one of the postsynaptic processes had pale matrix and numerous vesicles; it returned one or several punctate synapses onto the bipolar ending. These features are typical of wide-field, unistratified (WFU) rod amacrine cells. Most commonly, dyads resulted from the association of dendrites from NFB and WFU rod amacrines. In places, however, one of the postsynaptic dendrites belonged to other unidentified types of amacrine cells.

In addition to the reciprocal synapses, the axonal arborization of rod bipolars received a substantial input from a heterogeneous population of unidentified amacrine cell dendrites.

Thus, in both rabbit and cat retinas, transmission from rod bipolar to ganglion cells is mediated by an amacrine that is narrow-field and bistratified, and is modulated by the input of other varieties of amacrine cells onto the rod bipolar.

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108.2 MORPHOLOGY AND DISTRIBUTION OF SYNAPSES ONTO A LARGE-FIELD GANGLION CELL (TYPE 1.2) IN THE RETINA OF THE GOLDFISH. Peter F. Hitchcock, The University of Michigan, Department of Ophthalmology, W.K. Kellogg Eye Center, Ann Arbor, MI 48105

As the retina of the goldfish grows by expansion, new synapses are continually added to the inner plexiform layer (Fisher and Easter (1979) J. Comp. Neurol., 185: 373-380), and the dendritic arbors of ganglion cells grow interstitially (Hitchcock and Easter (1986) J. Neurosci., 6: 1037-1050). I have established the morphology and dendritic distribution of synapses ending on the Type 1.2 ganglion cell in preparation for quantitatively determining the relationship between dendritic growth and synapse addition for this cell.

Type 1.2 cells were stained using retrogradely transported horseradish peroxidase, drawn with a camera-lucida attachment and a light microscope, embedded in plastic, and thin-sectioned parallel to the plane of the dendritic arbor. (The dendrites of these cells are unistratified within the outermost zone of the inner plexiform layer.) Every fifth section through the dendritic arbor was photographed at scanning magnification with the EM. With the low-power photmicrographs serving as guides, the HRP-filled dendrites were inspected at 8200 X magnification, and the type and location of each synapse ending on the dendritic arbor was marked on the low-power photomicrograph. From these photomicrographs, the dendritic location of each synapse was determined and marked onto the camera-lucida drawing of the cell.

Preliminary results (3 cells; 468 synapses) show that this cell receives synaptic contacts from ribbon and 3 morphologically distinct types of conventional synapses. The ribbon synapses (n=115) were found in large terminals with relatively electron dense cytoplasm, evenly distributed vesicles, and numerous mitochondria. The HRP-filled dendrites were generally one of a pair of post-synaptic elements at each ribbon synapse. For the conventional synapses (n=353), ninety-six percent were in relatively small, oval, relatively electron lucent terminals that contained a few unevenly distributed vesicles, and few mitochondria; three-percent were found in small terminals that had relatively electron dense cytoplasm, densely packed vesicles and generally no mitochondria; and slightly less than one-percent were en passant synapses, made by large, electron lucent processes that lay adjacent to the HRP-filled dendrites.

With respect to dendritic distribution, both the ribbon and the conventional synapses were distributed homogeneously throughout the dendritic arbor of these cells. Both types of synapses made contacts on all orders of dendrites, as well as onto dendritic appendages. No synapses were observed to contact somata.

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108.3 MORPHOLOGY AND DISTRIBUTION OF DENDRITIC BEADS IN THE MAMMALIAN RETINA. Cesare USAI*, Silvia Bisti°* and Silvana Vallerga* (SPON: A.E.Del Bo). Istituto di Cibernetica e Biofisica, CNR, Genova and °Istituto di Neurofisiologia, CNR, Pisa, Italy.

The presence of beads on dendrites appears to be a general characteristics of neuronal morphology in the retinae of different animals. A detailed analysis of the properties of dendritic beads has been reported only for the amacrine cells of the fish retina.

Here we report results concerning the presence and distribution of beads in mammalian retinae. HRP or Golgi stained retinae of rat, cat and monkey were analyzed.

Almost all retinal neurons in the three species present dendritic beads differing in size and linear occupancy according to cell type and dendritic architecture. The same class of retinal neuron shows interspecies similarity in bead distribution.

An interesting exception are the ganglion cells. The type I ganglion cells of the rat retina and the α-type of the cat retina have dendrites devoid of beads, whereas all ganglion cells in the monkey retina are richly beaded.

This observation provides further evidence that the cat α-cells do not have any correlate in the ganglion cell populations of the monkey retina.

The results we present indicate that dendritic beads are indeed an outstanding feature also in mammalian retina, and they can be used as a tool to determine analogies among retinal neurons in different species.

108.4 AXO-DENTRITIC POLARITY IN RETINAL GANGLION CELLS. L. Maffei* and V. H. Perry* (SPON: V.K. Nielsen). Dept. of Experimental Psychology, Univ. of Oxford, England and Istituto di Neurofisiologia CNR, Pisa, Italy.

We have studied the relation between axons and primary dendrites of ganglion cells in wholemount retinae of rats, cats and monkeys. Ganglion cells were backfilled following injection of horseradish peroxidase (HRP) either in the optic tract or optic nerve. The retinae were prepared as wholemounts and the HRP revealed with a modification of the Hanker-Yates method (Perry and Linden, Nature, 297:683, 1982).

It is well known that retinal ganglion cells are polarized in the vertical plane, since the optic fiber layer lies vitread and the inner plexiform layer sclerad to the cell body.

We have found that the trajectories of the primary dendrites are biased away from the axon initial segment (AIS) also in the plane of the ganglion cell layer. This axo-dendritic polarity is also evident in that the majority of primary dendrites originate from the side of the cell body opposite to the axon initial segment. Higher order dendrites do not show this bias, so that the whole dendritic tree presents only a weak tendency to be oriented away from the AIS.

The axondendritic polarity is present in all the three species studied, suggesting that this could be a general rule in the organization of ganglion cells.

We then looked at the AIS and its orientation with respect to the optic disk. We found that in the cat the vast majority of ganglion cells have AISs directed towards the optic disk. This orientation is less clear in rats and monkeys.

The axondendritic polarity of retinal ganglion cells could be correlated to other cytological features of the cells. No clear relationship was observed between the position of the cell nucleus and the AIS either in adult rat and monkey, where the nucleus is eccentric, or in the adult cat, where the nucleus is at the center of the cell body. In the kitten however, the nucleus lies opposite to the AIS during the first 3 weeks of life and then moves to its adult central position. This period corresponds to a time of rapid growth of the ganglion cells axon terminals.

108.5 MORPHOLOGICAL CLASSIFICATION OF GANGLION CELLS IN RABBIT RETINA. E. V. Famiglietti. Dept. of Anat. and Lions' Sight Ctr., Univ. of Calgary, Calgary, AB, Can. T2N 4N1.

Ganglion cells (GCs) were studied in a large collection of whole flat-mounted and sectioned, Golgi-impregnated rabbit retinas. In the region of the visual streak 34 distinct morphological types could be recognized, and it is estimated that the number of types has an asymptote near 40. The following criteria were applied in order of importance: 1) dendritic morphology (a. branching pattern, b. contour, c. appendages), 2) dendritic stratification (a. sublamina a or b, b. stratum 1-5, c. substratum - 3 per stratum), 3) dendritic field size (DFD) and its regional variation, 4) cell body size and axon diameter (generally covarying). GCs were grouped in 4 classes. Class I GCs have large cell somata, large DFD, regular radiate branching with few appendages, and type a and b counterparts [3 types]. Class II GCs have medium somata, medium DFD, branching pattern like class I, a & b types [4 types]. Class III GCs have medium to small somata, small to large DFD, a heterogenous variety of dendritic morphologies, and a & b types [10 types]. Class IV GCs have small to large somata, small to large DFD, heterogenous dendritic morphologies, and include unistratified, bistratified, multistratified, and broadly stratified GCs with no type a & b counterparts [17 types]. The 4 part classification reflects a bias toward single-unit physiological studies using elementary visual stimuli. Class I is presumed to include physiological Y cells, large-field units and possibly some X cells, class II cells to include the majority of X cells, class III cells to correspond to "sluggish-concentric" GCs, and class IV to include the GCs with "complex" responses, such as direction and orientation selective GCs, and local edge and uniformity detectors. Some correlations in the last group have already been made (e.g. Amthor et al., '84).

It is unlikely that this diversity of types signals imprecision in the organization of the visual system, particularly in view of the complex but orderly arrangements of their neurotransmitter-selective presynaptic amacrine cells. At least 3 possibilities may underlie such diversity among rabbit GCs. GCs with similar receptive field (RF) properties could have unique sets of neurotransmitter-specific inputs, which give rise to unique responses under selected physiological conditions. GCs cells with similar RFs, but different morphology and slightly different inputs, may well project to different parts of the visual brain, or to different sets of neurons within the same target regions. Diversity might thus support the divergence of similar, but not identical information to different brain regions, and the convergence of pathways mediating slightly disparate information for integration at common targets in higher visual centers.

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108.6 **INVESTIGATION BY INTRACELLULAR RECORDING OF PUSH-PULL MICROCIRCUIT IN CAT RETINA** Michael A. Freed and Ralph Nelson Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bethesda, MD 20892

Electron microscopic investigation of the cat retina has yielded a detailed wiring diagram for synaptic input to the on-beta ganglion cell of the cat retina. About half of this input is from two types of cone bipolar: type b_1 is depolarizing to light on but hyperpolarizing to light off and type b_2 is hyperpolarizing to light on but depolarizing to light off. The push-pull hypothesis states that during light on in the on-beta receptive field center the type b_1 confers excitation (the push)upon the on-beta cell while the type b_2 confers disinhibition (the pull); thus a synergy of excitatory and inhibitory inputs causes a depolarizing response in the on-beta cell. During light off in the on-beta receptive field center, type b_2 confers inhibition, causing a hyperpolarization in the on-beta cell.

In order to investigate the light-evoked resistance changes within this microcircuit we have recorded intracellularly from four on-beta cells in a perfused eyecup preparation using sharp high-impedance (400 MΩ) microelectrodes. The dark membrane potential was changed in steps by injecting currents (-1 to 1 nA, 0.1 nA steps) into the cells and then bars of light (100-200 μm wide) were shown over the receptive field center for 1 second. The photic response was recorded for each membrane potential step. All responses were recorded under mesopic conditions. Each cell was stained with horseradish peroxidase after recording and identified in the light microscope by well-accepted anatomical criteria. For three different times during the photic response the slope resistance was calculated (dV/dI) and the reversal potential was extrapolated. The three times were: 1) at the peak of the depolarization in response to light on (On-transient) 2) during the subsequent sustained depolarization (On-sustained) and 3) at the peak of the hyperpolarizing response to light off (Off-transient).

All three times were associated with a small resistance decrease (mean = 3.4 MΩ) compared to the dark resistance (mean = 45 MΩ). The reversal potential for each time period varied widely between cells; this may be due to variation in the strength of excitatory and inhibitory inputs which contribute to each response. There was, however, a consistent relation between reversal potentials: the On-transient reversal potential (-31 to 66 mV) was more positive than the On-sustained reversal potential (-56 to -40 mV), which was more positive than the resting potential (-21 to -58 mV). The Off-transient reversal potential (-69 to -53 mV) was more negative than the resting potential.

These results expand on the push-pull hypothesis by suggesting predominant excitatory input at light on and predominant inhibitory input at light off. These results do not demonstrate, however, the source of these inputs; assuming that the conductances measured are synaptically controlled these inputs may originate from either bipolar or amacrine cells. The reversal potentials lie inside the range of conventional Nerst potentials, which suggests some antagonism (strongest at the On-sustained time) between excitatory and inhibitory inputs. Our finding of antagonism differs from the original push-pull hypothesis which suggested synergy between such inputs. The source of this antagonism is uncertain: it may reflect the presence of the surround mechanism in the center of the receptive field.

108.7 LOCALIZATION OF SOMATOSTATIN-LIKE AND NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE TURTLE RETINA AND CHOROID. Charles L. Zucker and Alan R. Adolph. Eye Research Institute of Retina Foundation and Harvard Medical School, 20 Staniford St., Boston, MA 02114.

Immunoreactivity for both somatostatin (SS-like IR) and neuropeptide Y (NPY-like IR) has been described in retinal amacrine cells of a wide variety of vertebrate species (Tornqvist et al. 1982; Bruun et al. 1986). In most species, the processes arising from SS-like IR cell bodies give rise to three, more or less evenly spaced plexuses in sublamina 1,3 and 5 of the inner plexiform layer (IPL). Several distinct cell body morphologies are usually evident in any one species. Similarly, NPY-like IR cells typically give rise to a tri-laminar pattern of processes in the IPL. Additionally, NPY-like IR fibers have been observed to innervate the choroidal vasculature in the Guinea-Pig (Bruun et al. 1984) In the present study, using several well characterized antisera to each peptide, we have identified several unique features of SS-like and NPY-like IRs in the choroid and retina of the turtle (Pseudemys scripta). In the choroid, NPY-like IR fibers are seen throughout the stroma with extensive contacts onto the vasculature. In the retina, SS-like IR is seen only in cell bodies of the ganglion cell layer and a very restricted plexus at the proximal-most IPL. Since no immunoreactive axons were observed, we could not discern if these are ganglion or displaced amacrine cells. NPY-like IR staining showed amacrine cell bodies giving rise to plexuses at about the 30%, 60% and 90% levels in the IPL as has also been described by Adolph and Bruun (1987). In addition, a fourth, less prominent but very consistent plexus occurs in the ganglion cell layer. Within this most proximal layer, several NPY-like IR varicosities are evident in juxtaposition to most cell bodies in the ganglion cell layer. These juxtapositions are highly suggestive of synaptic contacts onto ganglion cells, especially since a greater number of cells appear to be contacted than can be accounted for by displaced amacrine cells. Electron microscopical immunohistochemistry is being used to confirm the exact nature of these contacts. The relationship of both SS-like IR and NPY-like IR to the ganglion cell layer is suggestive of a neuromodulatory role of these two peptides in ganglion cell function; this is a possibility we are currently investigating.

108.8 THE PHYSIOLOGY OF SOMATOSTATIN IN THE RABBIT RETINA. R.A. Zalutsky* and R.F. Miller (SPON: A.I. Cohen). Dept. of Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110.

Somatostatin (SS) is present in amacrine and, possibly, interplexiform cells of the rabbit retina (Sagar et al., 1986). Since this neuropeptide has a wide spectrum of actions in the CNS and periphery, we have examined its effects on rabbit retinal neurons using an in vitro eyecup preparation designed for peptide pharmacology. The effects of SS and related peptides on neurons' light responses were monitored with ERG, single unit extracellular, intracellular, and whole cell recording.

SS affects virtually all types of ganglion cells in one or more of three ways. The first effect is a general excitation with a latency a few seconds longer than that of fast transmitters. The threshold concentration is less than 100 nM. The second effect is a simultaneous suppression of spontaneous activity and augmentation of light evoked spiking, resulting in a substantial increase in the ratio of light evoked to spontaneous firing. The third effect is a strong shift in center-surround balance toward a more dominant center. The latter two effects have threshold concentrations of less than 1 nM, latencies of tens of seconds, and durations of tens of minutes.

SS does have direct effects on ganglion cells evaluated during synaptic block with cobalt. The peptide also affects most amacrine cells causing a slight hyperpolarization and a pronounced increase in the amplitude of light evoked responses. The amacrine cell effects of SS also persist during synaptic block and are accompanied by an increase in input impedance. SS does not noticeably alter the behavior of horizontal cells. However, SS substantially reduces the influence of horizontal cells upong ganglion cells as demonstrated with dual intracellular-extracellular recordings of horizontal cell ganglion cells pairs with current injections into horizontal cells.

SS 28 has qualitatively and quantitatively similar effects to SS 14. The non-degraded SS analog SMS 201-995 is approximately equipotent with SS suggesting that SS may "act at a distance" in the rabbit retina. Cysteamine, which specifically depletes SS in rabbit retina (Sagar and Martin, 1982) increases the sensitivity of retinal neurons to SS.

We suggest that SS may be a neuromodulator in the rabbit retina with effects at multiple levels of the retinal circuitry, converging to produce a coordinated change in retinal output.

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108.9 THE EFFECTS OF APB ON THE CONTRAST SENSITIVITY OF CAT RETINAL X CELLS. E. P. Chen* and R. A. Linsenmeier. Departments of Biomedical Engineering and Neurobiology and Physiology Northwestern University, Evanston, IL 60201

Slaughter and Miller demonstrated in the mudpuppy retina that 2-amino-4-phosphonobutyric acid (APB), a glutamate analog, is very selective in mimicking the effect of the cone transmitter on the ON bipolar cells (Science, 219:1230-1232, 1981). This selectivity offers a unique opportunity for studying the contribution of ON bipolar cells to the receptive field circuitry of more proximal visual neurons. Our primary interest is the cone pathway to X-type cat retinal ganglion cells. Bolz et al. (Neurosci., 12:875-885, 1984) found that APB modifies the maintained discharge rate of cat retinal ganglion cells differently depending on the cell's center polarity; it reduced the discharge of the ON-center cells and increased that of OFF-center cells. In addition, the center response of ON-center cells to light was reduced in the presence of APB. The effect of APB on the light response of OFF-center cells, however, was small and variable. We have reexmined the effects of APB on the center mechanism of ON- and OFF-center X cells and studied its effect on center-surround spatial summating properties.

Our experiments were performed on the intact retina of anethetized cats with double-barreled electrodes. An X cell's activity was monitored extracellularly at the soma by one barrel, while 2-50 mM APB was delivered from the other by pressure ejection. The spatial summating properties of each X cell were characterized by using 2 Hz stationary or drifting sinusoidal gratings of various spatial frequencies at a photopic mean luminance, allowing us to estimate the strength and summating radius of the center and surround. Then APB was ejected until its effect reached a steady-state. At this time, the activity of the X cell was quantified in a similar fashion. Using this protocol, we were able to compare the strength and summation radius of the two mechanisms before, during, and after the application of APB. Our results confirmed Bolz et al.'s observations on APB's effects on X cells' maintained discharge. APB, however, reduced the responsivity of the center mechanism of not only ON-center, but also OFF-center X cells. There was no change in the size of the center mechanism. We also found an APB-induced phase advancement in the center response of both types of X cell, which may reflect a modification of the dynamic properties of these cells. APB seems also to reduce the surround responsivity of X cells, but further studies are required to determine whether center-surround balance or the surround radius have been altered by this agent. Supported by R01 EY00206 to C. Enroth-Cugell, R01 EY05034 and training grant T32 NS07223.

108.10 SPATIAL CONTRAST SENSITIVITY DEFICITS PRODUCED BY ON-CHANNEL BLOCKADE IN THE RHESUS MONKEY. E.L. SMITH III, G.C. DUNCAN, R.S. HARWERTH, AND M.L.J. CRAWFORD. College of Optometry, University of Houston and Sensory Sciences Center, University of Texas at Houston, Houston, Texas 77004.

One of the presumed advantages of processing information in parallel ON and OFF pathways is that the combined activity within these reciprocal systems facilitates the detection of simultaneous contrast. Logically it would be expected that inactivation of either the ON or the OFF pathway would result in a decrease in spatial contrast sensitivity. To examine this hypothesis, 2-amino-4-phosphonobutyric acid (APB) was used to reversibly inactivate the retinal ON channel and psychophysical procedures were employed to determine the effects of the ON-channel blockade on the rhesus monkey's spatial contrast sensitivity function.

The behavioral paradigm was a temporal interval detection task in which the subjects were required to respond to the appearance of a vertical sinusoidal grating pattern. Contrast detection thresholds for spatial frequencies between 0.25 and 16 cycles per degree were measured using a descending method of limits. Approximately three hours before the behavioral experiments, the retinal ON channel was inactivated by intraocular injections that produced estimated vitreal APB concentrations that ranged from 200 to 500 uM. The effectiveness of the APB injections was confirmed via the electroretinogram.

In agreement with a previous report by Schiller et al. (Nature, 322:824, 1986), APB-induced, ON-channel blockade resulted in a reduction in spatial contrast sensitivity. We found, however, that the magnitude of the contrast sensitivity deficit varied as a function of spatial frequency. The smallest changes in contrast sensitivity were observed for the lowest spatial frequencies; there was a systematic increase in the contrast sensitivity deficit as the spatial frequency of the grating pattern was increased.

108.11 SPATIAL PROPERTIES OF CONTRAST SIGNALLING BY Y RETINAL GANGLION CELLS OF CAT. John B. Troy, Christina Enroth-Cugell and John G. Robson. Biomedical Engineering and Neurobiology & Physiology, Northwestern University, Evanston, Illinois 60201 and Physiological Laboratory, University of Cambridge, U.K.

Since the middle sixties, it has been known that spatial summation within the receptive fields of Y retinal ganglion cells of cats is nonlinear (Enroth-Cugell, Ch. & Robson, J.G. J. Physiol. 187: 517-552, 1966). The elegant studies of Shapley and his colleagues have since provided us with first a quantitative model of the spatial properties of the Y cell's receptive field primarily founded on the cells' responses to single sinusoidal gratings (Hochstein, S. & Shapley, R.M., J. Physiol. 262: 265-284, 1976), and secondly a description of the dependence of the temporal transfer function on contrast (Victor, J.D. & Shapley, R.M., J. Gen. Physiol. 74: 671-689, 1979).

While Shapley and his coworkers have, in these earlier studies, examined the responses of Y cells to a number of non-sinusoidal spatial patterns, their investigations were focused more on the temporal properties of the Y cell receptive field than on its spatial properties. We have lately studied the responses of Y retinal ganglion cells to spatial patterns formed from more than one sinusoid; one of which was of high enough spatial frequency that it produced no first harmonic response from the cell. We found that the cell's response could not be predicted from a linear combination of the responses to each sinusoid presented alone, as might have been expected from Hochstein and Shapley's classic model. In fact, the presence of a second spatial sinusoid shifted the response versus contrast relationship of the Y cell for stimulation with another sinusoidal pattern along the logarithmic contrast axis. These results extend the description of contrast gain control in the retina provided by Shapley and Victor. It is clear that considerable work remains to unravel the mysteries of the spatiotemporal properties of the receptive field of the Y cell. Supported by NIH R01-EY00206 and R01-EY06669.

108.12 SPATIAL FREQUENCY ANALYSIS OF CAT RETINAL W-CELLS. M.H. Rowe and J.F. Cox*. Dept. Zoology & College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

An important first step in understanding the afferent circuitry of retinal ganglion cells is the identification and characterization of distinct receptive field components. Although a great deal is known about the spatial components of X-and Y-cells of the cat retina, relatively little is known about the structure of W-cell receptive fields. We have begun to use spatial frequency analysis to identify spatially distinct components of cat retinal W-cells.

Our sample to date includes on and off tonic and phasic, as well as directionally selective and one suppressed by contrast cell. Both on and off tonic and phasic cells appear to have spatially linear center mechanisms, i.e. their response to a stationary, sinusoidally counterphased sine wave grating contains a fundamental component whose amplitude is a sinusoidal function of spatial phase. The responses of all phasic W-cells and some tonic W-cells also contain a second harmonic component whose amplitude is independent of spatial phase. Furthermore, in these cells drifting gratings at a spatial frequency above the resolution of the center elicit an unmodulated elevated maintained discharge. Thus, these cells appear to receive input from excitatory rectifying subunits similar to those described for Y-cells.

The spatial resolution of the center mechanism, measured with drifting sine wave gratings, varies considerably among W-cells, but can be as high as 2.5 c/d in phasic W-cells, 1.5 c/d in directionally selective cells and over 3 c/d in tonic W-cells. Second harmonic components, when present, invariably have higher spatial resolution than the center. Most cells in both groups show a clear fall off in contrast sensitivity at low spatial frequencies, indicating the presence of a surround. Contrast thresholds for phasic W-cells were often an order of magnitude higher that for X- or Y-cells in the same animals. Thresholds for tonic W-cells were much lower, but still higher than for X- or Y-cells.

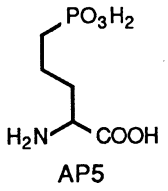
The response of the suppressed by contrast cell to drifting gratings included both a modulated and an unmodulated suppression which were proportional to contrast. The modulated suppression was dominant at low spatial frequencies and the unmodulated suppression at high spatial frequencies, suggesting that the unmodulated suppression represents inhibitory subunits with higher spatial resolution than the center.

109.1 THE N-METHYL-D-ASPARTATE ANTAGONIST MK-801 POTENTLY INTERACTS WITH THE BRAIN PHENCYCLIDINE/σ RECEPTOR. R. Sircar, M. Rappaport*+, R. Nichtenhauser* and S.R. Zukin, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, +University of Cincinnati School of Medicine, Cincinnati, OH.

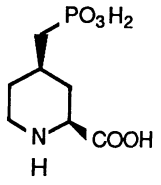
MK-801 ((+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d] cyclohepten-5, 10-imine maleate) is a novel anticonvulsant agent reported to antagonize CNS actions of the excitatory amino acid N-methyl-D-aspartate (NMDA) non-competitively (Wong et al., Proc. Nat. Acad. Sci. USA, 83:7104, 1986). The question arises of the mechanism underlying the anti-NMDA effects of MK-801. In this study we show MK-801 to be an extremely potent inhibitor of the binding of [^{3}H]N-(1-[2-thienyl] cyclohexyl) piperidine ([^{3}H]TCP) to brain PCP/σ receptors. Its IC_{50} value of 3.8 ± 0.8 nM in this assay ranks it as the most potent known ligand of the brain PCP/σ receptor (MK-801 > PCE > dexoxadrol > PCP > ketamine > levoxadrol). Addition of 1 μM MK-801 altered the apparent K_d of [^{3}H]TCP binding from 20.8 nM in the control situation to 291.3 nM in the presence of MK-801 without altering the apparent B_{max}. MK-801 strongly inhibited the binding of [^{3}H](+)N-allylnormetazocine ([^{3}H](+)SKF10,047) to the PCP/σ receptor but its effect on [^{3}H](+)SKF10,047 binding to the non-PCP haloperidol-sensitive σ binding site was weaker by several orders of magnitude. Functionally, MK-801 mimicked the inhibitory action of PCP on NMDA-stimulated [^{3}H]norepinephrine ([^{3}H]NE) release from rat hippocampal slices. For inhibition of NMDA-stimulated neurotransmitter release, MK-801 was significantly more potent than PCP; half maximal inhibition with MK-801 was observed at 1.47 nM against 75.9 nM for PCP. Our findings are consistent with those of a recent study (*Ibid.*) in which it was reported that MK-801 antagonized NMDA-induced depolarization in the rat cortical slice preparation, and that [^{3}H]MK-801 labeled a single population of binding sites from which it could be displaced by PCP and ketamine. All of those results can be interpreted on the basis of our determination that MK-801 exerts its anti-NMDA effects by potently binding to PCP/σ receptor. In fact, MK-801 appears to be the best available molecular probe of the PCP/σ receptor.

109.2 A NEW POTENT NMDA-TYPE RECEPTOR ANTAGONIST - CGS 19755. J. Lehmann, A.J. Hutchison, S.E. McPherson, C. Tsai, C.M. Sinton, D. Murphy, M. Williams, D.J. Steel, and P.L. Wood. Neuroscience/Cardiovascular Research, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ 07901.

CGS 19755 (1-(cis-2-carboxypiperidine-4-yl)methyl-1-phosphonic acid) potently inhibited N-methyl-D-aspartate- (NMDA-)evoked, but not KCl-evoked, [^{3}H]acetylcholine release from slices of the rat striatum. The concentration-response to NMDA was shifted to the right by CGS 19755, consistent with a competitive interaction with NMDA-type receptors. CGS 19755 inhibited [^{3}H]CPP binding to NMDA-type receptors with an IC_{50} of 67 nM, making it the most potent NMDA-type receptor antagonist yet reported. CGS 19755 did not affect the binding of 21 other ligands to their receptors. In crude P_2 fractions, no evidence was obtained to suggest that radiolabeled CGS 19755 is taken up by an active transport system. Furthermore, CGS 19755 failed to affect the uptake of [^{3}H]L-glutamate, or to interact with aconitine-induced inhibition of [^{3}H]L-glutamate uptake, the latter finding consistent with a lack of membrane-stabilizing or local anesthetic properties. CGS 19755 selectively antagonized the excitation by iontophoretically applied NMDA in the red nucleus of the rat, without affecting the excitation by quisqualate. CGS 19755 inhibited the harmaline-induced increase in cerebellar cGMP levels completely at a dose of 4 mg/kg i.p., with a duration of action exceeding 2 hours. CGS 19755 therefore is a competitive NMDA antagonist which is active in vivo and may be a useful therapeutic and investigative agent.



AP5



CGS 19755

109.3 [3H]-CPP BINDING TO NMDA RECEPTORS IN HIPPOCAMPAL MEMBRANES. David T. Wong and Penny Threlkeld*. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

CPP, 3-((±)-2-carboxypiperazin-4-propyl-1-phosphonic acid, has been identified as a potent and selective antagonist of NMDA receptors (Davies et al, Brain Res. 382:169, 1986). We describe here the specific binding of [3H]-CPP with properties similar to those of the N-methyl-D-aspartate-sensitive glutamate receptors in hippocampal membranes (P. Threlkeld, P. Ornstein and D. Wong, this volume, 1987). Crude synaptosomal preparations (P2 fraction) isolated from hippocampus of rat brain were suspended in 50 mM Tris-acetate (pH 7.4) containing the detergent Triton X-100 in a final concentration of 1%. After standing at 4°C for 15 min, the suspension was centrifuged at 50,000 x g for 10 min. The detergent was removed by repeating the process of suspension and centrifugation 5 times. The resulting membranes in 150 µg protein were incubated at 25°C for 15 min in a medium containing 50 mM Tris-acetate buffer (pH 7.4) and various concentrations (1-50 nM) of [3H]-CPP. L-Glutamate at 10 µM was included in separate samples to assess nonspecific binding at each concentration of [3H]-CPP. Specific binding represented 67-73% of total binding. [3H]-CPP binding was saturable with a maximum density (Bmax value) of 3.4 pmole/mg protein and a dissociation constant (Kd value) of 22 nM. L-Glutamate at 25 nM lowered the Bmax and Kd values by 54 and 28%, respectively, suggesting a noncompetitive nature of inhibition. L-Glutamate was most potent as an inhibitor with concentration required to lower 50% of [3H]-CPP binding at 25 nM (IC50 value). D-AP5 and D-AP7 were stereospecifically more potent (IC50's of 47 and 320 nM, respectively), than their corresponding L-isomers by 45 and 11 times. Glutamate diethylester, homocysteate, l-aspartate and NMDA also inhibited [3H]-CPP binding, with IC50's of 180, 240, 1000 and 1700 nM, respectively, but quisqualate, kainate and quinolinate inhibited only at 10,000 nM or higher concentrations. [3H]-CPP binding was inhibited by the guanine nucleotides GTP, GDP and GMP, with IC50's of 4.1, 10 and 100 µM, respectively. The adenosine nucleotides ATP, ADP and AMP were inert at 10 µM. Thus, these studies show that [3H]-CPP is an ideal 3H-ligand for studies of the NMDA receptors of excitatory amino acids in vitro.

109.4 **ANATOMICAL AND PHARMACOLOGICAL EVIDENCE FOR TWO CLASSES OF NMDA RECOGNITION SITES.** D.T. Monaghan[1], C. Chung*[1], H. Olverman*[2], J. Watkins*[3], and C.W. Cotman[1]. Dept. Psychobiology[1], Univ. California, Irvine, CA 92717, Dept. Pharmacology[2], Univ. Edinburgh, Edinburgh, U.K. and Dept. Pharmacology[3], The Medical School, Bristol BS81TD, U.K.

The excitatory amino acid receptor which is selectively activated by N-methyl-D-aspartate (NMDA) plays a critical role in synaptic transmission, neuronal plasticity, epileptiform activity, and excitotoxicity. Using electrophysiological and radioligand binding methods, pharmacological studies of the NMDA receptor generally indicate that this receptor represents a homogeneous population exhibiting a unique pharmacological profile. However, there is some evidence that there may exist multiple receptor populations. The relative potency of quinolinic acid as an NMDA agonist varies between regions. In radioligand binding experiments, agonists are more potent displacers of L-[^{3}H]glutamate binding (at the NMDA site) than of [^{3}H]3-((±2-carboxypiperazine-4-yl)propyl-1-phosphonic acid ([^{3}H]CPP) which also binds at the NMDA site. In contrast, antagonists are better displacers of radiolabelled antagonist binding.

To evaluate the possibity of multiple NMDA sites we have compared the distribution of NMDA-sensitive L-[^{3}H]glutamate binding sites to that of [^{3}H]CPP binding sites in the rat brain with the use of quantitative autoradiography. Autoradiograms of NMDA-sensitive L-[^{3}H]glutamate and [^{3}H]CPP binding sites were prepared as previously described (P.N.A.S. 83 (1986) 7532, Eur. J. Pharmacol. 131(1986) 161).

Relative to L-[^{3}H]glutamate, [^{3}H]CPP binding is low in the striatum, septum and cerebellar granule cell layer and relatively high in the thalamus. If two populations of NMDA binding sites exist, then the slower dissociating ligand L-[^{3}H]glutamate might label both populations in autoradiographic preparations, and various glutamate analogues may display regional variations in their ability to displace NMDA-sensitive L-[^{3}H]glutamate binding. We have found that D-2-amino-5-phosphonopentanoate, 2-amino-7-heptanoate, and D-aminoadipate more potently displace L-[^{3}H]glutamate binding from the cerebral cortex than other brain regions and are relatively weak at displacing binding in the cerebellar granule cell layer and the medial striatum. In contrast, the agonists, NMDA, quinolinate, and L-aspartate were more potent displacers of binding in the medial striatum than in other regions, and relatively weak displacers of binding in the thalamus. Thus, binding sites in the thalamus preferentially recognize NMDA antagonists while binding sites in the cerebellum and medial striatum preferentially recognize NMDA agonists.

These results suggest that there exist either two distinct NMDA receptor types or two states of the NMDA receptor which differ by their relative affinity for agonists and antagonists. Thus it remains to be determined whether these sites can interconvert or are distinctly different receptor populations.

Support: ARO grant DAAL 03-86-K-0067 and the MRC.

109.5 MODULATION OF EXCITATORY AMINO ACID TRANSMISSION BY PHENCYCLIDINE AND GLYCINE IN THE RAT CEREBELLUM STUDIED IN VIVO. W. Danysz*, J.T. Wroblewski*, G. Brooker* and E. Costa. (SPON: M. Miyata). FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Med. Sch., Washington, D.C. 20007.

It has been shown previously that excitatory amino acid responses induced by N-methyl-D-aspartate (NMDA) as measured electrophysiologically, biochemically and behaviorally can be attenuated by administration of phencyclidine (PCP). Our recent studies have demonstrated that ionotropic (G_C) and metabolotropic (G_P) excitatory amino acid receptors are present in primary cultures of cerebellar granule cells. One subclass of each receptor category (G_{C1} and G_{P1}) undergoes negative allosteric modulation by ligands acting at PCP recognition sites. This interaction may be of a physiological significance since an endogenous ligand for PCP recognition sites has been identified (Quiron et al., Peptides, 5, 967-973, 1984). Recent electrophysiological findings suggest that excitatory responses to NMDA can be modulated in a positive manner by glycine, acting at specific strychnine-insensitive recognition sites, possibly associated with G_C receptors (Johnson and Ascher, 1987, Nature, 325, 529-531).

In the present study, we have demonstrated that signal transduction at excitatory amino acid receptors present in the cerebellum can be modulated in opposite directions by PCP and glycine. For this purpose, male Sprague-Dawley rats (200 g) were implanted with plastic cannulae into the lateral cerebral ventricle. Four days after surgery, rats were used for the experiments. NMDA (G_{C1} and G_{P1} agonists) and PCP were injected i.c.v., while glycine was injected both i.c.v. and i.p. The animals were sacrificed by microwave irradiation 1-15 min after NMDA injections. The concentrations of cyclic GMP in brain tissues were measured by RIA.

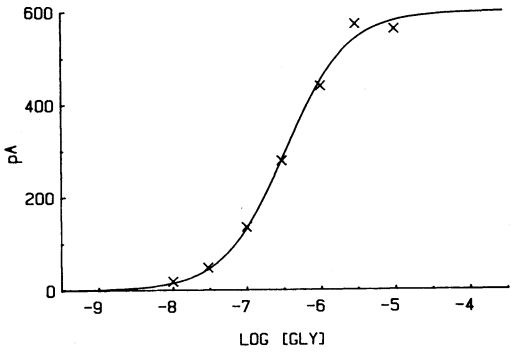
Injections of NMDA resulted in an increased content of cyclic GMP in the cerebellum, which was dose- and time-dependent. The co-administration of 2-amino-5-phosphonovaleric acid abolished this response confirming the specificity of NMDA action. PCP, injected (50 ug i.c.v.) together with NMDA inhibited the increase of cyclic GMP content elicited by NMDA. PCP also decreased cGMP content in the cerebella of saline-injected rats. Glycine, injected i.c.v. (1 ug) together with low doses of NMDA (1 ug) produced a 6-fold potentiation of NMDA action. The administration of glycine alone had no effect on cerebellar cGMP content.

These results indicate that PCP and glycine may act in opposite ways in modulating the responses of G_{C1} receptors. The possibility that modulatory sites for PCP and glycine may be located within the supramolecular complex of these receptors is in agreement with the present finding.

109.6 INTERACTION OF GLYCINE WITH THE N-METHYL-D-ASPARTATE RECEPTOR. J.W.Johnson* and P.Ascher* (SPON: C.KORDON) Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris.

The potentiation by glycine of the response activated by NMDA was studied using patch-clamp techniques on cultured brain neurons from mouse embryo. Dose-response curves for glycine were fit with the Hill equation by using a simplex algorithm to find the best values for n (Hill coefficient), Km (apparent dissociation constant), and maximum response. In the example shown below ([NMDA]=10 uM, [Mg]=0, V(h)=-50 mV) the value found for n was 1.04 and for Km 340 nM.

The high affinity of glycine for its receptor suggests that the glycine unbinding rate may be relatively slow. When the solution bathing a neuron is quickly changed from NMDA + glycine to NMDA alone, the decay of the response requires several seconds. The rate of this slow decay should approximate the glycine unbinding rate if NMDA channel closing rate is fast relative to both channel opening and glycine unbinding rates, as is suggested by data from outside-out patch recordings. Similar experiments in which a change is made from NMDA to NMDA + glycine (with glycine concentration below its Km) should allow estimation of the glycine binding rate. The validity of this simple approach can be tested by comparing the glycine affinity deduced from dose-response curves with that calculated from the on and off rates of glycine binding.



109.7 POTENT AND COMPETITIVE ANTAGONISM AT NON-NMDA RECEPTORS BY FG 9041 AND FG 9065. T. Honoré*[1], S.N. Davies*[2], J. Drejer*[1], E.J. Fletcher*[2], P. Jacobsen*[1], D. Lodge*[2] and F.E. Nielsen*[1] (SPON: L.H. Jensen). [1]Ferrosan Research Division, DK-2860 Soeborg, Denmark. [2]Royal Veterinary College, London NW1 OTU, UK.

Investigations of functions mediated by quisqualate receptors have been difficult due to lack of potent and selective antagonists. Here we report a new type of potent and competitive quisqualate antagonists.

FG 9041 and FG 9065 are potent inhibitors of ^{3}H-AMPA binding to rat cortical membranes with IC_{50}'s of 475 and 275 nM, respectively. Y-D-glutamyl aminomethyl sulphonic acid (GAMS), which has been reported to be a selective antagonist for non-NMDA-receptors gave an IC_{50} of 190,000 nM in the same assay.

FG 9041 and FG 9065 gave IC_{50}'s for the ^{3}H-kainate binding site of 2250 and 1700 nM, respectively and were very weak or inactive on NMDA-receptors as measured by ^{3}H-CPP binding (IC_{50} > 25 µM). No affinity of the compounds were detected for 5-HT-, NA-, Muscarinic-, DA-, opiate-, benzodiazepine-, GABA- or glycine-receptors.

In the following the antagonistic activities are exemplified for FG 9041, but generally FG 9065 has similar selectivity with a higher potency.

FG 9041 is a potent competitive antagonist of quisqualate induced ^{3}H-GABA release from cultured mouse cortical neurons (pA_2 = 6.25 corresponding to an EC_{50} = 560 nM). In this assay the EC_{50} of GAMS was > 200 µM. The EC_{50}'s of FG 9041 and GAMS as antagonists of kainate and NMDA induced ^{3}H-GABA release were 1300 and 4000 nM, and 185 and 235 µM, respectively. Detailed discussion of the activities of excitatory aminoacid antagonists on cell cultures will be presented elsewhere (Drejer et al. This meeting).

After electrophoretic application in rat spinal cord of FG 9041 to 15 cells the responses to quisqualate and kainate were reduced approximately equally with relatively little effect on the response to NMA. The effect of FG 9041 on the responses to L-glutamate and L-aspartate were tested on 11 cells which had selectivities to quisqualate and NMA as above. In these cells FG 9041 blocks the responses to L-aspartate most efficiently.

The above mentioned results indicate that FG 9041 and FG 9065 are selective and competitive antagonists of non-NMDA receptors with preference for the quisqualate receptors.

Acknowledgement. The close collaboration with Squibb Institute for Medical Research, Princeton, NJ 08540, USA is highly appreciated.

109.8 SINGLE CHANNEL PROPERTIES ESTIMATED FROM EXCITATORY AMINO ACID INDUCED CURRENT NOISE IN ISOLATED FISH HORIZONTAL CELLS. B.N. Christensen and T.J. O'Dell. Dept. Physiology and Biophysics, Univ. Texas Medical Branch, Galveston, TX. 77550.

Several of the excitatory amino acids produce increases in membrane permeability in fish horizontal cells. Glutamate (GLU), quisqualate (QA) and kainate (KA) are depolarizing agonists in both catfish (Ictalurus punctatus) and stingray (Dasyatis Sabina) whereas aspartate (ASP) and n-methyl-d-aspartate (NMDA) are effective only on catfish horizontal cells. We have used the fluctuations in the agonist induced current noise to measure the single channel conductance and mean channel open time in the presence of these agonists in an attempt to determine any differences that may exist between these two preparations of the agonist action.

Following enzymatic isolation, horizontal cells were voltage clamped at the resting membrane potential using patch electrodes. The cells were positioned by displacement of the electrode into a small isolation chamber with a volume around 25 uL to facilitate rapid exchange of solution containing the agonists to be studied. A background power spectrum was measured and subtracted from the agonist spectrum and this difference spectrum was used to estimate single channel conductance and mean open time. Open time was calculated from the corner frequency of a single Lorentzian fitted to the subtracted spectrum. Single channel conductance was calculated from the variance of the agonist induced current noise.

There was little difference in channel open time for the four agonists KA, QA, NMDA, or ASP in the catfish. They ranged from, 1.1 mSec to 1.3 mSec respectively (8 cells). The mean open times for KA and QA in stingray horizontal cells were about two times longer; 2.4 mSec for KA and 1.7 mSec for QA (12 cells). The single channel conductances were quite similar for the two preparations for the same agonist. KA produced the smallest conductance of 4.4 and 4.9 pS, QA the largest conductance of 9.9 and 10.1 pS for the two preparations. The single channel conductance for NMDA and ASP in the catfish was 6.7 and 5.0 pS. The single channel conductance measured under these conditions are similar to those measured in other CNS neurons except for the NMDA channel which has been reported to be as large as 50 pS in some preparations (Cull-Candy and Usowicz, Nat., 325, 1987 and Jahr and Stevens, Nat., 325, 1987). However, these authors reported that the NMDA channel had substates with conductances as low as those reported here. Our results suggest that under the conditions of these measurements, the large NMDA channel conductance state does not exist in the catfish horizontal cells.

Acknowledgements: This research was supported by grant EY-01897 from the Department of Health and Human Services.

109.9 INTERACTION OF EXCITATORY AMINO ACIDS AT A SINGLE NON-NMDA TYPE RECEPTOR IN ISOLATED CATFISH HORIZONTAL CELLS. T.J. O'Dell* and B.N. Christensen. (SPON: J. Blankenship) Dept. of Physiology and Biophysics, Univ. Texas Medical Branch, Galveston, TX. 77550.

While the excitatory amino acids glutamate (GLU) and quisqualate (QA) are generally considered to be agonists, these compounds can inhibit kainate (KA) responses in horizontal cells isolated from fish retina. Although the mechanism responsible for this inhibition is unknown, it has been suggested that uncompetitive kinetics are involved (Ishida and Neyton, P.N.A.S., 82, 1985). To better determine the mechanism(s) responsible for the GLU and QA inhibition of KA responses we have studied the actions and interactions of these compounds using horizontal cells isolated from catfish (Ictalurus punctatus) retina.

Whole-cell currents were recorded from enzymatically isolated cone horizontal cells using the single electrode patch-clamp technique to voltage-clamp the cells. Known concentrations of agonist were rapidly bath applied onto the cells using the "concentration-clamp" technique (Akike et al., J. Physiol., 379, 1986).

Dose-response curves for GLU, QA, KA, and alpha-amino-3-hydroxy-5-isoxazole-4-propionic acid (AMPA) were parallel to one another, suggesting that the agonists may be acting at a common site. The rank order affinity of the agonists was QA>AMPA>GLU>KA (EC50's = 2.3, 15.4, 23.0, and 85.0 (uM) respectively). Although KA had the lowest affinity of the agonists tested, at saturating concentrations KA produced 4-7 times more current than the other agonists. To examine the QA and GLU inhibition of KA responses, we applied 70 uM KA alone with concentrations of QA, GLU, or AMPA from 1 to 500 uM. QA, AMPA, and GLU all inhibited the KA response, the rank order potency for the inhibition being QA>AMPA>GLU (IC50's = 20.0, 88.0, and 150.0 uM respectively). Our results suggest that GLU, AMPA, KA, and QA act at a common receptor site. Although KA has the lowest affinity for this site it is more efficacious than the other agonists (i.e. produces the largest current response at saturating concentrations). When QA, AMPA, or GLU are applied with KA these agonists can displace KA from the receptor (due to their higher affinity) but they in turn produce much less current than KA. Thus the KA response appears to be inhibited by the other agonists. Our results suggest that the QA, GLU, and AMPA inhibition of KA responses arises from the interaction of agonists with different affinities and "efficacies" at a common receptor site.

Acknowledgements: This research was supported by grant EY-01897 from the Department of Health and Human Services.

109.10 NONCOMPETITIVE N-METHYL-D-ASPARTATE ANTAGONISTS AFFECT MULTIPLE ION CHANNELS. S.M. Rothman. Departments of Anatomy & Neurobiology and Pediatrics, Washington University School of Medicine, St. Louis, MO 63110.

Understanding the mechanism(s) of action of phencyclidine (PCP) and the related compounds ketamine (KET) and MK-801 has become an important problem in neuropharmacology. Considerable evidence suggests that these compounds are noncompetitive N-methyl-D-aspartate (NMDA) antagonists, while other experiments have demonstrated that at least PCP blocks voltage-gated potassium currents. We have examined the actions of PCP, KET, and MK-801 in cultures of dispersed rat hippocampal neurons. The whole cell patch clamp technique was used to record from single neurons under either voltage clamp or current clamp.

When neurons were stepped from -80 mV to -10 mV in buffer containing TTX and Cd and lacking added Ca, they produced a rapidly inactivating outward current which decayed by 100 msec (I_A) and a non-inactivating outward current which remained constant over 500 msec (I_K). The former current was partially blocked by 5 mM 4-aminopyridine (4-AP). PCP reversibly reduced both current in a concentration-dependent manner. At 100 µM, I_A decreased by 22.4±7.2% and I_K by 12.8±14.3% (n=12). At 3 mM, I_A and I_K diminished by 71.4±9.6% and 52.8±21.5% (n=5), respectively. Similar concentrations of KET or MK-801 produced equivalent reductions in I_A and I_K. However, the competitive NMDA antagonist D-2-amino-5-phosphonovalerate (APV) did not affect I_A and I_K.

Under current clamp, 4-AP increased the peak of the action potential and its maximum rate of rise while decreasing its rate of fall. PCP and MK-801 produced a concentration-dependent reduction in the action potential, its maximum rate of rise and rate of fall. Thus, both drugs likely block voltage-gated sodium channels as well as potassium channels.

At 100 µM, PCP and MK-801 decreased the inward current produced by a 200 msec application of NMDA (100 µM) by 74.5±6.2% and 71.2±8.9%, respectively (N=5). The NMDA blockade developed slowly but was at least partially reversible. It was present at holding potentials of -50 mV and +50 mV. APV (100 µM) produced an immediate and almost complete blockade of NMDA responses (92.0±6.1%, n=3). 4-AP had no effect on NMDA responses, even at 5 mM.

Potassium channels in intact hippocampal neurons are far more sensitive to 4-AP than neurons in culture. If there is a similar difference in their sensitivity to PCP, KET, and MK-801, then the behavioral effects associated with these drugs are likely due to actions at both potassium channels and NMDA-gated channels.

Supported by NINCDS (NS19988), the American Heart Association, and Monsanto Corporation.

109.11 **IONOPHORETIC MAPPING OF GLUTAMATE RECEPTORS ON CULTURED CHICK SPINAL NEURONS.** L.O. Trussell & G.D. Fischbach. Washington U. Sch. of Medicine, 660 S. Euclid, St. Louis, MO 63110.

There is now abundant evidence for the localization of neurotransmitter receptors at sites of synaptic contact in the neuromuscular junction. In contrast, little is known about receptor distribution on neurons, especially in regard to glutamatergic neuronal synapses. We have used micro-ionophoretic application of glutamate to map receptors over the surface of cultured chick spinal neurons. Neurons were voltage-clamped with patch electrodes containing Cs_2SO_4 to improve the spatial clamp. Spatially restricted application of 250 mM glutamate was achieved with 0.5-1.0 msec pulses of less than 100 nA and a retaining current less than 10 nA. The position of the electrode was monitored using a 40X objective and DIC optics.

With careful positioning of the electrode and control of ejection and backing current, a transient inward current was observed in response to a pulse of glutamate. This response ranged from 10-90 pA in amplitude, peaked within 1-3 msec, and decayed within 10 msec. In amplitude and timecourse, these fast responses strongly resembled spontaneous synaptic currents observed in the same cells. The fast responses were critically dependent on electrode position: movement as little as 1-2 um away from or along the neurite sharply reduced and slowed this response. These local areas of high sensitivity were most often observed where neighboring neurites crossed or ran parallel to the recorded neuron's processes or soma. The relative insensitivity of this glutamate-activated conductance to 2-amino-5-phosphonovalerate (APV) and to voltage implies that it is predominantly mediated by G2 (kainate/quisqualate) receptors. Its discrete localization suggests that such receptors are tightly aggregated on these neurons, possibly at synaptic sites.

Following the rapid response discussed above, a slow inward tail was often observed which decayed over hundreds of msec. This slow response was markedly reduced by 0.5mM APV and by holding potentials more negative than -40mV, suggesting that it reflects G1 (NMDA) receptor activation. The slow response was much less sensitive to electrode position, suggesting that such receptors may have a wider spatial distribution than the receptors producing the rapid response.

Other studies in the spinal cord and hippocampus show that fast and slow components of evoked glutamatergic transmission may correspond to G2 and G1 receptor activation, respectively. The present work suggests that this may result from a tight localization of G2 receptors and a diffuse distribution of G1 receptors both responding to synaptically released glutamate.

109.12 **THREE TYPES OF EXCITATORY AMINO ACID INDUCED CURRENTS IN mRNA INJECTED XENOPUS OOCYTES.** T.A. Verdoorn* and R.J. Dingledine. (SPON : Anthony T. Dren) Dept. Pharmacology and Curriculum in Neurobiology. Univ. of North Carolina, Chapel Hill, NC 27514.

Total mRNA was prepared from rat brain tissue and injected into Xenopus oocytes. The oocytes were incubated for at least 24 hours in modified Barths solution before voltage clamp experiments were done. Injected oocytes responded with inward currents to bath applied excitatory amino acid (EAA) agonists while uninjected or water injected oocytes had very small or no responses to these agonists. Three distinct types of inward currents were seen in the injected oocytes corresponding to the three subtypes of EAA receptors thought to exist in mammalian brain. The response to N-methyl-D-aspartate (NMDA) was smooth and nondesensitizing. Its physiological properties and sensitivity to modulators are consistent with the neuronal NMDA receptors studied previously (see Kleckner et. al., this meeting). The EC50 for NMDA was 39±13 µM (n=4) and D-2-amino-5-phosphonovaleric acid (10 µM) reduced the response to 100 uM NMDA by an average of 83 % (n=6). Ibotenate and glutamate could also activate this current.

Another type of response to EAA agonists was elicited by quisqualate and glutamate and consisted of a inward current with large current oscillations superimposed. The oscillations are thought to be due to a chloride channel and desensitize rapidly leaving only the smooth part of the response.

The kainate induced current represents the third type of EAA response that we see in mRNA injected oocytes. Kainate causes a smooth current that does not desensitize and is insensitive to Mg++ ions. The kainate conductance and EC50 do show a slight voltage dependency. The response can be reduced by the antagonist kynurenic acid (50 µM). The reversal potential for kainate is -5.3± 2.0 mV (n=15) which suggests that the kainate channel is nonselectively permeable to cations in the oocyte. At a holding potential of -60 mV the EC50s for kainate and its more potent analogue domoate were 89±8 µM (n=10) and 14± 0.5 µM (n=4) respectively. The EC50s (measured as the smooth response) for glutamate and quisqualate were found to be 14±4 µM (n=5) and 0.44±0.03 µM (n=4) respectively.

These experiments show that the oocyte can be used to study the properties and structural relationships of brain EAA receptors. In the future we will determine the potencies of the various EAA receptor antagonists with Schild analysis as well as begin to study the receptor inter-relationships by using mRNA purified by size fractionation.

Supported by NS-22249, NS-17771, NS-23804 and a Pharmaceutical Manufacturers Association Foundation predoctoral fellowship (TAV).

110.1 **HEPATIC PORTAL AND GASTRIC AFFERENT PROCESSING IN NUCLEUS OF THE SOLITARY TRACT OF THE RAT**

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Vagal gastric and hepatic mechano- and chemo- receptors monitor the entry of nutrients into the gastrointestinal tract and blood stream, respectively. This information, which is of crucial importance in the control of food intake, is relayed over subdiaphragmatic branches of the vagus, which ultimately synapse on neurons in the caudal and medial nucleus of the solitary tract (NTS). Given the importance that has been attributed to detection of changes in levels of blood glucose, and the possible peripheral monitoring of these changes, we wanted to assess the ways in which the central nervous system (CNS) processes changes in the glycemia, and how this is integrated with other afferent information, such as activity of gastric mechanoreceptors.

Chloralose/ urethane-anesthetized male rats instrumented for physiological recording were used in all studies. In order to devise a stimulus that would selectively influence peripheral (i.e. hepatic portal) glucose-sensitive structures, we employed simultaneous on-line recording of blood glucose levels in both the jugular and hepatic portal veins before, during, and after graded infusions of different volumes of glucose into the hepatic portal vein. A volume was found which produced significant transient increases in portal glycemia, but had no effect on systemic (jugular) levels.

In another series of experiments, this search stimulus was combined with graded distension of the gastric lumen (0-10cm H_2O). Single and multi-unit activity was recorded through glass microelectrodes in the caudal/medial NTS during application of these stimuli (and in some cases to electrical stimulation of the subdiaphragmatic branches). Chicago Sky Blue dye was iontophoretically applied at selected sites to permit histological reconstruction of the recording elctrode placements. Numerous units were found with tonic or phasic responses to gastric distension in the NTS regions receiving gastric branch afferents. Most were excited by distension, although a significant number (~10%) were inhibited. Units were also encountered that responded to increases in hepatic portal glucose concentrations--so far these responses have all been inhibitory. In addition, the cells receiving hepatic portal afferents also received convergent input from gastric mechanoreceptors. Cells that were inhibited by increases in portal glucose were excited by gastric distension.

This convergence of information at the first synapse in the vagal sensory pathway is consistent with our previously reported finding of extensive convergence of afferents in the hepatic and gastric branches of the vagus onto single NTS neurons. It is also consistent with the extensive integration of metabolic afferent information that is believed to take place in the hindbrain. (Supported by HL29033 and NS23432)

110.2 BRAINSTEM UNITARY RESPONSE TO ELECTRICAL STIMULATION OF VENTRAL AND DORSAL GASTRIC VAGAL BRANCHES IN THE CAT. W.D. Barber, C.S. Yuan* and T.F. Burks. Departments of Anatomy and Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Gastric vagal projections to the dorsomedial region of the caudal brainstem were examined electrophysiologically in cats anesthetized with halothane and nitrous oxide supplemented with oxygen. Single shock stimulations (0.3 msec, 500 uA, 0.5 Hz) were applied to the ventral and/or dorsal gastric vagal branches to activate C fibers which serve the proximal stomach. Single unit activity was recorded extracellularly from 290 neurons in both sides of the caudal brainstem during gastric vagal nerve stimulation. Based upon stereotaxic coordinates and electrophysiological correlates, the results showed that the units responding to gastric nerve stimulation were located in the region of nucleus solitarius, similar to the location of the responses to gastric distention reported previously in our laboratory (W.D. Barber and T.F. Burks, 1983). The latency of the unitary discharges ranged from 185 to 420 msec with a mean of 293 $\pm$ 52.7 msec. The conduction velocity was less than 1 m/sec based upon the latency of the brainstem responses. The majority of the unitary responses contained multiple spikes. Decreasing the stimulus strength reduced the number of spikes in the unitary discharges, with the first spike showing jitter, suggesting an orthodromic response. Geographically the brainstem projections of the ventral and dorsal gastric branch stimulations were similar. Out of a total of 290 responses recorded, the ventral and dorsal gastric branches projected to the same recording area in 53 cases, and projected to the same neuron in 42 cases. Of the latter 42 convergent projections, there were 30 excitatory and 12 inhibitory responses when the ventral and dorsal gastric branches were stimulated simultaneously. These data indicate that there is a substantial amount of convergence in the brainstem of vagally mediated sensory information from the proximal stomach. The specific nature of the information and its role in the regulation of gastric function remains to be resolved. However, these data have identified some very basic functional characteristics of brainstem neuronal mechanisms in response to electrical stimulation of gastric vagal afferent fibers. (Support by USPHS Grant AM 31804).

110.3 **PATTERNS OF FACILITATION AND INHIBITION IN SOLITARY TRACT (NTS) NEURONS IN CAT PRODUCED BY CONVERGING SOMATIC AND VAGAL AFFERENTS.** R. J. Person. Dept. of Physiology and Biophysics, Univ. Okla. Health Sci. Ctr., Oklahoma City, OK 73190

This laboratory reported data showing convergence of vagal and hindlimb somatic Group II-IV sensory afferents on NTS neurons (Neurosci. Abst. 12:1324). Described here are intra- and inter-afferent interactions producing facilitation and inhibition of sensory input defined by conditioning-test (CT) paradigms. NTS unit responses to afferent stimulation were recorded with tungsten (10 MΩ) microelectrodes in acute, artificially respired, α-chloralose anesthestized, pancuronium paralyzed cats. Recording and stimulating electrodes were placed on the cutaneous sural (S), muscular common peroneal (P), and cervical vagus (V) nerves. Compound action potentials were recorded in response to single 100 μs shocks to define threshold for each nerve. Stimulation was applied in units of threshold in the 1-30X range for Group II and III responses and in trains of five 500 Hz stimuli at 100X threshold for Group IV responses. Units were recorded and histologically localized to the NTS and DMV area contralateral to hindlimb stimulation. Peristimulus histograms (PSTs) were generated for both single and paired stimuli with interstimulus intervals (ISIs) for stimulus pairs of 10-2000 ms and various permuations of P, S, and V shocks as conditioning (C) or test (T) stimuli (eg., CTs of P-S, P-V, and P-P stimuli).

NTS unit responses to single somatic Group II and III stimuli have been described and conform, with an earlier latency, to single and multi-fiber somatosympathetic responses (cf, Terui and Koizumi, J. Auton. Nerv. Sys., 10:73). CT testing demonstrated a common period of inhibition of both subsequent vagal and somatic input at ISIs of 10-500 ms with maximal inhibition of input to 0-50% of control responses in an ISI range of 25-100 ms. At ISIs greater than 500 ms various patterns of facilitation and inhibition of T input were observed with a return to control T response amplitude only after 1500-2000 ms. In some units, early facilitation of T responses were seen particularly with sural C input. Inhibition of T input was often observed with C input excitation below threshold for unit action potential generation. Sensory afferents to NTS neurons therefore elicit a prolonged inhibition during which subsequent somatic and vagal input is substantially or completely blocked.

This central NTS period of inhibition corresponds to the peripheral sympathetic "silent" period and parasympathetic excitation elicited by somatic stimulation. The results suggest that Group II-IV somatic sensory afferents are capable of modulating NTS activity to match cardiopulmonary adjustments for movement and exercise with peripheral tissue demand for blood flow and substrate delivery and support the NTS as the central site of somato-autonomic reflex mediation. Supported by American Heart, Oklahoma Affiliate, OK-85-G-11.

110.4 **INFLUENCES OF VAGAL AFFERENTS ON RAPHESPINAL NEURONS IN THE CAT.** R.W. Blair, U.T. Oh*, S.F. Hobbs, and R.D. Foreman. Dept. Physiology and Biophysics, Univ. Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Excitation of vagal afferents often inhibits, and sometimes excites, thoracic spinothalamic (STT) and spinoreticular (SRT) neurons. One central site that may mediate these effects is the midline raphe complex; stimulation of raphe magnus inhibits STT, SRT, and other dorsal horn neurons. The purpose of this study was to determine the effects of electrical stimulation of cervical vagal afferents on raphespinal (RAS) neurons in raphe magnus. Fourteen cats were anesthetized with α-chloralose (40 mg/kg) and paralyzed with bolus doses of pancuronium bromide (0.3 mg). Adequacy of anesthesia was tested once paralysis wore off and before the next dose of pancuronium was given. A bipolar platinum electrode was attached to the right cervical vagus nerve after removal of the cervical sympathetic nerve. A laminectomy exposed the T_2-T_4 spinal segments, and concentric bipolar electrodes were placed bilaterally in white matter for antidromic activation of RAS cells. Pulses of 0.1 ms duration and 3-5 mA delivered at 1 Hz were used to search for antidromically-activated neurons. Individual RAS neurons were recorded extracellularly with tungsten microelectrodes. To date 18 RAS neurons have been tested for responses to vagal stimulation. Six neurons (33%) were responsive. All 6 cells were excited if single pulses or brief (10 ms) trains of high frequency (333 Hz) pulses were delivered at 1 Hz. However, if single pulses were delivered at higher frequencies, spontaneous cell activity was often inhibited; this pattern occurred for 3 neurons. A fourth cell's activity was unaffected if pulses were delivered at 20 Hz. The remaining two cells were not tested for responses to tonic trains of vagal stimuli. RAS neurons responsive to vagal stimulation had conduction velocities (CV) ranging between 43-97 m/s; unresponsive neurons had CV between 2-107 m/s. Results indicate that at low frequencies vagal stimulation may increase cell activity, but at higher frequencies inhibition occurs. About a third of RAS neurons respond to vagal afferent stimulation. Thus, vagal afferents can modulate activity of RAS neurons; this effect may then elicit altered activity of spinal neurons. (Supported by NIH grant # HL29618).

110.5 **LONGITUDINAL COLUMN OF GASTRIC BRANCH NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS IS COMPOSED OF SUBCOLUMNS CORRESPONDING TO DISTAL DIVISIONS OF GASTRIC BRANCH** T. L. Powley, E.A. Fox, E. Baronowsky*, D.L. Keller*, and H.-R. Berthoud. Lab. of Regulatory Psychobiology, Purdue University, W. Lafayette, IN, 47907.

The motor neurons of the abdominal vagus are organized into longitudinal columns within the dorsal motor nucleus of the vagus (dmnX) (Fox and Powley, Br. Res. 341:269, '85; Powley et al. AJP, in press). Specifically, the rat has five major columns, one corresponding to each of the primary branches of the subdiaphragmatic vagus. The possibility that these separate columns are composed of more punctate topographic elements has not been directly addressed. To evaluate this possibility and to characterize more fully the dmnX columnar organization, we mapped the central topographies associated with two secondary branches arising from the ventral (anterior) gastric branch.

Two complementary strategies were employed. In the first, either one of the two largest secondary gastric branches in the rat (cf. Prechtl and Powley, JCN, 235: 182, '85) as well as all other branches of the ventral vagus were cauterized, and then True Blue (TB) was injected IP (n = 15). [In this protocol all abdominal vagal terminals are exposed to TB, but only the intact secondary branch can transport it centrally--Powley et al., Soc. Neurosci. Abstr. 12, P. 1058, 1986.] In the second labeling strategy, one of the secondary gastric branches was cut and encapsulated in a small HRP-filled styrofoam cuff for a 48 hr survival period (n = 12 for each secondary branch).

Both TB and HRP experiments demonstrated that the ventral gastric branch column in the medial two thirds of the left dmnX was composed of separate, parallel, and longitudinal subcolumns. The axons of the secondary branch issuing onto the antrum/pylorus region of the stomach originated from a subcolumn constituting the medial half of the overall column. In contrast, the efferent fibers in the secondary branch issuing to the corpus/fundus region originated from a subcolumn forming the lateral half of the primary column. The subcolumns extended rostrocaudally throughout the full length of the dmnX. Like the major columns of the primary branches, the subcolumns were spatially more discrete in the caudal two thirds of the dmnX and overlapped more in the rostral pole of the nucleus.

Although to date we have only examined systematically the secondary branches of one of the five primary branches, it would seem parsimonious to hypothesize that the other major columns of abdominal efferents in the dmnX may also be composed of sets of finer subcolumns corresponding to secondary branches of the nerve. Further, the parallel longitudinal pattern of dmnX subunits, when considered in conjunction with the organization of the nucleus of the solitary tract, suggests that the specificity of vagal reflexes may be generated by the sensory-motor lattice which is formed by the orthogonal functional maps of the two nuclei. (NIH Grant DK27627)

110.6 EFFERENT PROJECTIONS OF THE NUCLEUS OF THE SOLITARY TRACT TO THE AMYGDALA, BED NUCLEUS OF THE STRIA TERMINALIS AND SUBFORNICAL ORGAN IN THE RAT EXAMINED USING THE PHA-L ANTEROGRADE TRACING METHOD. A.M. Zardetto-Smith and T.S. Gray. Dept. Anat., Loyola Univ., Maywood IL 60153.

The caudal, medial part of the nucleus of the solitary tract (NTS) receives the majority of general visceral afferent input carried by the vagus and glossopharyngeal nerves. Previous work in the cat has demonstrated that peripheral afferents terminate within the subnuclei of the NTS in a viscerotopic manner. The NTS projects to several forebrain areas in the rat, including the central nucleus of the amygdala (Ce). To examine whether NTS efferents terminate in a topographic manner within the Ce, small unilateral deposits of the sensitive anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) were iontophoretically placed within the caudal subnuclei of the NTS of male Long-Evans rats. After 14 d, the animals were overdosed with sodium pentobarbital and perfused according to the method described by Gerfen and Sawchenko ('84). Coronal sections (20 um) of the brainstem and forebrain were processed for visualization of PHA-L using the avidin-biotin immunoperoxidase technique.

Deposits of PHA-L were confined entirely within the NTS in some animals, or also included the NTS as well as the dorsolateral edge of the dorsal motor nucleus of the vagus in other animals. In the Ce, PHA-immunoreactive fibers were noted throughout all subdivisions of the Ce. The innervation of the ventral subnucleus was especially dense. At rostral levels, the bed nucleus of the stria terminalis contained labeled fibers throughout its medial and lateral subnuclei, with a particularly dense innervation present within the ventral lateral subnucleus. A new observation was the presence of PHA-L immunoreactive fibers within the subependymal and central parts of the subfornical organ. The same deposits of PHA-L resulted in widespread axonal labeling throughout other forebrain regions previously demonstrated to receive direct projections from the NTS. These areas included the paraventricular, supraoptic, dorsomedial and lateral hypothalamic nuclei, median and medial preoptic nuclei.

The present study demonstrates that the same population of cells within the caudal medial NTS send axons to terminate topographically within the Ce, bed nucleus, SFO and other areas of the forebrain. The relative density of terminal labeling within the ventrolateral bed nucleus suggests this region is a major forebrain target of the medial NTS. To our knowledge, the present study is the first to demonstrate direct efferent connections from the NTS to the subfornical organ. This projection may simultaneously relay blood pressure and body fluid volume changes to forebrain autonomic regions. Funded by NS 20041.

110.7 **EVIDENCE FOR DIRECT INHIBITION OF VISCERAL SENSORY NEURONS BY NORADRENERGIC SYMPATHETIC NEURONS.** D.L. Kreulen, and T.L. Anthony* (SPON: J. Angevine). Dept. Pharmacology, Univ. Arizona, Coll. of Med., Tucson, AZ 85724.

Sensory neurons (mechanoreceptors) located in the wall of the large intestine of guinea pig synapse on neurons in the inferior mesenteric ganglion. Transmission in these pathways is both cholinergic and noncholinergic, giving rise to fast, nicotinic excitatory postsynaptic potentials (e.p.s.p.s) and slow noncholinergic depolarizations. In this study we investigated the relationship between the patterns of contractile activity in the colon and the synaptic activity in the IMG. IMGs attached by mesentery and nerve supply to a 2 cm segment of distal colon were removed from guinea pigs and placed in a two-chambered recording bath which allowed separate superfusion of the ganglia and colon. Intracellular recordings in principal ganglionic neurons of responses to colon distension and subsequent contractile activity provided a means to evaluate the effects of postganglionic nerve stimulation and norepinephrine administration on the activity of colonic mechanoreceptors. Rapid distension of the colon to a basal intraluminal pressure of 20 cm H_2O results in an increase in the frequency and amplitude of fast e.p.s.p.s and a slow noncholinergic depolarization in neurons in the IMG. Distension also induced vigorous segmenting activity in the colon which was interrupted 4-7 times/min by sustained propulsive contractions of the entire colonic segment. 73 % of neurons tested received fast cholinergic e.p.s.p.s associated with colonic contractions. In all of these neurons the sustained colonic contractions following distension were associated with cessation of fast cholinergic e.p.s.p.s and partial repolarization of the slow noncholinergic e.p.s.p. Repetitive stimulation of postganglionic nerve trunks that innervate the colon (lumbar colonic nerves) during the distension, resulted in a decrease in the frequency and amplitude of fast cholinergic e.p.s.p.s and cessation of sustained propulsive contractions. Administration of norepinephrine (10^{-5}M) to only the colon had similar effects on both the colon and synaptic input to ganglionic neurons. The inhibitory effects of norepinephrine were antagonized by phentolamine (10^{-5}M). Administration of the smooth muscle relaxant papaverine ($5x10^{-5}$M) also resulted in cessation of sustained propulsive contractions of the colon but synaptic activity to the ganglion was not diminished. These experiments suggest that sympathetic noradrenergic outflow to the colon directly inhibits colonic mechanoreceptors so that they become less sensitive to colon distension. Support: HL 27781, DK 36289.

110.8 **CONVERGENCE AND COLLATERALIZATION OF NON-CHOLINERGIC AFFERENT NEURONS IN THE INFERIOR MESENTERIC GANGLION OF THE GUINEA PIG.** K.D. Keef*, P. Crowe* and D.L. Kreulen. Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

The purpose of these studies was to determine the pattern of non-cholinergic afferent input to neurons of the inferior mesenteric ganglion (IMG). IMG neurons of control or surgically altered guinea pigs (175-275 g) were studied. Under halothane anesthesia IMGs were decentralized and two of three peripheral nerves sectioned. Animals were allowed to recover for 7 days to allow time for nerve degeneration. The IMG was removed and the intracellular responses to nerve stimulation compared. Repetitive stimulation (20 Hz, 4 s) gave rise to a non-cholinergic slow EPSP. In surgically altered ganglia, slow EPSPs were obtained with stimulation of each nerve trunk regardless of the nerve trunk left intact. The amplitudes of these slow EPSPs were significantly less than those recorded in control ganglia. These data suggest that non-cholinergic neurons arise in the periphery, synapse on principal ganglion cells and send collaterals out other nerve trunks. Thus two possibilities exist for the origin of synaptic activity in a single cell in response to stimulation of different nerve trunks, namely; 1) Collateralization and/or 2) Convergence.

Given the extent of collateralization demonstrated in the surgically altered IMG, we proposed that all activity recorded from a single cell in control ganglia with stimulation of the four nerve trunks arises from neurons entering the ganglion from a single nerve trunk (i.e., via collateralization). To test this hypothesis, two nerve trunks were stimulated simultaneously while recording the activity from single cells in control ganglia. Supramaximal stimulation (17 V, 0.2 ms dur) of two nerve trunks never produced slow EPSPs of larger amplitude than that recorded from stimulation of one nerve trunk. Thus each neuron exhibited a maximum level of activation. Simultaneous stimulation with submaximal stimulus intensities (1-6 V) produced variable results ranging from no summation to summation to maximum response. Lack of summation was predicted if neural input from the different nerve trunks occurred exclusively via collateralization whereas summation suggests convergence of neurons originating from different locations.

The experiments provide evidence that the apparent convergence of afferent synaptic input to ganglion cells in the IMG results from 1) the collateralization of axons of afferent neurons and 2) the convergence of multiple afferent neurons, coursing in different nerve trunks, on an individual ganglion cell. Convergence of sensory input on to single IMG neurons from distinctly different regions of the periphery has important physiological implications in terms of the reflex control of viseral function. Support NIH HL27781, HL34449.

110.9 **BIDIRECTIONAL CONNECTIONS BETWEEN ROSTRAL VENTROLATERAL MEDULLA (RVL) AND AUTONOMIC NUCLEI IN THORACIC SPINAL CORD.** *R.L. Stornetta, D.A. Ruggiero and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Adrenergic (C1) neurons of the RVL project bilaterally to the intermediolateral (IML) and intermediomedial (IMM) cell columns of thoracic spinal cord and are believed to be crucial to the regulation of arterial pressure (Ross et al., *J. Comp. Neurol., 228*:168, 1984). Neurons of the C1 area receive afferents from dorsal horn neurons of lumbar cord presumably mediating the pressor response to somatic stimulation. It is not known if C1 neurons also receive projections from segments of thoracic cord containing sympathetic preganglionic neurons (SPN's). We examined the projections from thoracic spinal cord to C1 area by injecting anterograde tracers (^{3}H-amino acids or wheat germ agglutinated horseradish peroxidase (WGA-HRP)) into thoracic spinal cord of anesthetized rats. Tissues were processed by autoradiography or WGA-HRP histochemistry and, in double-label studies, C1 neurons were identified with antibodies against phenyl-ethanolamine-N-methyltransferase (PNMT). Anterogradely labeled terminal fields were localized in autonomic brainstem areas including NTS, both RVL and caudal ventrolateral medulla, the A5 area and parabrachial complex. In the C1 area of RVL, spinal afferents intermingled with PNMT labeled neurons and dendrites. The cells of origin of spino-bulbar afferents from thoracic cord were retrogradely labeled following pressure injections of 5% WGA-HRP into the RVL of anesthetized rats. Retrogradely labeled cell bodies and anterogradely labeled terminals were confined bilaterally to laminae VI, VII and X of thoracic cord, i.e. those laminae which contain SPN's in IML and IMM and which do not receive primary sensory afferents. This pattern of retrograde labelling contrasts with projections from lumbar cord where retrogradely labeled cells are concentrated in laminae I, II, IV and V of dorsal horn, areas which receive primary sensory afferents (Stornetta et al., *Neurosci. Abstr. 12*: 1156, 1986). To determine whether the spino-bulbar neurons in IMM and IML were SPN's, the SPN's were identified by dipping the proximal end of the transected cervical sympathetic trunk into a microvial of Fast Blue (FB) in anesthetized rats. 48 h later, rats were reanesthetized and 5% WGA-HRP was pressure injected into RVL. Another 48 h later, rats were killed and tissues processed with WGA-HRP histochemistry. WGA-HRP labeled neurons and terminals were simultaneously visualized with FB labeled SPN's. While some SPN's were seen in juxtaposition to WGA-HRP retrogradely labeled cells in IMM and IML, no double labeled cells were seen. Bulbo-spinal projections from RVL exactly overlapped SPN's bilaterally. We conclude (a) the C1 area of the RVL receives projections from neurons which are not SPN's in the vicinity of the IMM and IML (b) these same spinal neurons are in turn innervated by neurons of RVL. This two-way anatomical connection between spinal and bulbar autonomic centers raises the possibility that information from SPN's is conveyed to RVL neurons. (Supported by HL 07379 & HL 18974)

110.10 NEURALLY EVOKED ELECTRODERMAL RESPONSES (EDR) ARE INHIBITED BY CLONIDINE BY AN ACTION AT THE SPINAL LEVEL. M.C. Koss and J.A. Hey. Departments of Pharmacology and of Ophthalmology and Dean A. McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Clonidine inhibits function in a variety of autonomic systems by acting in the central nervous system. A spinal cord site of action for sympatho-inhibition in the cardiovascular system is well documented. A spinal locus of action has also been reported for centrally evoked electrodermal responses (Koss, Europ. J. Pharmacol. 37:381, 1976). In a recent series of articles it has been reported that clonidine inhibition of sudomotor responses is unique, in that the primary site of action is at the level of the sympathetic ganglion (Walland, Europ. J. Pharmacol. 102:39, 1984; 102:47, 1984). The present experiments were designed to determine the neural site(s) of action for clonidine inhibition of sympathetic-cholinergic electrodermal responses in anesthetized cats by comparing the effects of clonidine on electrodermal potentials when administered by a variety of routes (intravenous, intra-arterial to the stellate ganglion, and intrathecal). In these experiments electrodermal responses were evoked by stimulation of the posterior hypothalamus as well as from the pre- and postganglionic peripheral nerves. Administration of clonidine (0.3-3.0 µg i.a.) directly to the stellate ganglion did not significantly decrease the amplitude of responses evoked by submaximal hypothalamic stimulation but did inhibit EDR when administered intrathecally at the C_6 to T_2 spinal levels. Administration of clonidine to the ganglion, however, did depress EDR evoked by the nicotinic ganglionic stimulant DMPP (10 µg i.a.). Intravenous clonidine (1-30 µg) also reduced EDR amplitude evoked by single pulse stimulation of both the pre- and postganglionic sympathetic nerves with responses elicited from both sites inhibited to an equal extent. Yohimbine (0.5 mg/kg i.v.) uniformly antagonized clonidine's depression of EDR regardless of the site or mode of activation. These results indicate that clonidine depresses centrally evoked sudomotor responses by activation of alpha$_2$-adrenoceptors in the spinal cord and to a limited extent by direct action at the neuroeffector junction. Although a possible DMPP-clonidine interaction takes place at the level of the sympathetic ganglion, it is unlikely that ganglionic blockade contributes significantly to clonidine inhibition of EDR evoked by electrical activation of the nervous system. (Supported by NSF Grant GII-8610676)

110.11 PRESYNAPTIC α_2-ADRENOCEPTORS MEDIATE SUPPRESSION OF THE PERIPHERAL SYMPATHETIC-CHOLINERGIC SYSTEM. T. Ito* and M.C Koss (SPON: J.P. Farber). Dept. of Pharmacology, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

The sweat glands have a unique sympathetic-cholinergic innervation, activation of which is associated with electrical potential changes of the skin or electrodermal responses (EDRs). The present study was undertaken to investigate adrenergic control of the peripheral sympathetic-cholinergic system by utilizing peripherally evoked EDRs in rats anesthetized with pentobarbital and chloral hydrate (40 mg/kg and 200 mg/kg, i.p., respectively). EDRs evoked by electrical stimulation of the sciatic nerve were recorded from the hindfoot pads with Beckman miniature biopotential skin electrodes. Drugs were administered through a cannula inserted into the jugular vein. Electrical stimulation of the sciatic nerve (4-16 V) produced frequency dependent EDRs with threshold frequency below 1 Hz and maximal response at 16-32 Hz. In the following experiments stimulations of 8-12 V and 2-4 Hz were used to obtain submaximal electrodermal responses of approximately 40-80% of maximal amplitude. dl-Epinephrine (dl-Epi, 0.3-30 µg/kg, i.v.) suppressed evoked EDRs in a dose dependent manner, totally abolishing the response at the highest dose and with an ED_{50} of 4.4 µg/kg. l-Epi, d-Epi and l-norepinephrine also inhibited EDRs in a dose-related fashion with ED_{50}s of 1.7, 28.5 and 31 µg/kg, respectively. Isoproterenol (0.03-30 µg/kg, i.v.) partially decreased EDR amplitude, however, the maximal inhibitory effect obtained was only 34%. Yohimbine (0.75 mg/kg, i.v.) shifted the dose-response curve for dl-Epi to the right with the maximal inhibitory effect of 30 µg/kg of dl-Epi being 26%. Prazosin (0.3 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.) did not alter the effect of dl-Epi. The selective α_2-adrenergic agonist B-HT 920 (1-1000 µg/kg, i.v.), suppressed EDRs with an ED_{50} of 66 µg/kg. This action of B-HT 920 was antagonized by the selective α_2-adrenoceptor blocker, idazoxan (0.1 mg/kg, i.v.) but not by prazosin (0.3 mg/kg, i.v.). In order to directly stimulate the postjunctional muscarinic receptors, methacholine (1 µg) was injected into the femoral artery. Methacholine evoked EDRs of about 1 mV with l-Epi (1-3 µg/kg, i.v., 30-60 sec before methacholine, i.a.) having no inhibitory effect. Taken together, the present results suggest that presynaptic α_2-adrenergic mechanisms are involved in the suppression of the peripheral sympathetic-cholinergic system in the rat. (Supported by NSF Grant GII-8610676. T. Ito was supported by fellowships from Uehara Memorial Foundation and Kao Corp.)

110.12 LOWER BRAINSTEM REGULATION OF ROSTROCAUDAL SPECIAL VISCERAL FUNCTION. B. Graber. Dept. of Psychiatry, Univ. of Nebraska Med. Ctr., Omaha, NE 68105.

A synthetic explanation of essential caudal evacutive processes including micturition, defecation and ejaculation is now possible due to rapid advances in neuroscientific techniques including labelling and tracing of individual neurons and pathways. The traditional passive structural model of neuroanatomy are being revised by a more functional, active, neurochemcial explanations of vital processes. In this presentation I propose to extend and defend my hypothesized synthetic model of anogenitalurinary function described in "A Thirteenth Cranial Nerve: The Cloacal Nerve" (Medical Hypotheses, 1987, in press). Strict adherence to Sherrington's spinal reflexology and Langley's craniosacral fragmentation of the autonomic nervous system has delayed or distracted such a synthesis in spite of accumulating evidence. Put simply, both ontogeny and phylogeny provide evidence that the caudal sphincteric mechanisms seen in cloacal derivatives are anatomically and physiologically similar to rostral special visceral efferent (SVE) branchiomeric derivatives and that the function of these rostral and caudal structures may be at least partially integrated. In this presentation I will utilize this hypothesis and supporting evidence to explain some of the continuing controversies about human sexual function by elaborating on "orgasmic dimorphism".

111.1 BEHAVIORAL CHOICE IN THE ISOLATED LEECH NERVOUS SYSTEM: SHORTENING VS. SWIMMING. G. Wittenberg and W. B. Kristan, Jr. Dept. of Biology, University of California at San Diego, La Jolla, CA 92093.

The leech, *Hirudo medicinalis*, responds to moderate tactile stimuli by producing one of several behaviors, among which are whole-body shortening and swimming. Motor patterns characteristic of these two behaviors can be recorded from segmental nerves of brainless, isolated nerve cords, and can be elicited by electric shocks to a small patch of attached body wall. Such stimulation results in a reproducible train of sensory cell action potentials. Using simultaneous intracellular recording of identified neurons, we are investigating how identical sensory activity leads to the selection of one or another behavior or a sequence of the two. This may lead to an understanding of how a simple nervous system makes a behavioral choice.

Whole-body shortening is a defensive withdrawal in which the leech's length is rapidly reduced by contraction of both dorsal and longitudinal muscle fibers in several segments, whereas swimming is produced by antiphasic contractions of dorsal and ventral longitudinal fibers. The intensity and duration of a shortening response depends on the number of shocks in the stimulus train, and shortening is produced by stimuli below the threshold for swimming. As expected, several of the excitatory motorneurons innervating both dorsal and ventral longitudinal muscle fields are active during whole-body shortening, while the inhibitory motorneurons to those muscles are inhibited. Surprisingly, the L cells, motorneurons which are effective at exciting all the longitudinal muscle around an entire segment, are inhibited during both swimming and shortening.

Cell 204, a swim-initiating interneuron, also increases its activity during a shortening response, but not to the same degree as when swimming is produced. Direct intracellular stimulation of cell 204 that is subthreshold for swim initiation excites many of the same motorneurons which participate in shortening. Thus, depending on its level of activity, this neuron may help to produce both behaviors. In addition, increased cell 204 activity causes a lasting increase in the probability that the isolated CNS will produce a swim pattern in response to a stimulus that previously elicited only shortening. Besides the effect of cell 204, serotonin is known to increase the likelihood of swimming and several serotonin-containing neurons can initiate swimming. Bath-applied 10^{-5} M serotonin also affects the shortening response by increasing the intensity, duration, and intersegmental extent of the motorneuron bursts. Thus two different forms of escape locomotion appear to share common neuronal elements and mechanisms of modulation.

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111.2 **IDENTIFIED CELLS CAUSE HETEROSYNAPTIC FACILITATION AND HETEROSYNAPTIC DEPRESSION OF THE LEECH LOCAL BENDING REFLEX.** S.R. Lockery and W.B. Kristan, Jr. Dept. of Biol., Univ. of Calif., San Diego, La Jolla, CA 92093.

Focal mechanical stimulation of the leech *Hirudo medicinalis* causes a local withdrawal from the stimulus (local bending). The reflex can be elicited in a semi-intact preparation that consists of a segment of body wall innervated by two mechanosensory neurons, two motor neurons and a small number of interneurons from a single ganglion. Repeated stimulation of mechanosensory neurons causes a decline in reflex strength (habituation). Excitation of a nociceptive pathway (by pinching or stimulation of the nociceptive N cell) increases response strength (sensitization). We used this preparation to investigate the possible role of heterosynaptic modulation in these simple forms of learning.

The sensitizing effect of N cell stimulation is mimicked by bath application of serotonin, and the N cell excites two pairs of identified serotonin-containing neurons, cells 61 and 21. We therefore tested the strength of local bending before and after stimulation of single serotonin cells (8 Hz for 10 s). Activation of cell 61 did not elicit local bending. However, immediately following stimulation of cell 61, the local bending response was significantly increased. This effect was associated with an increase in the response of the motor neurons. Response strength was again normal 1 min after cell 61 stimulation. Activation of cell 21 produced similar results.

The opposite effect was achieved by stimulation of Leydig cells which, in a closely related species, contain and release octopamine. A single Leydig cell was impaled and hyperpolarized to stop tonic firing. Following a 10 min rest, the cell was fired at 5.0 Hz for 8 min. Leydig cell stimulation itself did not produce local bending. However, this treatment produced a 75% decline in the strength of the local bending response, and the decline outlasted Leydig stimulation by more than 25 min. The effect was detectable at frequencies as low as 0.5 Hz and was associated with a decline in the motor neuron EPSP's elicited during production of behavior. Spontaneous firing in unimpaled Leydig cells (recorded extracellularly from somata) ranged from 0.1 to 0.9 Hz. Thus the Leydig cell may exert tonic control over reflex strength. Repeated trains of sensory cell stimulation sufficient to cause habituation increased Leydig activity. We are currently testing the hypothesis that the Leydig cell contributes to habituation.

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111.3 CENTRAL CORRELATES OF TASTE-DISCRIMINATION IN THE TERRESTRIAL SLUG *LIMAX MAXIMUS* *K. Delaney and †A. Gelperin *Dept. of Biology, Princeton Univ., Princeton, NJ 08544 and †Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974

Using an *in vitro* preparation consisting of lips, cerebral ganglia (CG) and buccal ganglia (BG) we have identified a group of cerebral to buccal interneurons (CB's) which have powerful initiating and modulating effects on fictive feeding rhythm (Delaney and Gelperin, 1986;Gelperin, 1986;King et al., 1987). CB's receive synaptic input from lip mechanoreceptors and chemoreceptors, feedback from the BG during fictive feeding and strong inhibitory input from stimuli which elicit withdrawal in the whole animal such as electric shock of the foot. Thus, they provide neural loci from which to study convergence and processing of sensory information in a situation where the result of this processing has implications for the control of motor output by the central pattern generator for ingestion.

Fictive feeding is monitored *in vitro* with suction electrodes attached to several BG nerves. Application of potato extract, which is attractive to intact slugs, to chemoreceptors on the lips, triggers fictive feeding. When potato extract is applied to the lip and BG are attached CB_1, CB_{EC}, CB_3, CB_4 and CB_{EPSP} receive a mixed but predominantly excitatory input before and during the initiation of fictive feeding. Application to lips of 0.7% quinidine sulfate (QSO_4), a bitter tasting substance which produces withdrawal responses as opposed to ingestion in intact slugs, produces a predominantly inhibitory response in these CB's. With the BG removed there is no fictive feeding pattern but the response to potato is strongly excitatory with few inhibitory inputs apparent while there is no response or inhibition to QSO_4. In contrast, CB_{ST} receives strong excitation to potato extract when BG are attached and fictive feeding is initiated but is weakly or not at all excited by potato when BG are removed. CB_{ST} is also unique in that without BG attached it is strongly excited by QSO_4. Several putative lip motor neurons are excited by QSO_4 with and without BG attached. Effects of QSO_4, including inhibition of spontaneous excitatory inputs in CB_{EPSP} and tonic excitation of CB_{ST} can persist for tens of minutes following a single 30 second application.

We have also begun to study a group of 5 CG interneurons presynaptic to CB's which have contralateral projections via the sub-cerebral commissure. Responses of these neurons to potato and QSO_4 are similar to the responses of $CB's_{1,3,4}$ and CB_{EC}. We hope to use this system to examine both central mechanisms of discrimination as well as for studies of associative and non-associative conditioning *in vitro*. Delaney, K. and Gelperin A. 1986 Soc. Neurosci. Abstr. 12: 39, Gelperin 1986 TINS 9(7) 323-328, King, M.S., Delaney, K. and Gelperin , A. 1987 MS submitted.

111.4 A CLEANING REFLEX AND ITS MODIFICATION BY EXPERIENCE IN DROSOPHILA. G. Corfas* and Y. Dudai, Department of Neurobiology, Weizmann Institute, Rehovot 76100, Israel.

Mechanical stimulation of thoracic bristles in Drosophila melanogaster elicits a characteristic reflex in which a leg cleans the sensory field covered by the stimulated bristles (Vandervorst and Ghysen, Nature 286:65, 1980). We have set out to analyze and quantify this reflex and its modification by experience. Stimulation of immobilized decapitated flies was performed by a series of air puffs (280 msec each, 0.2 pulses/sec) directed at the tegular, antero-notopleural (ANP) and humeral bristles, which elicit cleaning response by the ipsilateral foreleg. Leg movement was observed under a dissecting microscope. The quantified variable was the number of stimuli required for the fly to stop responding (SSR). SSR in naive flies was 40.5±2.4 (n=62). Following a resting period (1-15 min), SSR was measured again. Response after 1,5 and 15 min was 33.9±5.1%, 15.1±5.1% and 69.1±12.9% of the naive fly's SSR, respectively. When a fly that has stopped responding after training was immediately stimulated by a different stimulus (4x1 sec trains of 20 pulses/sec each, every 3 sec, directed at the thoracic dorso-central area), and then tested with the original training stimulus, its response, quantified as SSR, recovered. The experience-dependent modification of the cleaning reflex satisfies accepted criteria for habituation (Thompson and Spencer, Psychol. Rev. 73:16, 1966). Thus, in addition to 1) Response decrement with repeated stimuli (SSR), 2) Spontaneous recovery and 3) Dishabituation, we observed that 4) Higher frequency stimuli produced quicker habituation and 5) Weaker stimuli led to more rapid habituation. The experimental advantage of studying the cleaning reflex lies in the ability to identify sensory and motor neurons mediating the response and to study the reflex by neuroanatomical and electrophysiological methods. We have characterized by HRP backfilling the fine anatomy of a single sensory neuron which is involved in the reflex by mediating information from the ANP bristle to the thoracic ganglion, and found that a mutation that affects the cAMP cascade (rut) increases the number of axonal varicosities while a mutation in a presumptive K^+ channel (Sh^{133}) decreases the number of varicosities. We have also initiated recording of evoked field potentials from the responding leg. The cleaning reflex thus yields itself to multidisciplinary research methods that might contribute to out understanding of the effect of mutations on plasticity of identified cellular components underlying behavioral alteration in Drosophila. (Supported in part by the US-Israel Binational Science Foundation, Jerusalem, and the US-Israel Binational Agricultural Research and Development Fund (BARD)).

111.5 PHARMACOLOGIC MODULATION OF HERMISSENDA CRASSICORNIS PHOTORECEPTOR IONIC CURRENTS. C.E. COLLIN, D.L. ALKON (SPON: W.J. Adelman, Jr.). Laboratory of Cellular and Molecular Neurobiology, NIH-NINCDS, Rockville, MD 20892

Previous biohemical and electrophysiological studies of Hermissenda photoreceptors (Heldman et al., J. Neurophysiol. 42, 153, 1979) have implicated acetylcholine (ACh) as a neurotransmitter mediating their inhibitory interactions. Isolated Hermissenda eyes synthesized and accumulated ACh but not other neurotransmitters. Carbachol (CCh) applications produced potential changes which mimicked naturally evoked inhibitory postsynaptic potentials (IPSP's) and shared the same reversal potential. Histochemical staining revealed the presence of acetylcholinesterase in the photoreceptor endings where synaptic interactions occur; and physostigmine blocked the IPSP's. Evidence was obtained by histochemical techniques that adrenergic substance-(s) are also endogenous to the Hermissenda visual system, specifically within a cluster of 2nd order neurons called optic ganglion cells. Other results suggested that an excitatory synaptic feedback from 2nd order visual cell(s) onto the type B cell is mediated by an adrenergic agonist. There was no indication of other endogenous neurotransmitters anywhere in the Hermissenda visual system (Sakakibara et al., J.Neurochem, 48:405, 1987). In the present experiments we investigated the effects of cholinergic agonists on the same soma membrane ionic currents and the possible interactions of these cholinergic substances with α_2 adrenergic agonists.Isolated medial type B photoreceptors of Hermissenda were impaled with 2 microelectrodes and voltage clamped at -60mV in darkness. Bath application of CCh (1mM) induced an increase of I_A of 10% ± 3.2 (N=5) and I_C of 20% ± 2.7 (N=5) at step commands to 0mV. This effect was clear and sustained after 5 min. of drug application. A voltage - dependent inward sustained calcium current $I_{Ca}2+$ was also measured at 0mV with elevated extracellular K^+ concentration (300mM). Applications of CCh (1mM) also induced an increase of $I_{Ca}2+$ with the same time course of the changes in I_A and I_C. The simultaneous applications of CCh (1mM) and the α_2 adrenergic agonist clonidine (1mM) largely prevented any effect of CCh on I_A and I_C. Serotonin at 1 mM induced a transient increase of I_A and I_C, which reversed spontaneously after 10 min. The results here indicate that modulation of Type B ionic currents could interact with other physiologic conditions (e.g. prolonged depolarization and elevation of intracellular Ca^{2+}) to generate long lasting changes in excitability during classical conditioning. We suggest that the role of endogenous neurotransmitters (both cholinergic and adrenergic) may have more relevance for classical conditioning mechanisms than substances not demonstrated to function in physiologic contexts.

111.6 PKC CLOSES SINGLE VOLTAGE DEPENDENT K CHANNELS IN HERMISSENDA TYPE B PHOTORECEPTORS. J. Farley, D. Resnick*, and S. Auerbach. Prog. in Neurosci., Princeton Univ., Princeton, NJ 08544.

Previous results (Farley & Auerbach, Nature, 1986) have demonstrated that injection of exogenous PKC into Type B cells, as well as activation of endogenous PKC by phorbol esters, mimic the increase in the steady-state depolarizing generator potential and resting input resistance that results from associative training. Conventional two micro-electrode voltage clamp analysis revealed that PKC activation reduced two K^+ currents (I_A and I_{K-Ca}) and enhanced a calcium current (I_{Ca}).

Recently, (Farley, Neurosci. Letter 1987, in press) it has been shown that an intermediate-sized (∿110 pS), calcium- and voltage-dependent K^+ channel, which is TEA- and 4-AP resistant, is likely to underlie the macroscopic I_{K-Ca} current recorded in previous voltage-clamp studies. Here, this channel was studied in ripped-off patches in a bath which contained (in mM): 300 KCl, 10 NaCl, CaCl, 0.15 EGTA, 170 sucrose, 2 Mg-ATP [free calcium was 0.85 mM; ph=7.6]. In the absence of the PKC-activator diacylglycerol (DiG), purified PKC resulted in a transient closure of the intermediate-sized (∿95 pS) K^+ channel in 4 of 4 separate experiments. The duration of PKC's closure, defined as the time required for single-channel activity to return to >50% of the pre-PKC baseline level, was 3.74 ± 4.06 min. In the presence of DiG, PKC addition produced a complete closure of channels in 5 of 5 separate experiments. The average duration of channel closure was 12.25 ± 8.06 min. DiG addition (without PKC) was without consistent effect, although in 3 of 5 experiments a clear closure of channels was observed.

We also observed that PKC consistently closed a smaller (∿ 23 pS) voltage-dependent K^+ channel, whose calcium-, 4-AP, and TEA sensitivities have not yet been determined. In contrast, a large (> 220 pS) calcium-dependent K^+ channel, seen infrequently in B cells, was not affected by PKC. In summary, PKC activation is sufficient to reduce the activity of single calcium-dependent K^+ channels in Type B photoreceptors. The results suggest that these effects are mediated by protein phosphorylation, and that the phosphorylation site is closely associated with the channel itself.

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111.7 A PKC-INHIBITOR PREVENTS IN VITRO CONDITIONING-PRODUCED CHANGES IN HERMISSENDA TYPE B PHOTORECEPTORS. E.M. Schuman and J.Farley, Program in Neuroscience and Behavior, Princeton University, Princeton, N.J.,08544.

It has been suggested that training-produced increases in intracellular calcium might play a causal role in both short- and long-term changes in Hermissenda Type B photoreceptor excitability. It has further been proposed that these effects of calcium are mediated by the activation of calcium-dependent phosphorylation pathways. In the present experiments we have assessed the respective roles of two distinct Ca2+-dependent phosphorylation pathways as mediators of short-term neural correlates of learning in Type B photoreceptors during in vitro conditioning. The two pathways are Ca2+/calmodulin-dependent protein kinase (Ca2+/CAM PK) pathway(s), and a calcium-dependent phospholipid-sensitive protein kinase (PKC) pathway.

We have injected relatively specific inhibitors of each pathway (trifluoperazine,TFP, for Ca2+/CAM PK; imipramine for PKC) into Type B photoreceptors and have assessed the effects of the inhibitor upon in vitro conditioning produced changes in B cell excitability. In control experiments, pairings of light and intracellular current stimulation of a statocyst caudal hair cell (in vitro conditioning) resulted in a cumulative depolarization and increased input resistance of Type B photoreceptors (Table 1). Ionophoresis of imipramine into a Type B photoreceptor, prior to in vitro conditioning, prevented these changes. Ionophoresis of TFP failed to affect the in vitro conditioning-produced cumulative depolarization and increased input resistances of Type B cells. Neither imipramine nor TFP had any discernible effects on B cell membrane excitability or light response prior to in vitro conditioning. Biochemical analysis confirmed previous suggestions that imipramine is a more potent inhibitor of PKC than TFP, over the concentration range of 10^{-5} to 10^{-4} M. These results suggest that activation of PKC -- not a Ca2+/CAM-dependent PK -- is of primary importance in mediating the changes in Type B cell membrane excitability which result from associative training.

Table 1

Condition	n	Cumulative depolarization(mV) (sec post-conditioning) 30	60	90	120	Input resistance (% increase)
control	6	5.3*	4.9*	4.3*	3.9*	+25.0
imipr.	6	-0.7	-1.3	-1.7	-1.5*	-02.3
TFP	6	6.2	5.3	5.2	5.2	+15.6

* p <.05, relative to control

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111.8 INHIBITION OF PROTEIN SYNTHESIS BLOCKS LONG TERM PLASTICITY IN IDENTIFIED B-PHOTORECEPTORS IN HERMISSENDA. T. Crow and J. Forrester*. Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Pairing light with direct application of 5-HT to the exposed nervous system of otherwise intact Hermissenda produces a long-term reduction in normal positive phototactic behavior (Crow and Forrester, 1986). Control groups that received unpaired light and 5-HT do not exhibit a significant change in phototactic behavior. Associated with the light-5-HT application are both short-term and long-term changes in the light evoked generator potentials of identified medial and lateral type B-photoreceptors. Previous results suggest that short-term and long-term memory show differential effects of the inhibition of protein synthesis. Protein synthesis appears to be essential in the formation of long-term memory in mammals (Davis and Squire, 1984). Research in Aplysia has indicated that inhibitors of protein synthesis blocked long-term facilitation but not short-term facilitation (Montarolo et al., 1986). In order to determine if short-term and long-term plasticity intrinsic to B-photoreceptors depends upon protein synthesis, we examined the effects of the protein synthesis inhibitor anisomycin on light responses of medial and lateral B-photoreceptors at two different times (1 hr & 24 hrs) following the paired and unpaired application of light & 5-HT.

Anisomycin did not block the short-term enhancement in the generator potentials of medial and lateral B-photoreceptors produced by paired and unpaired light and 5-HT. Surgically isolated lateral and medial B-photoreceptors from the paired group (N=11) and the unpaired group (N=11) exhibited a significantly larger generator potential at the end of a 2 min light step compared to controls that received anisomycin without 5-HT application (N=7) (paired X=28.1mV, unpaired X=28.7mV, anisomycin control X=23.9mV; P < .05). Light responses were examined 1 hr after the application of light and 5-HT in the presence of anisomycin (10^{-6}M). The anisomycin was added to the chamber 30 min before the application of light and 5-HT and was washed out 30 min after the conclusion of the light-5-HT session. In contrast to the results from the short-term experiments, anisomycin blocked the long-term enhancement of the light responses of lateral B-photoreceptors observed 24 hrs after pairing light and 5-HT. Delaying the application of anisomycin 1 hr following the pairing of light and 5-HT or applying a derivative of anisomycin (deacetylanisomycin) (10^{-6}M) that does not inhibit protein synthesis, did not block the enhanced light response produced by paired light and 5-HT (paired & deacetylanisomycin X=29.4mV; paired & delayed anisomycin X=30.1mV; paired & anisomycin X=25mV; P < .01).

Thus short-term plasticity produced by pairing light with 5-HT does not depend upon protein synthesis for its expression. However, the long-term changes in the light response of lateral B-photoreceptors produced by pairing light with 5-HT depends upon protein synthesis. The formation of long-term plasticity in B-photoreceptors appears to have a narrow time window for protein synthesis since delaying the application of anisomycin by 1 hr resulted in the full expression of the enhanced generator potential.

111.9 cAMP LEADS TO LONG-TERM (24HR) CHANGES IN MEMBRANE CURRENT IN APLYSIA TAIL SENSORY NEURONS: A POSSIBLE MECHANISM FOR LONG-TERM SENSITIZATION. K.P. Scholz and J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77225.

Sensory neurons that innervate the tail of Aplysia undergo a long-term (24 hr) reduction in net outward currents following sensitization of the animal (Scholz and Byrne, 1986, 1987). This modulation of membrane current appears to contribute to the storage and read-out of long-term memory. In an earlier study, Ocorr et al (1986) found that sensitizing stimuli caused a rapid elevation of cAMP within these same neurons. Since cAMP has been implicated in the regulation of gene expression in other cells, it seemed possible that its transient elevation by sensitizing stimuli might lead to the induction of the membrane effects associated with long-term sensitization. In the present study, we tested this hypothesis by examining the membrane currents of sensory neurons 24 hrs after injecting them with cAMP.

Sensory neurons in the pleural ganglion were impaled with one microelectrode that contained 30 mM fast-green and 200 mM of either cAMP or 5'-AMP. The compounds were injected by passing hyperpolarizing current into the cell (1 nA; five 20 sec trains and one 420 sec pulse). The ganglion was then placed in organ-culture. One day after injection, the same cells were voltage-clamped and their current-voltage relationship obtained over a range of membrane potentials from -80 to +25 mV. The cells injected with cAMP showed significantly less net outward currents at depolarized potentials compared to the cells which had been injected with 5'-AMP (10 ganglia, 36 cells).

The properties of the outward current that was reduced 24 hrs after injection of cAMP were studied by digital subtraction of experimental from control traces. The cAMP-sensitive current was only mildly voltage-dependent and had kinetics and an I-V relationship very similar to the S-current (a current that is regulated in the short-term by cAMP-dependent phosphorylation). Furthermore, outward tail-currents measured at -50 and -80 mV following depolarizing pulses indicated that the cAMP-sensitive current reverses near E_K. There appeared to be no modulation of the delayed K current. Finally, in both kinetics and voltage sensitivity, the currents modulated 24 hrs after injection of cAMP were nearly identical to those modulated 24 hrs after sensitizing stimulation. These results parallel findings in cultured sensory neurons where there is a 5-HT- and protein synthesis-dependent long-term enhancement of transmitter release and excitability (Montarolo et al, 1986, Dale et al, 1986).

Thus, cAMP-induced long-term regulation of a K current appears to be involved in the storage of long-term memory for sensitization in Aplysia.

111.10 EXOGENOUS CYCLIC AMP ANALOG PRODUCES LONG-TERM FACILITATION OF APLYSIA SENSORIMOTOR SYNAPSES IN CULTURE THAT IS BLOCKED BY THE PROTEIN SYNTHESIS INHIBITOR ANISOMYCIN. S. Schacher, V.F. Castellucci and E.R. Kandel. Ctr. for Neurobiol. & Behav. and HHMI, Columbia Univ., College of P&S, and NYS Psychiatric Institute, NY, NY 10032.

Both short-term and long-term sensitization of the gill and siphon withdrawal reflex in Aplysia are associated with an increase in efficacy of sensory neuron excitatory postsynaptic potentials (EPSPs) on follower cells. Short-term facilitation of sensory neuron synapses is primarily due to a transmitter mediated increase in cyclic AMP (cAMP) levels within the sensory cells. A single application of serotonin (5-HT), a transmitter that increases cAMP levels in sensory cells, can evoke short-term facilitation. When applied repeatedly over a 1.5 hour period, 5-HT can also produce long-term synaptic facilitation of sensorimotor synapses in culture (Montarolo et al., 1986). To determine whether this long-lasting facilitation produced by 5-HT is mediated by a cAMP-dependent mechanism, we examined the long-term effects of exogenous cAMP analog 8-benzylthio 3'-5' cAMP on sensory neuron-motor neuron EPSPs.

Single L7 motor cells were co-cultured with one to three sensory cells for five days. We then measured the amplitude of the EPSPs before exposing the cultures for two hours to 10^{-4} M cAMP in the presence of 5×10^{-4} M IBMX (a phosphodiesterase inhibitor). When these cultures (n = 5) were reexamined 24 hours later, we found a significant increase of 52% $\pm$ 5 ($p < .001$) in the amplitude of the EPSPs compared to a change of -8% $\pm$ 4 in untreated control cultures (n = 5), and a change of -9% $\pm$ 8 in cultures treated with IBMX alone (n = 5).

Since the long-term facilitation induced by 5-HT, but not the short-term, is blocked when 5-HT is applied in the presence of protein synthesis inhibitors (Montarolo et al., 1986), we examined the effects of cAMP analog in the presence of anisomycin, a reversible protein synthesis inhibitor. After measuring the amplitude of the EPSPs, the cultures were treated for three hours with 10^{-5} M anisomycin starting 15 minutes before incubation with cAMP-IMBX until 45 minutes after the two-hour exposure to cAMP-IBMX. Whereas cAMP-IBMX treatment of a second group of cultures (n = 5) evoked a significant increase of 44% $\pm$ 18 ($p < .01$) in the amplitude of the EPSPs measured 24 hours later, cultures exposed to cAMP-IBMX in presence of anisomycin failed to show an increase in synaptic strength; -8% $\pm$ 7 (n = 5). Cultures exposed to anisomycin alone for three hours showed a similar change of -9% $\pm$ 8 (n = 5).

These results are consistent with the idea that the induction of long-term facilitation of sensorimotor synapses by 5-HT may involve the same intracellular second messenger system that mediates short-term facilitation. However, unlike short-term facilitation, which utilizes cAMP-dependent mechanisms to modify pre-existing macromolecules (Kandel and Schwartz, 1982), the role of cAMP in the long-term increase in synaptic strength may reflect some regulatory processes in the synthesis of necessary macromolecules.

111.11 LEARNING ASSOCIATED PROTEINS (LAPs) FOLLOWING LONG-TERM SENSITIZATION OF THE GILL AND SIPHON WITHDRAWAL REFLEX IN APLYSIA. V.F. Castellucci, E.R. Kandel, T.E. Kennedy and P. Goelet*. HHMI, Columbia P&S, NY, NY 10032.

Long-term memory for sensitization of the withdrawal reflex in Aplysia, produced by 4 days of training, is associated with an increased synaptic efficacy of the connection between the sensory and motor neurons (Frost et al., 1985). This training is also accompanied by neuronal growth; there is an increase in the number of synaptic varicosities per sensory neuron, and in the number of active zones (Bailey and Chen, 1985, 1986). Such structural changes may require increased synthesis of certain proteins. In addition, a simplified five-trial one-day protocol in isolated reflex preparation or in dissociated cell culture, given over a 2-hour period, also produces long-term sensitization and synaptic facilitation when assayed 24 hrs later. Long-term sensitization and synaptic facilitation in these cases are blocked by inhibitors of protein synthesis if they are applied during the training period. By contrast, short-term sensitization and synaptic facilitation are unaffected (Montarolo et al., 1986). These results suggest that long-term memory for sensitization requires the expression of proteins and genes not required for the short-term memory.

We therefore searched for proteins whose rates of synthesis were altered during either the acquisition or maintenance phase of long-term memory for sensitization by using computer-assisted quantitative two-dimensional gel analysis (PROTEIN DATABASES, INC., NY). These methods have not allowed us, as yet, to detect significant changes during the 2 hr acquisition phase, but they have allowed us to identify proteins whose rates of synthesis were altered in the maintenance phase as assayed 24 hrs after either one day or four days of training. We focus here on a comparison of animals trained for four days to produce maximal long-term sensitization (n=11) and a control group (n=11). One day after training, we labeled abdominal ganglia from the sensitized and control group for 2 hrs with ^{35}S-methionine. We dissected out the region of the sensory and motor neurons and prepared protein extracts from each animal for high resolution two-dimensional gel electrophoresis.

We initially found changes in about 20 proteins, but focused on four candidates whose relative rates of synthesis were both significantly increased in the sensitized animals compared to controls and whose positions on the gels were relatively easy to distinguish from surrounding protein spots. The approximate molecular weight, isoelectric point, and the magnitude of the increase for these four proteins were: LAP A, 15.8 Kd, pI 5.3, 24-fold increase; LAP B, 16.2 Kd, pI 7.3, 3.5-fold increase; LAP-C, 79.4 Kd, pI 5.2, 2-fold increase; LAP D, 56.4 Kd, pI 4.7, 2-fold increase. By mixing these labeled samples with protein extracts from the total nervous system, we found, by silver staining, that these proteins are present at levels that may permit their isolation and characterization in molecular and behavioral terms.

111.12 SEROTONIN PRODUCES LONG-TERM FACILITATION AT PERIPHERAL SYNAPSES WHEN APPLIED SELECTIVELY TO SYNAPTIC REGIONS OF APLYSIA SIPHON SENSORY CELLS. G.A. Clark and E.R. Kandel. HHMI; Ctr. for Neurobio. & Behav., Columbia Univ.; & NYS Psych. Instit., NY, NY 10032.

RNA and protein synthesis inhibitors selectively block long-term but not short-term presynaptic facilitation at central connections of siphon sensory neurons in dissociated cell culture and in the semi-intact preparation, suggesting that certain gene products are required for the long-term but not the short-term process. We now ask: Must serotonin act directly on presynaptic somatic regions, which contain the RNA and protein synthetic machinery, to produce long-term facilitation? Or can it act when application is restricted to synaptic regions, either via retrograde transport of an intracellular signal, or through local and/or postsynaptic processes? To address this question, we have examined long-term facilitation at peripheral synapses of siphon sensory cells. In contrast to the central sensory neuron synapses in the abdominal ganglion, these peripheral synapses are located approximately 3 cm from the sensory neuron cell body, so it is possible to apply serotonin selectively onto synaptic regions of the sensory cell without applying it onto somatic regions. We previously found that bath application of serotonin onto both central (somatic) and peripheral (synaptic) regions produces long-term facilitation at peripheral synapses, relative to untreated controls. We now report that application of serotonin restricted to synaptic regions can also produce long-term facilitation at these distant synapses.

The abdominal ganglion and attached siphon nerve with associated peripheral siphon motoneurons were dissected from Aplysia and maintained in organ culture. The synaptic potentials from several different siphon sensory cells onto peripheral siphon motoneurons were then tested to establish a pre-serotonin baseline. Serotonin (20-50 uM) was then bath-applied onto the peripheral nervous system (containing the synaptic regions of the sensory cells, as well as the postsynaptic peripheral siphon motoneurons). Serotonin treatment consisted of 5 applications of 5 min duration, separated by a 15 min washout period. When the same synaptic connections were retested the following day, preparations that had received peripheral (synaptic) applications of serotonin exhibited significant facilitation at peripheral synapses (+52%; $p < .02$). These findings indicate that application of serotonin onto the synaptic cellular regions is sufficient to produce long-term synaptic facilitation.

We now plan to ask whether long-term changes induced by serotonin at these distant synapses are blocked by central (somatic) or by peripheral (synaptic and postsynaptic) application of protein synthesis inhibitors. In addition, we plan to compare effects of central and peripheral application of serotonin to determine whether long-term changes are synapse-specific.

111.13 LONG-TERM HETEROSYNAPTIC DEPRESSION OF APLYSIA SENSORIMOTOR SYNAPSES IN CULTURE IS PRODUCED BY THE PEPTIDE FMRF-AMIDE. P.G. Montarolo*, E.R. Kandel and S. Schacher. (SPON: E. Holtzman) Ctr. for Neurobiol. & Behav. and HHMI, Columbia Univ., College of P&S, and NYS Psychiat. Instit., NY, NY 10032.

The sensorimotor synapses of the gill- and siphon-withdrawal reflex in Aplysia can undergo heterosynaptic facilitation or depression both in-vivo and in-vitro. In culture, a single application of serotonin (5-HT) produces facilitation lasting minutes, while repeated applications of 5-HT causes facilitation lasting more than 24 hours (Montarolo et al. 1986). Short-term depression of sensory cell synapses can be evoked by a single application of either dopamine or the peptide FMRF-amide (Abrams et al., 1984; Belardetti et al., 1987). We have now examined whether repeated applications of FMRF-amide (10^{-7} M) or dopamine (10^{-6} M) can produce long-term heterosynaptic depression of sensorimotor synapses in culture.

After measuring the strength of the connections and the extent of short-term depression produced by the first 5-minute application of the inhibitory transmitters, the cells were exposed to 3 additional applications lasting 5 minutes, separated by 30 minutes of washing. The repeated applications of FMRF-amide caused a significant decrease ($p < .01$) of $-31 \pm 6\%$ ($n = 6$) in synaptic strength when measured 24 hours later. In contrast, dopamine, which can depress connections for a short duration, failed to reduce synaptic strength 24 hours later; $5 \pm 10\%$ ($n = 5$).

Since sensorimotor synapses can be depressed with low frequency stimulation of the sensory cells, we examined whether long-term depression can also be evoked by homosynaptic mechanisms. Sensory cells were given 10 stimuli at 30-second intervals during four sessions separated by either 30-minute or 90-minute rest intervals. The decrease in EPSP amplitude during each session and the extent of recovery with each rest interval was comparable to that observed in the intact nervous system. When the connections were retested 24 hours later, they were not changed significantly from their initial values from the previous day: $-7 \pm 6\%$, $n = 5$ and $-8 \pm 8\%$, $n = 5$ for the groups separated by 30- and 90-minute rest intervals, respectively. These changes were also not significantly different from the change obtained for control cells that received a single stimulus on the first day; $-2 \pm 11\%$, $n = 7$.

The results on long-term depression of sensory synapses with FMRF-amide, coupled with those on long-term facilitation with 5-HT, suggest that long-term heterosynaptic modulation can be produced in both directions by means of specific modulatory neurotransmitters. The inability to produce long-term homosynaptic depression in culture with stimulation protocols analogous to those used to evoke long-term habituation of the gill-withdrawal reflex (Carew et al., 1972) suggests the possibility that long-term habituation, unlike the short-term, may require heterosynaptic modulatory influences perhaps produced by transmitters such as FMRF-amide. To test this idea, one needs to identify the appropriate modulatory neurons that produce synaptic depression and see whether these cells are activated during long-term habituation training.

111.14 INDIVIDUAL APLYSIA SENSORY NEURONS VISUALIZED OVER TIME IN CELL CULTURE: ATTEMPTS TO CORRELATE LONG-TERM SYNAPTIC AND MORPHOLOGICAL CHANGES. D.L. Glanzman, E.R. Kandel and S. Schacher. HHMI, Ctr. for Neurobiol. & Behav., Columbia Univ., College of P&S, and NYS Psychiat. Instit., NY, NY 10032.

Studies on the nervous systems of vertebrates and invertebrates suggest that long-term synaptic changes during both development and learning are mediated, in part, by morphological changes. To determine whether synaptic changes that occur during learning and development share common mechanisms and whether they require interaction between the presynaptic cell and its postsynaptic target, we have applied to sensory neurons of Aplysia in dissociated cell culture the methodology for visualizing the structure of a single neuron over the course of days recently developed by Kater and Hadley (1982) and Purves and Hadley (1985).

We chose the Aplysia in-vitro culture system since sensory neurons can form stable synaptic connections with identified motor cells over a period of 4 days. Moreover, once stable, these connections can undergo long-lasting facilitation following repeated applications of serotonin (Montarolo et al., 1986). Sensory neurons were placed into culture either alone or together with motor cell L7. After 2-5 days in culture, synaptic strength was assayed electrophysiologically, and sensory neurons were stained with intracellular injections of the fluorescent dye 5(6)-carboxyfluorescein and viewed with a fluorescence microscope coupled to a low-light level video camera. In some experiments, the same sensory cells were reinjected with dye 24 hrs later.

We found that when sensory neurons were cultured alone, they were much less complex structurally than when they were co-cultured and formed synapses with the motor cell L7. The co-cultured sensory neurons had more processes and more varicosities than sensory neurons cultured alone. These data suggest that some interaction with the motor neuron regulates the growth of the sensory neurons' processes. Consistent with this correlation, there were concomitant increases in the complexity of the processes of sensory neurons as synaptic strength increased during the first 4 days. Specifically, there was elongation of sensory neuron branches and an increase in the number of varicosities. By contrast, after four days in culture, when the strength of the sensorimotor connections had stabilized, there were only minor morphological changes in sensory neurons over the next 24 hrs. In these stable cultures, there was a rough correlation between the complexity of a sensory neuron's processes and the strength of its synaptic connection. We have begun attempts to determine whether repeated applications of serotonin, which produce long-term facilitation of stable sensorimotor connections, can also produce morphological changes like those seen in developing sensorimotor cultures. In addition, we plan to examine whether such changes are cell autonomous or require the presence of the motor cell.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM II

112.1 SUPPRESSION OF OKN WITHOUT RETINAL ERROR SIGNALS: EFFECTS OF ATTENTIONAL MODE AND STIMULUS FREQUENCY J. Pola, H. J. Wyatt and M. Lustgarten. Schnurmacher Institute for Vision Research, SUNY State College of Optometry, 100 East 24th St., N.Y., N.Y. 10010.

We can suppress OKN by looking at a target. It is usually thought that such suppression depends on the pursuit system holding the eye on target against the influence of an optokinetic background. In recent studies we showed that this is not necessarily so, since viewing a target stabilized at the fovea (no retinal slip) results in suppression of OKN (Vis Res '84). Furthermore, the characteristics of this suppression depend on how subjects attend to the target (Neurosci Abstr '86).

We have now explored the influence of attending to a target on suppression of OKN over a wide range of frequencies of sinusoidal background field motion. As in previous studies we stabilized the target at the fovea to eliminate effects of target retinal slip. There were three conditions: "gaze", "look" and "hold". In the "gaze" condition, subjects regarded the target without attending to it; in the "look", subjects actively attended to the target; and in the "hold", subjects attempted to maintain the target at a location directly in front of them. In all three conditions, the frequency of field motion varied from 1/32 to 2 Hz. (Using field alone, we also studied OKN at these frequencies.)

Regardless of condition there was substantial suppression of OKN. In the "gaze" condition, the eyes moved roughly in phase with field motion at all frequencies, but much less than during OKN when viewing the field alone. In the "look" condition, the eyes moved counterphase to the field motion (what we call "hyper-suppression"), with amplitude of counterphase motion increasing and then decreasing over frequency, peak amplitude occurring at about 0.5 Hz. For the "hold" condition, the eyes remained relatively stationary across all frequencies.

We also asked subjects to imagine a target and to "hold" it at a fixed position in front of them against the field motion. In this case we did not find suppression of OKN at any frequency.

In summary, these findings show that the mode of attending determines how OKN is suppressed over a wide frequency range. The "hold" (no target) condition, however, indicates that the presence of a target is essential. Since there was no retinal slip in these experiments, it would seem that the presence of a target and attending to it are primary in modulating the optokinetic effect of the field on the oculomotor system. This may involve attenuating (as in "gaze") or even eliminating (as in "look" or "hold") the influence of the field.

(Supported by NSF BSN-85-19267)

112.2 THE ROLE OF THE NUCLEUS OF THE OPTIC TRACT IN PRODUCTION OF OPTOKINETIC NYSTAGMUS (OKN) AND AFTER-NYSTAGMUS (OKAN) IN THE MONKEY. D.Schiff*, B.Cohen & J.Buettner-Ennever* (SPON: H. Krieger). Depts. of Neurol, Mt Sinai Sch of Med, CUNY & Neuropath, Univ of Munich.

The pretectal nucleus of the optic tract (NOT) processes retinal slip information that leads to OKN and OKAN in the cat, rat and rabbit. The purpose of this study was to determine if this was also true in the monkey. Eye positions were recorded with a magnetic scleral search coil. The region of NOT was monopolarly stimulated through tungsten microelectrodes with 0.5 ms, 30-40 microamp pulses, delivered over 10 to 40 s, at frequencies of 50 to 500 Hz. Horizontal nystagmus with ipsilateral slow phases was elicited in darkness but not in light. Eye velocity rose slowly to a steady state level and was followed by after-nystagmus at the end of stimulation. The time constant of rise of the nystagmus was similar to the slow rise of slow phase eye velocity during OKN and to the charge of OKAN. The saturation velocity of the induced nystagmus was statistically the same as that of OKAN, and the after-nystagmus declined over the same time course. Activity induced by NOT stimulation could enhance, prolong or block the slow component of OKN and OKAN depending whether slow phases were to the same or opposite side and interacted with vestibular nystagmus as would OKN and OKAN. Interpreted in terms of the model of OKN and OKAN (Cohen et al. 1977), these data indicate that the stimulus had activated the velocity storage mechanism in the vestibular system through the indirect pathway to produce the slow component of OKN and OKAN.

Positive stimulation sites for inducing nystagmus were located in regions of NOT that were interstitial to fibers in the brachium of the superior colliculus, from the dorsal terminal nucleus (DTN) and from a fiber bundle in the pulvinar that extends from the cortex to NOT. Muscimol injections into NOT caused spontaneous nystagmus with contralateral slow phases and attenuated the slow rise in ipsilateral slow phases of OKN and OKAN. Kainic acid injections into NOT and DTN had the same effect. After lesions the slow rise in OKN with ipsilateral slow phases and the corresponding OKAN were lost. The rapid rise in OKN and vestibular nystagmus were unaffected. Ocular pursuit appeared intact. Partial electrolytic lesions of the cortico-pretectal tract resulted in a reduction of peak velocity of OKN and a longer rising time constant.

The lesion data suggest that after NOT lesions the velocity storage integrator could no longer be activated by retinal slip to produce ipsilateral slow phases. This indicates that the direct and indirect pathways for producing OKN and OKAN are separate at the level of NOT, and that NOT is part of the anatomical substrate for the indirect pathway for horizontal OKN and OKAN in the monkey as in cat, rat and rabbit. These data also indicate that inhibition in NOT is mediated by GABA, acting through GABA-A receptors.

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112.3 VISUALLY INDUCED ADAPTIVE CHANGES IN POST-SACCADIC DRIFT IN NORMAL HUMANS. Z. Kapoula*, L. M. Optican* & D. A. Robinson. Lab. of Sensorimotor Res., National Eye Institute, Bethesda, MD 20892, Johns Hopkins Hospital, Dept. of Ophthalmol., Baltimore, MD, USA.

Post-saccadic ocular drift occurs when the brain fails to program the correct level of innervation to hold the eyes in their new final position. Such drifts are evident in cases of extraocular muscle weakness, and can be corrected by a cerebellar-dependent adaptive mechanism (Kommerell et al., Invest. Ophthalmol. 15:657, 1976; Optican and Robinson, J. Neurophysiol. 44:1058, 1980). This adaptation can be elicited optically in normal monkeys, and depends on the time constant of the adapting stimulus (Optican and Miles, J. Neurophysiol. 54:940, 1985). We examined the ability of normal humans to adapt to optically-induced post-saccadic retinal slip.

Four subjects sat in front of a white hemisphere (2 m diam) onto which a random dot pattern (180 x 60 deg) was projected. A computer detected the end of spontaneous saccades (within ±3 ms) and drifted the visual display by 25% of the horizontal component of that saccade. The scene could drift in the same direction as, or opposite to, the saccade, with a time constant of 25, 50 or 100 ms, depending on the experiment. Subjects were exposed to this visual experience for 3 hrs, producing 10,000-16,000 saccades. During training, eye movements were monitored with an EOG. Pre- and post-training binocular recordings were made with the magnetic-field/search-coil method. At first, the subjects followed the drift of the visual scene with a delay of 40-100 ms. After 1 - 3 hrs of training all 4 subjects developed consistent, zero-latency, compensatory ocular drifts, even after spontaneous saccades in the dark. The drift waveform was roughly exponential. The amount of drift was 15-20% in the presence of the adapting stimulus and 6-10% in the dark. The time constant of the ocular drift in the dark varied with subjects (52-149 ms) and was not influenced by the time constant of the stimulus used to train the subject. There were no significant differences in the adaptive changes in drift between the two eyes. However, significant differences were found between abductions and adductions: subjects trained to backward drift developed compensatory drift mostly in the abducting eye while subjects trained to onward drift developed drift mostly in the adducting eye. This asymmetry between the two eyes was evident mainly in the dark; in the light the drift was more conjugate.

The persistence of ocular drifts in the dark, and their exponential waveform, suggest that central readjustment of the basic saccadic innervation had occurred. The asymmetry present in the dark suggests that the adaptive mechanism is able to adjust the saccadic neural signals differently for each eye.

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112.4 EFFECTS OF MONOCULAR AND BINOCULAR PATCHING UPON OCULAR ALIGNMENT AND SACCADE METRICS IN RHESUS MONKEYS. T. Suzuki*, S. Das* and D.S. Zee. Johns Hopkins University, Baltimore, Md 21205.

We investigated ocular alignment and postsaccadic gaze stability in monkeys after patching of one or both eyes. We measured the position of both eyes using search coils. Ocular alignment was assessed by measuring the phoria: the relative position of the two eyes with one fixing the target and the other under cover. Postsaccadic drift was inferred from the ratio (P:S) of the rapid portion of the saccade, the pulse (P), and the final resting position of the eye, the step (S). P:S of both eyes were measured under bi- (BV) and monocular (MV) viewing. Four paradigms were used: 10 days, right eye patched (REP), left eye patched (LEP), patch alternated daily (AP), and 5 days both eyes patched (BEP).

Phoria: Prior to patching both animals had < 0.35 deg of phoria but after each patching paradigm they developed an esophoria (2.0-2.25 and 0.6-2.05 deg for animals 1 and 2, respectively). Animal 1 also developed a left hyperphoria (.7-1.1 deg.) after LEP and REP.

Postsaccadic drift: Prior to patching, animal 1 had no postsaccadic drift (0.99 < P:S > 1.01). After either LEP or REP postsaccadic drift appeared with a duration < 100ms. The P:S of the habitually-patched eye decreased (0.95) for abducting saccades and increased (1.04) for adducting saccades; horizontal saccades were always followed by temporally-directed drift. During MV the habitually-viewing eye also showed postsaccadic drift (P:S = 0.98 for abduction and 1.03 for adduction) when it was the eye under cover but not when it was the fixing eye. Following BEP, P:S decreased (0.97-0.98) for saccades in both directions causing onward drift after horizontal saccades. For vertical saccades P:S of the habitually-patched eye increased (1.05) for down saccades and decreased (0.97) for up saccades so that postsaccadic drift was always upwards. No changes in vertical P:S were noted after BEP. For animal 2, prepatch, there was backward drift (P:S, 1.06) after horizontal saccades by the left eye. Postpatch the changes in P:S for horizontal saccades for REP and BEP were similar to those of animal 1 but little change was seen after LEP. For vertical saccades onward drift developed (P:S, 0.94-0.97) for upward saccades only, primarily after REP and primarily in the right eye when it was under cover.

Our results indicate that simultaneous binocular visual inputs are necessary to maintain ocular alignment. Absence of visual inputs also leads to postsaccadic drift, but the pattern of drift cannot be attributed solely to the associated phoria. Important, too, is whether one or both eyes had been patched and which eye viewed the target during testing. Since the drift was brief and immediately followed the saccade, we infer that mechanisms other than immediate visual feedback account for the decrease in postsaccadic drift when the eye is viewing rather than covered.

112.5 **EFFECT OF PERIPHERAL VISUAL FIELD ON VESTIBULO-OCULAR REFLEX (VOR) GAIN PLASTICITY AND VISUAL-VESTIBULAR INTERACTION.** J. L. Demer, J. Goldberg, F. I. Porter*, and K. Schmidt*. Cullen Eye Institute and Clayton Neurotology Laboratory, Baylor College of Medicine, Houston, Texas. 77030

When telescopes are mounted in spectacles for use as aids for the visually impaired, only the central visual field is magnified and the periphery is unmagnified. To maintain retinal image stability during head movements in this situation, the *gain* (eye velocity/head velocity) of compensatory eye movements required in central vision is equal to telescope magnification, while the required gain for the periphery is 1.0. We investigated the effect of this telescopic spectacle configuration on VOR gain plasticity and visual-vestibular interaction in 8 subjects, selected for high VOR gain plasticity from a group of 35 normally sighted subjects.

Eye movements were recorded by digitally sampled electro-oculography during sinusoidal whole-body rotation in a servo driven chair in darkness (**VOR**) and in light (visually augmented VOR, **VVOR**). Gains were measured for 0.1 Hz rotations (amplitude 30°/s in light, 60°/s in dark) as subjects wore 4X binocular telescopic spectacles having a magnified visual field of 10°. The peripheral field was either occluded or unobstructed. After initial measurements, subjects underwent training by sinusoidal rotation (0.1 Hz, amplitude 20°/s) for 15 min while viewing a distant video display. Gain measurements were then repeated.

Initial VOR gains were 0.65±0.08 (mean±SD, n=8) with the periphery occluded and 0.67±0.05 without occlusion. After plasticity training, each subject exhibited a significant ($p<0.05$) VOR gain increase with the periphery occluded, so that VOR gains increased on average by 21% to 0.78±0.07. Without occlusion of the periphery, only 5 subjects exhibited a significant VOR gain increase, so that the VOR gain for all subjects after plasticity training was 0.71±0.05; the mean gain increase was 7%. With peripheral occlusion, mean initial VVOR gain was 1.93±0.30, significantly greater than the value of 1.27±0.30 measured without occlusion. VVOR gain was not significantly changed after training for either condition.

These results indicate that the unmagnified peripheral field inhibits VOR gain plasticity and immediate modification of VVOR gain by magnified central vision. This finding supports a role for peripheral retinal slip in inducing VOR gain plasticity in humans.

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112.6 DIRECTIONAL TUNING OF BURST NEURONS IN THE riMLF OF ALERT MONKEYS IN RESPONSE TO VESTIBULAR AND VISUAL STIMULATION. V. Henn and K. Hepp*. Neurology Dept. University and Physics Dept. ETH, 8091 Zürich, Switzerland.

Rapid eye movements have 3 degrees of freedom in the context of vestibular and optokinetic nystagmus when the head is rotated about arbitrary axes in 3-dimensional space. With the head erect and stationary, visually elicited saccades obey Listing's law and have 2 degrees of freedom. Bilateral lesions of the riMLF lead to a syndrome in which rapid eye movement generation is confined to the horizontal plane only. We conjectured that the riMLF constitutes an interface in synergy with the PPRF between the 3-dimensional vestibular input, the 2-dimensional visual input, and the 3-dimensional oculomotor output for rapid eye movement generation.

The riMLF contains predominantly burst neurons which we systematically studied during visual and vestibular stimulation in alert monkeys. Eye movements were measured with two orthogonally implanted search coils, unit activity was recorded with varnished tungsten electrodes. Animals were placed in a gimbal system which allowed rotation in 3 dimensions.

Rapid eye movements were analyzed in terms of 3-dimensional rotation vectors which are the linear approximation to a description in terms of rotation matrices. This description is independent of the initial eye position and was found adequate in the peri-primary range. In first approximation saccade trajectories in 3-dimensional space can be described as straight lines with the duration determined by the movement amplitude.

Burst neurons were either of short- or long-lead type. Analysis was performed to obtain 3-dimensional tuning curves. On-directions of neurons were closely aligned to canal and muscle planes, and activity attenuated in other directions according to a cosine function. The firing patterns of burst neurons could be interpreted in the sense that they were excited by ipsilateral canal input and excitatory on ipsilateral motoneurons. Thus, the basic input-output organization was found to be similar to horizontal burst neurons in the PPRF. Saccades in Listing's plane, or rapid phases of nystagmus along specific canal planes, or pure torsional nystagmus (about the line of sight) involved the same burst neurons which were activated in different ratios according to the specific movement direction.

Burst neurons in the riMLF with on-directions along vertical canal planes together with horizontal burst neurons in the PPRF constitute the final common pathway for saccade generation in 3 dimensions.

112.7 THE EFFECT OF IBOTENIC ACID LESIONS OF THE OMNIPUASE NEURONS ON SACCADIC EYE MOVEMENTS IN THE MONKEY. C.R.S. Kaneko and A.F. Fuchs. Reg. Primate Research Center and Department of Physiol. & Biophysics, University of Washington, Seattle, WA 98115.

In the mid 1970's, D.A. Robinson and D.S. Zee and colleagues formulated the "local feedback model" of the neuronal circuits that generate saccades. This model and several more recent variations have been difficult to evaluate because of the tight coupling of their cellular components and the consequent inability to affect them independently. The development of techniques to produce small, axon-sparing chemical lesions and the localization of a critical element, the omnipause neurons (OPNs), to a morphologically distinct pontine nucleus have made it possible to attack this problem. Specifically, we wanted to test which of the alternatives of the local feedback models best predicted the effect of loss of OPN activity: would OPN lesions produce saccades of increased duration and decreased velocity (Scudder, J. Neurophysiol., in press) or ocular flutter and opsoclonus (Zee & Robinson Ann. Neurol. 5:405-414, 1979).

Two rhesus monkeys were trained to track a back-projected spot for food reward. OPNs were located by their distinctive discharge in relation to saccades and their extent was mapped and marked by bracketing electrolytic lesions. Ibotenic acid was injected via a 30 gauge stainless cannula in concentrations of 15 µg/µl while OPNs were recorded via an attached microelectrode.

In the first animal, a 1.0 µl injection destroyed cells from 200 µm to the right of the midline to the rootlets of the left 6th nerve and from just rostral to the abducens nucleus 1.5 mm rostrad. Virtually the entire OPN nucleus was destroyed with some damage to adjacent areas but there was no apparent damage to the rootlets or any of the nearby fiber tracts. The second animal has had a single 0.2 µl injection and its histology is not yet available as other small injections are planned.

Both animals showed qualitatively similar deficits but those of the first animal were more severe. The saccade duration increased by an average of 500% and recovered to 300% of the original value as indicated by the slope of the regression line relating the saccade duration to its amplitude. Recovery was more complete for vertical components or vertical saccades averaging only 114% increase at 3 weeks post-lesion. Likewise, the peak velocity (i.e. the slope of the peak velocity/saccade amplitude relation) decreased, on average 73% and recovered to only 76% of the original value. The range of saccadic amplitudes decreased transiently but recovered for all but the horizontal direction in the animal with the large lesion in which it shrank from 38° left and 47° rightward to 6° left and 11° rightward.

In general, our results support the Scudder variation of the local feedback model. Simulations are being conducted to test the model's ability to predict, in detail, our lesion results.

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112.8 RETICULAR PRESACCADIC INPUT TO THE PRIMATE SUPERIOR COLLICULUS. A.K. Moschovakis, A.B. Karabelas and S.M. Highstein, Dept. of Otolaryngology, Washington University, and the McDonnell Center for Studies of Higher Brain Function, St. Louis, Missouri 63110.

Intraaxonal recording and HRP injection were employed in alert naive, squirrel monkeys to identify superior colliculus (SC) neurons carrying presaccadic signals. In addition to tectal long lead burst neurons (TLLBs), (Moschovakis et al., Soc. Neurosci. Ab. 12:1185, '86), our sample includes axons of mesencephalic reticular formation (MRF) cells that project to the SC.

In contrast to TLLBs, that are active before saccades within a movement field of limited size, reticulotectal long-lead burst neurons (RTLLBs) discharge before most contraversive and some ipsiversive saccades. The latency of the high frequency portion of RTLLB presaccadic activity (1-46msec, x=18.75, S.D.=7.97) is statistically indistinguishable from that of TLLBs. There is a significant correlation ($0.50 < r < 0.84$, $p<0.01$) between the number of spikes in a burst (N_b) and the cosine of the angle between saccade vector and on-direction of the cell as well as between N_b and the amplitude of the saccadic component in the on-direction of the cell (approximately horizontal contraversive). The cell bodies of RTLLBs are located in the MRF, bordering the rostral 1mm of the SC dorsally, the periaqueductal gray medially, and the interstitial nucleus of Cajal ventrally. This area of the MRF has been shown to contain cells firing before contraversive rapid eye movements and its electrical stimulation elicits contraversive saccades (Cohen et al., Progr. Brain Res. 64:243, '86).

RTLLB axons emerge from the cell body and turn dorsally towards the deeper layers of the SC, where they ramify extensively, mainly in the stratum griseum intermedium but also in the stratum opticum, i.e. in the layers of the SC that contain the cell bodies of TLLBs. They then cross the midline in the intertectal commissure to terminate in the deeper layers of the contralateral SC. Terminal fields of RTLLBs are distributed to progressively more rostral and medial portions of the ipsilateral SC, as the cell body of origin is located more dorsally and the on-direction of the neuron is upward biased. RTLLBs may be both presynaptic and postsynaptic to TLLBs. (Supported by NEI EY-05433).

112.9 TECTO-RETICULO-SPINAL-NEURONS HAVE DISCHARGES CODING THE VELOCITY PROFILES OF EYE AND HEAD ORIENTING MOVEMENTS. D.P. Munoz and D. Guitton, Dept. Neurol.& Neurosurg. McGill Univ. Montreal, Canada.

Tecto-reticulo-spinal neurons (TRSNs) form a major descending projection from the superior colliculus (SC) to contralateral brainstem and spinal cord premotor areas involved in the control of eye and head movements. These neurons mediate the SC's influence in controlling orienting behaviour. We have already provided preliminary evidence that the velocity of gaze shifts and the intensity of TRSN phasic bursts covary and depend on the behavioral context within which the orienting movement is made (Brain Res. 398 185-190, 1986). In this abstract we provide further evidence on this unexpected result. TRSNs were studied in alert head-free or fixed cats trained to orient to targets that were either visible or whose location could be predicted based on knowledge gained from previous trials. RESULTS: The peak velocities of the eye, head, and therefore gaze movements were fastest and the durations shortest, when the cat oriented to visible targets. In the visible target condition the gaze velocity profle had a symmetrical, bell-shaped appearance. In the predicted target condition the gaze velocity profile was somewhat irregular, almost trapezoid-like in appearance. The instantaneous firing frequency profile of a TRSN, averaged over several trials, roughly approximated the mean gaze velocity profile in both target conditions. The target sometimes appeared immediately before the onset of an orienting movement to the predicted target. The shape of the initial part of the eye, head and gaze movement trajectories was characteristic of movements to the predicted target. However, there was a sudden acceleration of the eye and head during the course of these movements and the gaze velocity profile assumed a new shape more characteristic of movements to a visible target. Modification in the trajectory of the head movement occurred 20 ms after the reacceleration of the eye. TRSNs burst 10 ms and 30 ms before the increase in eye and head acceleration respectively. Microstimulation of the SC was used to mimic the effect of TRSN discharge on movement trajectory. Movements triggered by a high frequency train had greater peak velocities than identical amplitude movements triggered by low frequency stimulation. On some trials in which the low frequency train had evoked a movement, a superimposed high frequency train was introduced during the course of the gaze movement. The high frequency train triggered a reacceleration of the eye and head at latencies of 10 ms and 27 ms respectively. These results suggest that TRSN discharges can control both eye and head movements; and, in contrast to classical models of SC function, suggest that characteristics of TRSN burst activity predict the shape of the associated gaze trajectories.

112.10 THE OPTIC TECTUM ENCODES SACCADE MAGNITUDE IN A PUSH-PULL FASHION IN THE BARN OWL. S. du Lac and E.I. Knudsen. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Single unit studies and focal electrical stimulation have demonstrated that the superior colliculus contains a map of orienting movement vector. However, the mechanisms by which place coded activity in the colliculus determines the size and direction of saccadic orienting movements have not been resolved. Current models of saccade generation assume that saccade size is specified by the topographically weighted sum of excitatory drives from collicular neurons. If this assumption is true, then simultaneous stimulation of two collicular sites should produce a saccade with a magnitude that is the sum of those elicited by stimulating each site alone. Alternatively, the colliculus could specify saccade size in a push-pull fashion by a ratio of excitatory and inhibitory influence on the saccade generator, the value of which varies topographically. This hypothesis predicts that simultaneous stimulation of two collicular sites would produce a saccade with a magnitude intermediate to the magnitudes encoded by each site.

We sought to distinguish between these alternatives by electrically stimulating the optic tectum in barn owls. (Although barn owls make saccadic head movements instead of saccadic eye movements, the role of the tectum in specifying the metrics of head movements appears to be similar to that of the superior colliculus in encoding orienting eye movements.) Pairs of monopolar stimulating microelectrodes were implanted in the optic tecta of anesthetized owls, and sensory receptive fields were mapped. The following day, electrodes were stimulated singly and simultaneously in the alert owl. Stimulation at the visual representations of 8° and of 60° contralateral elicited saccadic head movements of $8° \pm 2°$ and $63° \pm 3°$, respectively. Simultaneous stimulation elicited a head movement of $40° \pm 2°$. The experiment was repeated for 10 additional pairs of electrode sites in 3 owls. In all cases, the size of the resulting saccade was intermediate to the saccade sizes elicited from stimulation of each site alone. (Similar results were reported by Robinson in monkeys: Vis. Res., 1970.) However, the initial velocity of the saccade (first 10-20 msec) always matched that of the saccade elicited from the caudalmost site (in barn owls, the elicited saccade velocity increases from rostral to caudal). These results support the hypothesis that the tectum specifies saccade size in a push-pull fashion and that initial velocity is specified separately from saccade size.

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112.11 EFFECTS OF FOCAL INACTIVATION OF SUPERIOR COLLICULUS ON SACCADES: SUPPORT FOR THE HYPOTHESIS OF VECTOR AVERAGING OF NEURONAL POPULATION RESPONSE. C. Lee, W. H. Rohrer* and D. L. Sparks. Dept. of Physiology and Biophysics, University of Alabama, Birmingham, AL 35294.

Cells in the deep layers of the superior colliculus discharge before saccadic eye movements with a gradient of response strength within a range of amplitudes and directions (Sparks, Holland and Guthrie, *Brain Res.*, 113:21, 1976). Since this range of movement (movement field) is broad, a large number of cells are active for any given saccade. We hypothesized that the amplitude and direction of a saccade are determined by the average drive of this active neuronal population, not by the drive of a single or a few cells with the strongest activity. To test this idea, we tried to manipulate the activity profile of the population response, and we measured the effect on saccades.

Three rhesus monkeys were prepared for chronic recording and for measurement of eye movements with the scleral search coil technique (Fuchs and Robinson, *J. Appl. Physiol.*, 21:1068, 1966). The monkeys were then trained on a saccadic task where the reward was contingent on successive acquisitions of a fixation target and a saccade target. Targets were selected from an array of LED's on a tangent screen. A metal-coated glass pipette (Malpeli and Schiller, *J. Neurosci. Methods*, 1:143, 1979), filled with lidocaine hydrochloride (2%), was placed in the deep layers of the superior colliculus. The movement associated with the strongest neuronal activity ("best movement" of the recorded site) was estimated by an on-line analysis of the number of action potentials associated with each saccade, and by movements triggered by electrical stimulation of the superior colliculus. After establishing normal saccades around the "best movement", we ejected 100 nl of lidocaine from the tip of the recording pipette into the recorded site.

As predicted, the nature of saccadic dysmetria following the lidocaine injection was target-specific. Saccades to targets with the same direction as the "best movement" but with smaller eccentricities fell short, while those with larger eccentricities overshot the target. Saccades to targets of the same amplitude as the "best movement" but in different directions deviated away from both the target and the "best movement". The amplitude and direction of saccades to the target of the "best movement" often remained the same. Thus, the pattern of saccadic errors was to shift the saccadic trajectory away from the "best movement". These changes are explained by modified profiles of population activity after focal inactivation which shift the average vector of population response toward directions corresponding to the observed saccadic errors.

These results indicate that the amplitude and the direction of a saccade are coded as the vector average of the population activity in the superior colliculus. The accuracy of a saccade is ensured by the contribution of a large number of active neurons that are broadly tuned, not by a few neurons that exibit the strongest activity. (Supported by NIH EY01189).

112.12 MEMORY CONTINGENT RESPONSES OF SUPERIOR COLLICULUS MOVEMENT CELLS D. M. Waitzman, T. P. Ma, L. M. Optican, and R. H. Wurtz. Lab. of Sensorimotor Res., National Eye Inst., Bethesda, MD 20892, USA.

Cells whose firing changed following the presentation of a flashed visual stimulus, to which monkeys would subsequently make a saccade, have been described in the substantia nigra pars reticulata (SNr) by Hikosaka and Wurtz (J. Neurophysiol. 49:1268-1301, 1983). These cells were termed memory contingent and many of them project to the superior colliculus (SC). The current experiments examined the response of intermediate and deep layer SC cells under similar memory contingent behavioral conditions.

We studied memory guided saccades by requiring the monkey to fixate a small spot of light in the primary position of gaze and indicated the location to be remembered by flashing a peripheral target for 50 ms. Eye position was maintained within a window (determined by the magnetic search coil technique) until the fixation point went off at a random time 500 to 1000 ms later. This offset signaled the monkey to look toward the location of the previously flashed target. Saccade responses were recorded to a set of locations which covered the movement field of the cell. Memory trials were randomly interspersed with trials which examined the discharge of the cell to visual stimuli without subsequent saccades, visually guided saccades, and spontaneous saccades.

Memory related activity was comprised of three parts: a phasic visual response, a sustained discharge, and a phasic movement discharge just before the remembered saccade. Cells had various combinations of these three parts. The phasic visual response to the flashed target had about 50 ms latency to onset and 50-100 ms duration. The peak activity either equaled or was less than the phasic response seen following a continuously presented visual stimulus. The sustained memory discharge began about 100 ms after the flashed stimulus and could continue until the monkey made a saccade to the target. The phasic movement discharge preceded the saccade by at least 50 ms. Those cells which discharged prior to remembered saccades also had phasic discharges preceding comparably sized visually guided saccades. Half of the cells with phasic movement activity discharged before spontaneous saccades made in total darkness.

These results indicate that there is a subset of saccade related neurons in the SC whose firing is complementary to the inhibition of the cells of SNr. The sustained memory discharges appear similar to those of the quasi-visual (QV) cells described by Mays and Sparks (J. Neurophysiol. 43:207-232, 1980). Moreover, as with the QV cells, both the sustained and phasic saccade related activity of memory cells could signal the change in eye position for intended eye movement in the absence of a visual target.

REGENERATION: SPINAL CORD I

113.1 THE SEVERITY OF LESION INDUCED HIND LIMB BEHAVIORAL DEFICITS AFTER CERVICAL FASICULUS GRACILIS ABLATION ARE AMELIORATED BY FETAL SPINAL CORD HOMOGRAFTS J.J. Bernstein and W.J. Goldberg. Laboratory of Central Nervous System Injury and Regeneration, Veterans Administration Medical Center, Washington, DC, 20422 and Departments of Physiology and Neurosurgery, George Washington University School of Medicine, Washington, DC.

Fetal spinal cord grafts survive for long periods of time in host spinal cord. Transplanted neurons express peptides and transmitters with limited axonal growth into the host. Astrocytes also mature and express GFAP. The present study explores the ability of fetal spinal cord grafts into C3 fasiculus gracilis (FG) to influence the expected deterioration of hindlimb performance following this lesion. Ten adult Sprague-Dawley rats were placed on a schedule of 23-h off and 1-h on water schedule. These subjects were then trained to traverse a narrow platform for a water reward. Criterion was established as 10 trials/day, completed in 15-s a trial, for two consecutive days. Animals were ranked for hind and fore limb performance utilizing slips, recovery and manner of traversing the platform. In a triple blind experimental design the animals numbers were coded and laminectomy performed at C3. The FG was bilaterally sectioned at the rostral and caudal borders of the segment and the FG aspirated. Five of the subjects were chosen at random for the implantation of two, one mm segments of E14 spinal cord from fetuses of timed pregnant Sprague-Dawley dams. Coded, lesion only and lesion-transplanted animals were then tested at 21,30,45,60 and 90 days later until criterion or for 4 days. Average ranked scores were coded and statistically analyzed by the non-parametric Friedman two-way analysis of variance a priori and the Mann-Whitney U test a posteriori. Hind limb performance of the lesion-only (ANOVA X^2=13.04, df=5; $p<0.05$) and lesion-transplant groups (ANOVA X^2=11.82, df=5; $p<0.05$) showed that the C3 fetal transplants significantly decreased ($p<0.05$) the severity of hind limb deficit at 21 days post lesion and significantly decreased ($p<0.05$) the expected foot slips and limb recovery to the platform that was expected and did develop in lesion-only animals at 90 days. There were no deficits in fore limb performance (ANOVAs $p>0.05$). There were a few small caliber nerve fibers in the FG at the level of C1. For this reason the transplant induced decreased severity of deficit is discussed stressing the trophic and tropic aspects of the transplant and the astrocytes that are known to migrate for centimeters from the site of transplantation in adult host spinal cord and cortex.

Supported by the Veterans Administration.

113.2 CULTURED FETAL SPINAL CORD ASTROCYTES MIGRATE INTO ADULT HOST CERVICAL CORD AND MEDULLA FOLLOWING TRANSPLANTATION INTO THORACIC SPINAL CORD. W.J. Goldberg and J.J. Bernstein. Laboratory of Central Nervous System Injury and Regeneration, Veterans Administration Medical Center and Departments of Physiology and Neurosurgery, George Washington University School of Medicine, Washington D.C.

We have previously demonstrated astrocytic migration from pressure-injected minced-piece transplants. Astrocytes migrated at a rate of 0.76 mm/day after a 14 day delay [Goldberg and Bernstein, J. Neurosci. Res. 17(4):in press]. In the presents experiments cell suspensions from 14-day gestation rat spinal cord, which had previously been soaked for 1 hour in a 2 ug/ml solution of Phaseolus vulgaris leucoagglutinin (PHAL), were cultured on collagen gels containing laminin for two weeks, at a density of 1500-2000 cells/mm^2. Pieces of the gel (0.5 mm^2) and attached cells (about 875) were then transplanted into the dorsal column of adult host thoracic spinal cord [Bernstein and Goldberg, Brain Res. 377(1986):403-406]. At 1, 2 and 3 months post implantation (MPI) animals were sacrificed and the spinal cords removed, embedded in paraffin and sectioned at 8 um for immunohistochemistry at the light microscopic level. Sections were double labeled for PHAL, utilized as a marker for transplant derived cells, and glial fibrillary acidic protein (GFAP), a specific marker for astrocytes. Transplant-derived astrocytes (PHAL-GFAP positive cells) migrated from the transplantation site both rostral and caudal and were observed within the host dorsal column ipsilateral to to transplantation site. At 2 months, lateral migration into the contralateral dorsal column and ipsilateral dorsal horn was observed. At 3 MPI transplant-derived astrocytes were observed in host nucleus gracilis, a distance of approximately 55 mm from the implantation site. This calculates into a migration rate of 0.72 mm/day, assuming a 14 day delay occurred as was observed with pressure-injected transplants. Astrocytes represent approximately 30% of the total cell population in this culture system, or about 260 cells. If these 260 astrocytes were evenly distributed over the 7 cm of lumbar to cervical spinal cord we would expect a final density of ca 4 astrocytes/mm, assuming that all of the astrocytes migrated in a single line. A single 8 um section may contain 15 or more astrocytes spread throughout the dorsal columns at 2 months post implantation, indicating that a significant number of cell divisions must have occurred. Since astrocytes in the original culture do not divide due to a lack of mitogens there must be factors present in the host dorsal column following the transplantation procedure which induce the division of astrocytes.

Supported by the Veterans Administration

113.3 SOME DORSAL ROOT AXONS REGENERATE INTO THE ADULT RAT SPINAL CORD. AN HRP STUDY. F.J. Liuzzi and R.J. Lasek. Bio-architectonics Ctr., Case Western Res. Univ. Sch.of Med., Cleveland, OH 44106.

It has been known since the time of Cajal that many regenerating adult mammalian dorsal root axons are stopped at the dorsal root transitional zone where the cellular environment changes from one of Schwann cells and collagen to one of oligodendroglia and astrocytic processes. EM of this zone has shown that the axons end among the reactive astrocytic processes. Indeed, our studies of this region in adult rats after dorsal root crush indicate that reactive astrocytes block axonal growth by activating a physiological stop pathway which is extant in all axons (Liuzzi and Lasek, Soc. Neurosci. Abst. 12:697, 1986 and in press). The questions remained, however, of whether any regenerating dorsal root axons get past the astrocytes and, if so, whether any reach the gray matter to arborize and synapse. We have used anterograde injury-filling of regenerating dorsal root axons with HRP to demonstrate the reinnervation of the adult frog spinal cord (Liuzzi and Lasek, J. Comp. Neurol., 232:456, 1985) and are now using the same technique to answer the above questions in the adult rat.

For these studies, the L5 dorsal root was crushed twice, 10 sec each, with a #5 Dumont, approximately 10mm from the cord. After survival times up to 60 days, the root was reexposed and cut 5mm from cord. The proximal stump of the root was isolated and chips of dried concentrated HRP (Sigma type VI) were applied. After one hour, excess HRP was flushed from the area, the wound was closed and the animal was allowed to survive for 24 hours. Following perfusion with 1.0% glut. and 3.0% para. in phosphate buffer, the cord was removed, fixed for 6 hours and stored overnight in phosphate buffered sucrose (30%). Sixty micron-thick, transverse, vibratome sections were processed for HRP visualization using the same protocol that had been used in the frog studies.

Examination of cresyl-violet counterstained sections consistently revealed a very small number of HRP-labelled axons in the spinal cord. In the white matter, a few labelled axons were seen, particularly at the edge of the cord. Deeper in the markedly gliotic dorsal funiculus, there were many fewer, tortuous axons. In the gray matter, some very small diameter axons (<1.0um) were seen. Some of these axons, which were confined to the dorsal horn, meandered through the gray matter and appeared to branch infrequently. Others grew fairly straight. Although these regenerated axons have not been examined at the EM level, many were punctuated by en passant varicosities suggesting that they have the capacity to reform synaptic contacts with targets in the gray matter. This work was supported by a grant to F.J.L. from the S.C.R.F. of the Paralyzed Veterans of America.

113.4 A CELLULAR MATRIX FOR STUDYING AXONAL REGROWTH IN ADULT RAT SPINAL CORD INDUCED BY CONTROLLED FREEZING OF THE DORSAL COLUMN. N.R. West and G.H. Collins. Departments of Pathology and Anatomy, S.U.N.Y. Health Science Center at Syracuse, Syracuse, New York 13210.

Mature axons of the mammalian central nervous system will regenerate when provided with an appropriate matrix. Previous experience with cryogenic injury in rat spinal cord has shown that a cellular matrix does develop into which axonal regrowth occurs (Collins, West, et al. J. Neuropath. Exp. Neurol. 45:742, 1986). The rostral limit of the matrix occurs approximately at the junction of the injury zone with the Wallerian zone and the amount of axonal regrowth beyond this point is severely limited. The purpose of these studies has been, therefore, to develop a matrix of greater length so as to provide a model of spinal cord injury in which the following can be studied: 1) Axonal elongation over an extended distance (1.0 cm.), 2) Sequence of cellular and molecular events associated with this growth (West and Collins, unpublished observations), and 3) The effects of experimental manipulation upon the process of regrowth.

In studies involving 10 animals we have found that temperatures and times varying from -5°C for 60 minutes to -7°C for 15 minutes when applied by a cryode measuring 0.4 x 7.0 mm through an atraumatic laminectomy at T7-T9 will produce a lesion in which nearly all axons are destroyed and a cellular matrix forms. The area of injury extends laterally into the dorsal horns and ventrally encroaches upon the corticospinal tract, thus being approximately 1.0 mm in depth, 1.5 mm in width and 1.0 cm in length. Within this area phagocytic cells remove breakdown products of axons and myelin sheaths so that by 10 days following the injury, most of the degeneration has been cleared and the macrophages sequestered in the dorsal region where the matrix is less compact and contains cells with a spindly configuration. A variable amount of cellular proliferation is present around blood vessels and beneath the pia mater. Based upon previous studies, this matrix is the site where axons will regrow in association with Schwann cell ensheathment. Ventrally, a broad band of matrix develops consisting of cells which have a glial morphology, plus large immature cells. We conclude that this matrix, which is identical to that which has been shown to support axonal regrowth and central type myelination, has the capacity to support the regrowth of axons over an extended distance and will thereby constitute a model for the study of axonal growth in adult mammalian central nervous tissue.

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113.5 GROWTH OF BLOOD VESSELS AND NEURITES INTO A COLLAGEN MATRIX PLACED BETWEEN THE CUT ENDS OF TRANSECTED RAT SPINAL CORD. J.B. Gelderd, Department of Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843.

The purpose of this study was to evaluate the capability of a sterile, cell-free, bovine, collagen matrix (Collagen Corp., Palo Alto, CA) to support ingrowth of blood vessels and neurites following spinal cord transection in rats.

Using an operating microscope, adult Long-Evans hooded rats underwent laminectomy at the T_8-T_{10} vertebral level. The dura mater was split longitudinally and the spinal cord transected with a scalpel. Using gentle suction and iridectomy scissors, spinal cord and meninges were removed to form a gap of 3-4 mm between the rostral and caudal stumps of spinal cord. Following hemostasis, the gap was filled with collagen matrix and the musculature and skin sutured shut in layers. Some animals were killed at weekly intervals from 2 through 6 weeks postimplantation. The implantation site was serially sectioned horizontally and processed for light microscopy (Bodian silver, Gomori's trichrome) or separated into quadrants and processed for electron microscopy. Other implanted animals underwent surgery at 6 weeks to expose the implantation site. Using an operating microscope, the rostro-caudal center of the collagen implant was identified and transected with a scalpel. A piece of filter paper impregnated with HRP (Sigma, Type VI) was placed in the transection site and the incision closed. Forty-eight hours later, animals were killed by intracardiac perfusion with fixatives. Brain, spinal cord and all dorsal root ganglia located within 3 spinal segments of the implantation site were processed for HRP visualization, using the TMB reaction method.

The implantation site revealed typical cavitation formation in the neuropil adjacent to the lesion. A well-vascularized, connective tissue capsule surrounded the collagen implant and the stumps of spinal cord. Many blood vessels and fibroblasts penetrated the implant from this capsule. In addition, small numbers of axons could be seen within the connective tissue capsule and the collagen implant. Within the implant, axons typically were seen near blood vessels and appeared as single entities or as small, tightly-packed fascicles. A few HRP labelled cells were found predominantly within lamina 7-9 from upper cervical through lower lumbar spinal cord. In addition, a small number of HRP labelled cells were seen in brain stem reticular formation and red nucleus. No HRP labelling was seen in any other areas of the brain or in dorsal root ganglia adjacent to the implantation site. Supported by Texas A&M grant #15707.

113.6 THE EFFECT OF AN APPLIED DIRECT CURRENT FIELD ON RECOVERY AFTER SPINAL CORD INJURY: A BEHAVIORAL, ELECTROPHYSIOLOGICAL AND ANATOMICAL STUDY M.G. Fehlings*, C.H. Tator, R.D. Linden* Div. of Neurosurgery and Playfair Neuroscience Unit, Toronto Western Hospital, Univ. of Toronto, Toronto,Ont, M5T 2S8.

Recent work has indicated that direct current (DC) fields may promote the regeneration of transected mammalian spinal axons. In the present experiment, the therapeutic value of an applied DC field was studied in 40 rats with clip compression injuries of the cord at T1. The rats were randomly allocated to one of 4 groups of 10 rats each: two groups received a 17 g cord injury, and two groups a 53 g injury. One group at each injury severity received a treatment (14 μA) DC stimulator and the other group a control (0 μA) stimulator. Clinical neurological function was assessed weekly by the inclined plane technique. At 8 weeks after injury, motor and somatosensory evoked potentials (MEP and SSEP) were performed, and the axonal tracer horseradish peroxidase (HRP) was introduced into the cord at T6. The total number of HRP-labelled cells was counted in every sixth coronal section through the brainstem and motor cortex. All outcome parameters were assessed blindly.

In the 17 g group, there were no significant differences in any outcome measure between control and treated rats. In contrast, in the 53 g group, the inclined plane scores, the amplitude of the MEPs and the numbers of labelled cells in the red nucleus, raphé nuclei, and vestibular nuclei were greater in treated than control rats. These data strongly indicate that an applied DC field can produce functional neurological and anatomical improvement in rats with acute spinal cord injuries.

113.7 REINNERVATION OF SKELETAL MUSCLE VIA VENTRAL ROOTS REPLANTED INTO THE LATERAL COLUMN OF THE RAT SPINAL CORD. J.K. Terzis[1], K.J. Smith[2], M. Eramus[3], and K.A. Carson[4], Depts. of Microsurgery[1], Anatomy[2], & Neurosurgery[3], Eastern Virginia Medical School, Norfolk Virginia 23501

Spinal cord injury often results in a lifetime of dependency due to paralysis of the muscles below the level of the lesion. We have examined the possibility of reinnervating the affected muscles by the direct implantation of ventral roots into the lateral columns.

Sprague-Dawley rats were anesthetized (Nembutal and halothane) and the spinal cord exposed beneath lamina T_{13} by a lateral approach. In 17 rats the L_2 or L_3 ventral root was severed near its exit from the spinal cord and the distal stump replanted into the lateral column via a stab wound. The site of replantation was 3-4 mm caudal to the original position of root exit. In 10 control rats the cord was stabbed, but the distal end of the severed root was displaced from the proximal stump and left free in the cerebrospinal fluid. Eight to twelve months later the affected roots were pale and translucent, the replanted roots were white. Electrical stimulation of thd distal stump of divided replanted roots routinely evoked a prominent contraction of the appropriate muscles, whereas stimulation of non-replanted roots evoked a much smaller contraction. Where anesthetic depth permitted a slight pinch withdrawal reflex, pinching the hindpaws routinely evoked efferent activity in the proximal stump of the replanted root, just as it did in the contralateral control root. In some preparations, light stroking of the ipsilateral hindlimb or tail evoked centrifugal, seemingly somatosensory, discharges in the proximal stump of replanted (but not normal) ventral roots. Stimulation of the proximal stump of replanted roots sometimes activated time-locked units in more caudal ventral roots, and vice versa. Replanted roots were found to contain many regenerated myelinated nerve fibers upon histological examination, while non-replanted roots contained few nerve fibers. Horseradish peroxidase applied to the proximal stump of replanted roots (acutely severed) heavily labeled motoneurons located within 1-2 mm from the replanted site. The results suggest that axons regenerated from the CNS to establish functional contacts with the denervated skeletal muscle. The results may be of value in the reinnervation of muscles below a site of transection or damage of the spinal cord.

113.8 CNS AXONS REGENERATE INTO INTRASPINAL FETAL SPINAL CORD GRAFTS THROUGH CO-GRAFTS OF PERIPHERAL NERVE J. D. Houle Depts. of Neurological Surgery and Neuroscience, Univ. of Florida, Gainesville, FL 32610

Axonal integration between the injured adult central nervous system (CNS) and intraspinal grafts of fetal spinal cord (FSC) tissue often appears limited by glial scarring at the interface between graft and host tissue. The present study examined whether peripheral nerve grafts could be used to circumvent the scar and thereby facilitate the regeneration and interaction of spinal axons with co-grafted FSC tissue.

Both ends of a pre-degenerated segment (2 cm) of autologous tibial nerve were apposed to intermediate gray regions of the adult rat thoraco-lumbar spinal cord. After 2-8 months a spinal hemisection cavity was prepared between the two ends of the PNS graft, the PNS graft was cut at its midpoint and the two new ends were apposed to a suspension of FSC tissue grafted into the cavity. PNS bridges were re-exposed 2-12 months later, cut at their midpoint and all ends soaked in horseradish peroxidase (HRP). Animals were sacrificed after 48 hrs. and the entire spinal cord processed by either the DAB or TMB histochemical reaction. Portions of the PNS grafts were fixed and processed for Epon embedding.

Retrogradely filled spinal neurons (X per animal=650) in Laminae IV-VII were found up to 3 cm away from each PNS graft insertion site into the host spinal cord. Injury-filled host axons extended beyond the PNS graft interface with the FSC transplant to spread throughout the dorsal half of the FSC grafts. Large and small caliber axons grew in a straight course of up to 2 mm within the graft and were mostly unbranched, although a few displayed an extensive arborization that included terminal bouton formation. Neurons in dorsal root ganglia whose roots entered near these sites were also labeled with HRP (X=525), while only a few retrogradely labeled neurons (X=4) were found in the matured FSC tissue. These results indicate that the ingrowth of regenerating host axons into FSC grafts can be promoted by the diversion of these fibers around scars at the graft-host interface by a piece of PNS tissue. The co-grafting paradigm described here provides a model for future study of the dynamics of host axon interaction with FSC grafts from both anatomical and functional perspectives. This work was supported by NIH Grant NS 22316.

113.9 USE OF NATURAL HYDROGELS TO REPAIR THE INJURED RAT SPINAL CORD. S. Woerly, R. Marchand and L. Bertrand, Laboratoire de Neurobiologie, Hôpital de l'Enfant-Jésus, Qué., Canada, G1J 1Z4

Anatomical repair of the transected adult mammalian spinal cord with the aid of biomaterials (tissular or non tissular) requires that the implant material fulfills two essential functions (i) it must provide a bridge that will reestablish a physical continuity between the stumps of the severed spinal cord (ii) it must provide a terrain, mechanically and chemically favorable to the growth of regenerating neurites from the injured ends of the cord. To achieve this issue, we used natural hydrogels as biomaterials replacing lost spinal tissue. Hemiresection cavities and the gap between the stumps of transected thoracic spinal cords of adult rats were infiltrated immediately after injury, either with an ice-cold neutral prepolymerised type I collagen solution (concentration 2.4 mg/ml, Vitrogen, Collagen Corp.) alone or mixed with basement membrane Matrigel (Collaborative Res. Inc.), a biomatrix derived from the EHS mouse sarcoma. In a few cases, fetal rat spinal cord microfragments (E13) were also incorporated in the semi-fluid bioimplants prior to their implantation. Implant recipients were sacrificed one week to 2½ months later and tissues were processed for Gomori trichrome, Luxol-PAS, cholinesterase (Karnowsky and Roots) and immunocytochemistry for neurofilaments. Our results show 1) that self-assembly of collagen fibrils occurs in situ at the temperature of the tissue and results in the formation of a gel-like material. The collagen-matrigel mix forms a denser material 2) the gel completely fills the space offered by the premade cavities and fuses tightly with the healthy spinal tissue yielding an anastomosis between the two extremities of the transected spinal cords 3) the gel is invaded by (i) mesenchymatous cells, astrocytes and a few macrophages, (ii) capillaries and blood vessels (iii) in a few cases, cholinesterase- and/or neurofilament-rich processes from the host tissue extends along the fibrillar structures of the matrices 4) when present, the fetal implants survive within the gel and are intensely stained for cholinesterase 5) after 2½ months, the bioimplants are still well tolerated by the host tissue, and compared to sham, there is no cystic degeneration at the level of the transection and in most cases no scar formation. Hence, the hydrogel-derived collagen polymers that set in situ fulfills the requirements for a suitable material for implantation into the cord since it has the capacity (i) to anchor to the healthy tissue and to bridge resection cavities (ii) to support the growth of both neural elements and blood vessels across the gap of transection. Moreover, such a hydrophylic gel resembles the neural tissue by its consistency. (Supported by the MRC and FRSQ).

113.10 QUAIL-CHICK SPINAL CORD CHIMERAS TREATED WITH ORAL CYCLOSPORINE: 2 CASES. B.G. Uzman, G.M. Villegas, and K. Suzuki. Nerve Structure Research Lab., V.A. Medical Center, Memphis, TN 38104 Inst. de Estud. Avanz., Caracas, Vz. 1015A and U. North Carolina, Chapel Hill, NC 27514

Two quail-chick chimeras (0-1, 0-2) were treated with daily oral administration, not intraperitoneal injection, of Cyclosporin A (Csp) (Sandimmune, oral solution 15 mg/kg) beginning on the 54th post-hatching day (phd). Compared to a series reported by Kinutani and Le Douarin[1] in which 20 of 21 chimeras died with signs of acute spinal cord rejection before 65 phd, with 1 of the 21 surviving 113 phd, the survivals to 106 (0-1) phd and 200 (0-2) dph of the oral Csp-treated chimeras represent significant extensions of expected longevity. In addition, in the series reported by Kinutani and Le Douarin, the first neurologic signs of rejection appeared at 36 phd ± 3 d and inability to stand at 46 phd ± 3 d.[1] Chimera 0-1, initially without detectable motor defects, exhibited drooping of wings at 97 dph and, thereafter, a rapidly deteriorating neurologic course, similar to the Kinutani and Le Douarin chimeras [1,2]. Chimera 0-2 exhibited difficulty in standing and ambulation at hatching and within 12 days could not stand, apparently due to an imperfect graft. At 80 phd 0-2 exhibited a remitting neurologic picture of trembling and difficulty eating (requiring dropper and hand feeding) with recurrence approximately every 20 days for 4-6 day periods. Simultaneously recurring tonic/clonic "seizures" were first noted at 104 phd. Light and electron micrographs of central and peripheral nervous systems of 0-1 and 0-2 show differing degrees and types of cellular infiltration as well as differences in spinal cord fiber tract degeneration. Similarities to and differences from experimental allergic encephalomyelitis or neuritis and multiple sclerosis are notable.

1. Kinutani, M. and Le Douarin, N.M., Devel. Biol. 111:243, 1985
2. Kinutani, M., Coltey, M., and Le Douarin, N.M., Cell 45: 307, 1986

3. The authors acknowledge the assistance of Mary Melvin and Donna Hall; and extend thanks to Prof. Le Douarin and Dr. M. Aimée Teillet for valuable discussions and instruction in quail/chick spinal cord chimera preparation, and to Dr. William Winter, Sandoz, Ltd, East Hanover, N.J. for a generous gift of Csp.

113.11 EFFECT OF DORSAL ROOT GANGLION NEURON PLUS SCHWANN CELL IMPLANTS ON GROWTH OF CORTICOSPINAL FIBERS AFTER SPINAL CORD INJURY IN NEONATAL RATS. K.R. Kuhlengel*, R.P. Bunge, H. Burton and M.B. Bunge (SPON: S. Goldring), Depts. Anat./Neurobiol. & Neurosurg., Washington Univ., St. Louis, MO 63110.

Injury sites were created in the dorsal thoracolumbar spinal cord of neonatal rats to interrupt the pathway of the developing corticospinal tract (CST). These lesioned cavities were filled with explants composed of rat dorsal root ganglion neurons and Schwann cells with or without vascular leptomeningeal cells prepared in tissue culture. The purpose of these implants was to facilitate axonal growth across the spinal injury site. Two surgical protocols were utilized. In one case, implants were placed at the time of lesioning at two days of age; in the second case, lesions were created on the first day of life and the implant was placed four to five days later. Implants placed at the time of surgery survived in 77.4% (48/62) of cases, whereas implants placed at the later time survived in 58.3% (35/60) of cases. Specimens were examined with light and electron microscopy and immunocytochemistry with antibodies against GFAP and laminin. These studies demonstrated that Schwann cells did not migrate into the host parenchyma but that astrocytes invaded the implants diffusely.

Studies were then performed using WGA-HRP to trace the CST and study the effects of these implants on the growth of this tract. In cases in which no implants were placed, CST fibers were either not seen distal to the lesion or were diffusely distributed throughout the gray matter caudally and did not return to their normal pathway in the ventral portion of the dorsal columns. In cases receiving implants at the time of surgery, diffuse labeling was present in the gray matter, with a fasciculated fiber pattern subjacent to the implant; this fasciculated fiber grouping continued caudally beyond the lesion in a corresponding gray matter location subjacent to the ventral portion of the dorsal columns. No fibers were seen passing through the implant. In cases of delayed implantation, a variable effect was seen in which in some cases there was mild fasciculation adjacent to the implant which persisted in this location caudally. In other cases, however, there was diffuse nonfasciculated fiber growth in the gray matter caudally, similar to that seen in non-implanted lesion controls. Thus, these implants did not function as tissue bridges, but appeared to influence the adjacent spinal cord parenchyma (dominated by astrocyte processes) to permit CST fiber fasciculation and passage beyond the lesion site. The effect of these implants was more marked if they were placed at the time of lesioning rather than later. One possible explanation for the accumulation of fasciculated fibers below the implant is the production of Schwann cell trophic factors which diffuse into the adjacent host cord and influence the expression of macromolecules in the glial border. (NIH grants NS09923, NS15070; NS07205.)

NEUROETHOLOGY I

114.1 NEURONAL AND HORMONAL CONTROL OF EGG LAYING BEHAVIORS IN THE POND SNAIL. G.P. Ferguson, A.W. Pieneman* and A. ter Maat*. Department of Biology, Free University, PO Box 7161, 1007 MC, Amsterdam, The Netherlands.

The freshwater pulmonate, *Lymnaea stagnalis* provides a useful model system for analysis of the neuronal and hormonal control of egg laying behaviors. In *Lymnaea* the critical event that determines when egg laying occurs is the discharge of the neurosecretory Caudo Dorsal Cells (CDCs). During this discharge the CDCs fire synchronously and release neuroactive peptides into the blood. One of these peptides is Caudo Dorsal Cell Hormone (CDCH), which causes ovulation of ripe oocytes from the ovotestis.

In the laboratory, egg laying occurs when the CDCs fire "spontaneously", or can be induced by either transferring animals into clean water, or using a fine wire electrode implanted on the CDCs to stimulate them electrically (in the intact animal) and selectively elicit a discharge. Under these conditions the overt egg laying behaviors have four distinct phases. During the first phase (resting phase), locomotion stops (for about 25 min) and the animal assumes a posture with the tentacles curved downwards, the foot contracted and the shell over the head. This is followed by the turning phase, the most active phase of egg laying. During this phase the animal cleans the area where the egg mass will be deposited by performing three appetitive behaviors simultaneously. There is clockwise bending and movement of the head-foot, counterclockwise rotation of the shell and an increase in the number of rasping movements made by the buccal mass. The subsequent oviposition phase (approx 12 min) is the consummatory egg laying behavior, the egg mass leaves the genital pore and is pressed onto the substrate by the mantle edge and pneumostome. Finally, the snail enters the inspection phase and brushes the egg mass with its lips and tentacles.

When ovulation was induced by injections of purified CDCH, the subsequent egg laying behaviors were found to differ from those following CDC discharges. Animals showed no resting phase. Instead, they continued locomotion and then entered directly into the turning phase. This indicates that the presence of only CDCH within the blood is not sufficient to produce the full complement of egg laying behaviors and suggests that the resting phase of egg laying may be dependent on the local action of CDC releasate within the CNS. The other egg laying behaviors of animals with CDCH injections were similar to those which occur after CDC discharges. The neural networks that control movements of the shell, foot and buccal mass of *Lymnaea* have been identified previously and studies are now in progress to determine how these networks are activated by CDC releasate and input from the eggs to produce coordinated egg laying behaviors.

114.2 SPECIALIZED VISUAL RECEPTORS RESPOND TO MAGNETIC FIELD ALIGNMENT IN THE BLOWFLY (CALLIPHORA VICINA). John B. Phillips* (SPON: J. Rosenbaum), Dept. of Biology, Indiana Univ., Bloomington, IN 47405

The optical-pumping model of magnetoreception (Leask 1977, Nature 267: 144) postulates that the magnetic field influences the response of a specialized visual receptor by changing the efficiency of energy transfer from an "antenna" pigment to the photopigment following excitation of the antenna pigment by light. Retinula 1-6 cells of the blowfly (Calliphora vicina) contain an antenna pigment that absorbs near-UV light and transfers energy by a radiationless process to a visual pigment absorbing at longer wavelengths. To determine whether energy transfer in R1-6 cells is influenced by an external magnetic field, the response to a standard flash of light was recorded intracellularly while varying the horizontal alignment of a 1.0 gauss magnetic field. In cells stimulated with 370 nm light (absorbed preferentially by the antenna pigment), responses that showed a slight decrement when compared to "zero field" control responses were consistently associated with alignments of the magnetic field that formed a bimodal or quadramodal pattern. Both patterns are axially-symmetrical (i.e., independent of the polarity of the magnetic field) which is a requirement of the optical-pumping model. In contrast, when R1-6 cells were stimulated with wavelengths of light absorbed by the photopigment directly, there was no consistent pattern of response. These findings suggest that the alignment of a 1.0 gauss magnetic field has a weak, but reproducible, effect on energy transfer in R1-6 cells. Similar bimodal or quadramodal patterns of response have been found in behavioral studies of magnetic orientation in several insects.

Under natural conditions, small changes in energy transfer efficiency would not be detectable in R1-6 cells because of masking due to direct excitation of the photopigment by light. However, in the R7(y) cell, also containing a UV-absorbing antenna pigment, sensitivity of the blue-absorbing photopigment to direct absorption of light is greatly reduced by an optically-dense screening pigment. Thus, the R7(y) photopigment is primarily sensitive to energy transfer from the antenna pigment. UV-sensitive R7(p) and blue-sensitive R8(p) cells provide additional inputs necessary to determine the proportion of UV light absorbed by the R7(y) antenna pigment that is transferred to the photopigment, and to factor out the component of R7(y) response due to direct excitation of the photopigment by light bypassing the screening pigment. These three cells are proposed to form a specialized receptor system that is responsible for magnetoreception in the blowfly, detecting small changes in the efficiency of energy transfer in R7(y) that indicate the relative alignment of the earth's magnetic field.

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114.3 CHANGES IN WING PARAMETERS IN TELEOGRYLLUS OCEANICUS DUE TO ULTRASONIC STIMULI. Michael L. May and Peter D. Brodfuehrer. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

This work describes three wing-related effects in the Australian field cricket (Teleogryllus oceanicus) in response to an ultrasonic stimulus. While previous work on the effects of ultrasound, delivered during flight, showed negative phonotaxis with emphasis on the abdominal "rudder," this study reveals characteristic changes in the movements of both fore and hindwings: 1) a lift (i.e., increased angle of elevation) in the forewing ipsilateral to the stimulus; 2) a drop in the forewing contralateral to the stimulus; and 3) a "hitch" or momentarily decreased beating frequency in the hindwings. Each of these responses is consistent with the hypothesis that negative phonotaxis represents an escape behavior from echolocating bats.

Both forewing lift and drop are linearly related to the sound level intensity as determined from photographs of flying crickets tethered upright although the lift is larger in magnitude than the drop. To the best of our knowledge, wing lift has only been described as a steering movement in a mantis species (Yager, D.D.and Hoy, R.R., Soc. Neurosci. Abstr. 12:202, 1986). The contralateral forewing drop is of a relatively small magnitude and may, therefore, be due largely to pronation. Such pronation in turning has been described in the locust (Dugard, J.J., J. Insect Physiol. 13:1055-1063, 1967) and suggested in T. oceanicus (Pollack, G.S. and Hoy, R., J. Insect Physiol. 27:41-45, 1981).

The hindwing hitch was observed via both a light detector method and electromyographic recordings from muscle # 129. Further, it is a bilateral event. In a flying tethered cricket beating its wings at approximately 25 Hz, an ultrasonic stimulus induces a momentary drop in beating frequency to about 10 Hz. Within 2-3 wing cycles, the original frequency is recovered. As this response habituates rather quickly with multiple stimuli, it may be a startle response.

These responses further substantiate the potential of negative phonotaxis as a bat avoidance behavior. A startle response, such as the hindwing hitch, is not uncommon in an animal presented with a noxious stimulus. Further, the forewing responses would likely induce a turn away from the source of the sound stimulus. Although it is presently undetermined, the linearity of the forewing responses is likely to produce a turn which is also graded with sound level intensity. (Supported by NIH Grant #5T32GM07469.)

114.4 Non-Synaptic Regulation of Sensory Feedback During Movement Ronald S. Goldstein*, Frederic Libersat*, Jeffrey M. Camhi Dept. Zoology, Hebrew University, Jerusalem, Israel (SPON: M. Devor)

Movements of an animal produce sensory feedback, not all of which is useful. Potentially perturbing feedback is sometimes inhibited synaptically, either peripherally or centrally.

In cockroaches, flying produces strong wind feedback to the cerci, paired wind-responsive sensory appendages. When not flying, the cockroach uses its cerci to evoke well studied escape responses to very gentle air currents generated by approaching predators. The wind feedback during flight could therefore habituate the escape response. While flying, the cockroach moves its cerci by 45-60° about their joint with the body, and this cercal displacement reduces the sensory response to wind, by a novel, apparently non-synaptic mechanism (Libersat et al., this volume). This abstract describes the effect of this sensory reduction on wind-responsive interneurons, some of which mediate the escape response.

Controlled wind puffs from the front, presented when the cerci were in their rest position, elicited repeatable responses from the medial cercal nerve, the nerve containing wind-sensitive afferents from the cercus. However, when the cercus was displaced by 45-60° and held parallel to the body, and the wind stimulator was rotated to retain the same angle of stimulation, the wind-evoked response was reduced. The reduction, a mean of 34% occured in each of the nine animals tested. This reduction was repeatable within the same animal for as long as was tested (3 hours) as determined by repeatedly displacing and releasing the cercus.

In order to determine the effect of this sensory reduction on wind-sensitive interneurons, we recorded from these interneurons simultaneously with the sensory axons. When the cercus was displaced and produced typical sensory reduction, the wind-evoked interneuron response was reduced by 40% (six animals).

We examined the possible contribution to the depression of the interneuron response from two other sources besides the displacement-induced reduction of sensory activity. First, a chordotonal organ sensitive to lateral cercal displacements can apparently produce large IPSP's in some of the wind-sensitive interneurons (Bernard J. et al J. Comp. Phys. 153:377). This sensory structure could therefore contribute to depression of the interneuronal wind response when the cercus is displaced. Second, some information about the cercal displacement may ascend the nerve cord and elicit known descending inhibitory signals (Daley D. and Delcomyn F. J. Comp. Phys. 138:231) reducing the interneuronal response. We tested these two possibilities by: 1) cutting the lateral cercal nerve containing the afferent fibers from the chordotonal organ, and 2) cutting the nerve cord above the abdominal ganglia to prevent descending inhibition. No significant changes in the reduction of either sensory activity or interneuronal activity were found with cutting these nerves in any combination.

It appears, therefore, that the non-synaptic suppression of sensory activity produced by cercal displacement can account for the entire reduction of wind-evoked activity in the nerve cord that occurs in the interneurons. We are now examining the possibility that this reduction serves to protect the escape response from habituation.

114.5 MECHANISM OF NON-SYNAPTIC REGULATION OF SENSORY ACTIVITY DURING MOVEMENT Frederic Libersat*, Ronald S. Goldstein*, Jeffrey M. Camhi (SPON:A.F.Mirsky). Dept. Zoology, Hebrew University, Jerusalem, Israel

When a cockroach flies, it generates strong winds. Such winds could habituate the escape circuitry, normally excited in a non-flying cockroach by gentle wind gusts. These escape-inducing stimuli are detected by the cerci, two sensory organs located in the posterior abdominal region. However, during flight, the cockroach displaces the cerci, a movement that causes a reduction in the sensory response to wind (Goldstein et al., this volume). This abstract examines the mechanism of this sensory reduction.

Controlled wind puffs from the front, presented when the cerci are in their rest position, elicit very repeatable responses of the wind-sensory cells. We moved a cercus in a manner resembling its movement during flight and rotated the wind-tube to retain the same angle of stimulation. Now the wind-evoked reponse was reduced by a mean of 34% or 48% (extra-cellular and intra-cellular recordings repectively).

The same reduction in sensory activity occured when the animal displaced its cerci in reponse to stimulation of the motor axons in the lateral cercal nerve. Moreover, when semi-intact preparations made rhytmic wing movements, accompanied by cercal displacement, the same reduction of sensory activity occurred.

The sensory reduction did not result from displacement of the cercus to a position where the wind was blocked by other body parts or by the recording apparatus; when a pin was placed medial to the cercus, preventing its movement upon stimulation of the lateral nerve, the same reduction still occured.

Inhibition emanating from the central nervous system can be ruled out as the source of the reduction in sensory activity; cutting near the last abdominal ganglion the two nerves that provide the only innervation of the cercus and surrounding tissues leaves the reduction unaffected.

Two experiments demonstrate that the reduction takes place near the point where the sensory nerve emerges from the cercus. First, recordings from the nerve within the cercus near its base show no significant reduction upon cercal displacement, while simultaneous recordings from the nerve inside the body cavity show a typical reduction. Second, when the cercal nerve was dissected free from its surrounding tissues near the cercal joint, the wind response recorded central to the joint was not reduced by displacing the cercus.

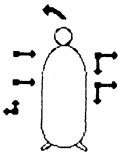
In order to test the possible role of "en-passant" synaptic inhibition on to the sensory axons, we recorded sensory activity using 0 Ca++ saline. In each of six experiments, this saline did not block the displacement-induced sensory reduction; yet, as a control, this saline did block sensory-to-giant interneuron synapses.

Intracellular recordings from sensory axons indicate that when the cercus is displaced, that the axonal core resistance and/or the extracellular resistance increase in the region of the cercal joint. This could contribute to the the blockage of sensory action potentials, and thereby to the observed sensory reduction.

In summary, this mechanism of sensory reduction is entirely peripheral, and apparently non-synaptic, being based upon mechanical stress on the sensory nerve that blocks some action potentials at the joint of the cercus with the body. The raises the possibility that body movements in general might impose similar stress on peripheral nerves, and thereby alter impulse traffic.

114.6 LEG MOVEMENTS AND THEIR CONTROL IN THE ESCAPE TURNS OF COCKROACHES J.M. Camhi and A. Levy*. Dept. Zoology, Hebrew University, Jerusalem, Israel.

Cockroaches respond to the minute wind gust made by the approach of a predator by turning rapidly away and then running. In spite of intensive work on the sensory and central control of this escape behavior, relatively little is known about the actual leg movements that produce the turn. We have filmed at 250 frames/sec turning responses of cockroaches to controlled wind stimuli, and carried out a computer graphics, frame-by-frame analysis of the movements. Our program calculates for each frame: 1) The body angle relative to wind direction; 2) for each leg, whether the tarsus is stationary or moving relative to the ground, (defining stance vs swing phases, respectively); 3) the vectorial anterior-posterior (A-P), and 4) medial-lateral (M-L) components of each leg's movement relative to the body.



Right front wind stimuli initially elicit usually a swing phase in the right hind leg and a stance phase in all five remaining legs. This differs from running, in which three legs are in stance and three in swing at any moment. This running pattern is established by the second or third step of the turn. The mean A-P and M-L movement vectors for each leg in stance during the starts of left turns evoked by wind from the right is shown in the figure.

These data suggest, first, that the initial movements of the hind legs (right leg swing, left leg small movements) contribute little to the strong left turn. This turn appears to result mainly from an inward pull by the left, and an outward push by the right, front and middle legs. In fact, if one computes the body angle that would result from only the M-L component of the front and middle legs, this accounts for over 75% of the angle of the turn. The left hind leg's action may serve to provide a pivot point, as is further suggested by this leg's remaining in stance for the first few stepping cycles of the other legs. The posterior movement of the right front and middle legs may represent largely a by-product of a left turn about a posterior pivot point, rather than a posteriorly directed force by these legs.

We have found that essentially the same initial leg movements and body turn are made in response to wind by cockroaches whose posterior abdomen is pinned in place and whose legs are free to move on a slick surface. This permits physiological recordings to be made during turning behavior carried out in place. However, dissected preparations show weak or no behavioral responses to wind stimuli. Therefore, we have developed techniques for implanting cuff electrodes around both abdominal and thoracic connectives and re-closing the body. In this way we record apparently normal interneuronal activity during turning behavior. In the thoracic connectives, the axon diameters of the much studied giant interneurons are small, permitting their small action potentials to be discriminated from larger action potentials of larger interneurons. Some of these latter are excited by the giant interneruons and may comprise a command for coordinated movement of all the legs. These studies are helping to define and analyze this command.

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114.7 NEURAL CONTROL OF FLEXIBLE BODY DYNAMICS DURING THE C-START. R. DiDomenico*, J. Nissanov*, and R. C. Eaton. Department of Biology, EPO Box 334, Univ. of Colorado, Boulder, CO 80309.

This study continues our functional analysis of a pair of identified medial reticulospinal neurons, the Mauthner cells (M-cells) in the brain stem of teleost fishes. These cells are involved in producing a rapid escape response (the C-start) to predatory attacks. In this study we used a matrix camera, a high-speed digital imaging device for rapid acquisition and quantification of multiple behavioral trials. Bilateral EMG recordings were taken from the body musculature as an indication of the neural commands underlying the production of the behavior in response to a displacement stimulus.

Our study shows that the following features are required for an adequate neural explanation of the C-start: 1) inherent flexibility of the behavior pattern, which is expressed by the variety of possible trajectories and response strengths, and 2) invariant coupling between the various mechanical stages of the movement pattern. There are minimally two neural signals to be considered. These are revealed by their EMGs. The first of these signals triggers the initial C-like contraction of the response known as Stage 1. When the M-cell is present, it seems to participate in the production of the Stage 1 signal but we know from previous studies in which the M-cell has been lesioned that it is not indispensible for this component. The Stage 1 EMG is variable in duration and provides a sustained impulse that seems to code for turning angle of Stage 1. A second signal appears later in time on the side opposite the initial contraction and appears to code for the onset of the propulsive component of the behavior, Stage 2. Of the Stage 1 and 2 signals, the M-cell is included in the first, but Stage 1 does not occur in isolation from Stage 2 in freely-behaving animals. Instead, the two signals are inextricably linked in the production of the behavioral response. This is the minimal naturally occurring unit of the behavior, and our study shows that it requires complex neural processing in addition to that triggered only by the M-cell.

Two hypotheses have been suggested for the role of the M-cell in the C-start. One view [Hackett and Greenfield (1986) Behav. & Brain Sci. 9:729-730] implies that the behavior is mediated by a rigidly stereotypic system that produces an invariant output. In contrast, the present paper suggests a dynamic regulatory process that tunes the continuity of the motor performance to a variable stimulus environment. [Supported by NIH grant NS22621]

114.8 A SEXUALLY DIMORPHIC SONIC (ACOUSTIC) MOTOR SYSTEM IN A TELEOST FISH: PERIPHERAL AND CENTRAL ELEMENTS. A. H. Bass and M. A. Marchaterre. Section of Neurobiology & Behavior, Cornell University, Ithaca, N.Y. 14853.

One system controlling the generation of acoustic communication signals is the sonic motor system of marine teleost fishes. In some species, the effector organ consists of striated muscles that form the lateral walls of the swimbladder. The contraction rate of the "drum" muscles, which is under the control of a central sonic motor nucleus, determines the spectral features of the acoustic signals. In the midshipman, Porichthys notatus, there is a behavioral sex difference in that only males are known to produce sounds during the breeding season. We have now discovered a dramatic sex difference in the gross and fine structure of the swimbladder drum muscles of adult specimens. There is a highly significant sex difference ($p<0.0001$, $T=7.61$, $DF=18.5$) in the mean value of the ratio of swimbladder weight to body weight (sb/bw=0.030 for males, 0.018 for females). The bladder of males is heart-shaped with two large bands of muscle along its lateral margins; the female bladder is more conical-shaped with two small dorsolateral bands of muscle. The muscle of males also has a deep red coloration while that of females has a pale yellowish-white tint. At an ultrastructural level, there is a sex difference in individual myofibrils of sonic muscle: the width of Z bands in males are 10-15 fold that found in females, or the body wall muscle of males and females, or the swimbladder drum muscles of other sonic teleosts such as toadfish, Opsanus and sea robins, Prionotus. The sarcoplasmic reticulum of the sonic muscle is also hypertrophied in male midshipmen in comparison to females.

Given the extreme sex difference in sound production and the peripheral sonic swimbladder and its intrinsic muscles, we want to know the central structure-function correlates associated with these differences. Both males and females have a sonic motor nucleus. At an ultrastructural level there are several classes of terminal boutons in both males and females: axosomatic gap junctions associated presynaptically with pleomorphic vesicles, axosomatic chemical synapses associated with round or pleomorphic vesicles; axodendritic chemical synapses associated presynaptically with round vesicles. So far, axodendritic synapses with pleomorphic vesicles have been found only in males. While a limiting factor in sound production may indeed lie with the sonic muscles, the central neurophysiological correlates of the behavioral and structural sex differences await definition.

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114.9 ELECTROLOCATION IN THE PRESENCE OF JAMMING SIGNALS: BEHAVIOR AND ELECTRORECEPTOR PHYSIOLOGY. J. Bastian. Dept. of Zoology, Univ. of Oklahoma, Norman, OK 73019. The electrolocation behavior of Apteronotus leptorhynchus was studied in the presence of various interfering or 'jamming' electrical signals. Electroreceptor afferent responses to the same moving electrolocation targets (metal cylinders) were also studied in the presence of the same jamming signals, allowing comparisons to be made of the effects of jamming on the behavior and on the receptors' responses. Broad-band noise, high frequency sinusoidal signals maintained at a given frequency difference (DF) from the fish's electric organ discharge frequency, and low frequency (5 or 50 Hz) sinusoidal signals were used to jam the electrosensory system. The latency of the fish's responses and the minimum distance maintained between the moving targets and the fish were used to estimate electrolocation performance.

Both noise and DF jamming had strong deleterious effects on electrolocation performance, and threshold amplitudes for DF jamming were about three-fold lower than thresholds for noise jamming. The rates of change of jamming effectiveness as a function of increasing jamming intensity were, however, not different for noise and DF. Response latency increased by 78 and 84 ms per 10 dB increase in the rms amplitude of noise and DF signals respectively, and minimum fish-target distance decreased by 0.21 and 0.24 cm per 10 dB increase in noise and DF respectively. Neither the 5 nor the 50 Hz stimuli, which are expected to primarily stimulate the ampullary receptor system, were effective in causing significant electrolocation response deterioration when presented alone. If, however, the 5 Hz stimulus was presented simultaneously with either the noise or DF stimulus, then the low frequency jamming augmented the response deterioration. This suggests that the ampullary system might provide supplementary information utilized by the fish when normal cues received over the tuberous system are interfered with.

Receptor afferent responses to the same electrolocation targets were more severely disrupted by the jamming signals, and both the threshold and the rate of increase of receptor response decrement as a function of increased jamming signal amplitude were significantly greater for DF jamming as compared to noise. Estimates of electroreceptor threshold distance decreased by 0.53 and 0.8 cm per 10 dB increase in noise and DF jamming respectively. 5 Hz jamming signals had no effect on tuberous receptor afferent responses, but this form of jamming did severely disrupt the responses of ampullary afferents to the galvanic potentials produced by the metal targets.

These results suggest that CNS mechanisms exist which improve the signal to noise ratio of relevant signals, and that these mechanisms are better able to reject disturbances created by DF jamming as compared to those resulting from noise. Supported by NIH grant NS12337.

114.10 SUPPRESSION OF SELF-GENERATED ELECTROSENSORY INTERFERENCE VIA A COMMISSURAL MEDULLARY PATHWAY IN THE LITTLE SKATE J.G. New and D.Bodznick, Dept. of Biology, Wesleyan University, Middletown, CT 06457.

Common mode electrosensory interference generated by respiratory activity in skates and rays may pose a significant neuroethological problem when the animal attempts to locate and orient to weak environmental electric fields. Previous studies demonstrate that while activity of electrosensory afferents in the anterior lateral line nerve (ALLN) is strongly modulated during ventilatory movements, ascending efferent neurons (AENs) within the dorsal octavolateralis nucleus (DON) are only weakly affected by ventilation (Montgomery, 1984; New & Bodznick, 1986). Direct evidence for active suppression of ventilatory electrosensory interference in the skate DON has been previously reported (New & Bodznick, 1986). The current study provides evidence that in skates this suppression is achieved by a commissural common-mode rejection mechanism and not by a ventilatory efference copy mechanism.

Extracellular recordings of ALLN fibers and AENs were made in little skates, Raja erinacea, that were decerebrate and either completely paralyzed by curare or spinalized to permit normal ventilation. AENs were identified by antidromic responses to lateral mesencephalic nucleus stimulation. In curarized animals AEN activity and responsiveness were examined for evidence of modulation by a ventilatory efference copy signal. The ventilatory motor burst in the hyomandibular nerve served to indicate the timing of fictive ventilation. However, in 18 AENs no modulation of ongoing activity phase related to the ventilatory burst was observed. Furthermore, AEN responses to weak electric fields were the same regardless of which point in the respiratory cycle stimuli were presented. Thus an efference copy mechanism is not supported.

In spinalized animals, transection of the contralateral ALLN caused a mean 93% increase in AEN modulation during ventilation. Furthermore, the responses of AENs to weak electric fields were diminished by a mean of 50% when the transected contralateral ALLN was driven via a train of stimulus pulses. These experiments argue that the commissural pathway mediates the suppression of common-mode electrosensory interference. The involvement of the commissural pathway in interference suppression may prove a useful comparative feature in investigations of medullary processes. Supported by a Sigma Xi Grant-in-aid to JGN and an NIH grant to DB.

114.11 A VERTEBRATE COMMUNICATORY BEHAVIOR ELICITED IN VITRO: CHIRPING IN THE WEAKLY ELECTRIC FISH, APTERONOTUS. J. Dye. Dept. of Neurosciences & Neurobiology Unit, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

Apteronotus is a weakly electric gymnotiform fish possessing a 'wave-type' electric organ discharge (EOD). The EOD is maintained continuously at a remarkably stable frequency. In addition to subserving electrolocation, the electrosensory system is a medium of intraspecific communication. Several modulations in the EOD frequency that are performed by these fish have been characterized at the behavioral and physiological levels. Chief among these is the jamming avoidance response (JAR), a slow, smooth shift in frequency away from that of a nearby conspecific to prevent impairment of electrolocation. These animals also have a repertoire of communications involving modulation of EOD frequency. The most salient of these is the chirp, a large, rapid acceleration used as a signal during aggression and courtship. Chirping, like the JAR, can be elicited in an animal during acute electrophysiological recording.

The EOD is commanded by a nucleus on the ventral aspect of the medulla, the pacemaker nucleus. It is composed of pacemaker cells, which are interneuronal and possess underlying pacemaker potentials, and relay cells, which project axons down the spinal cord to trigger each EOD cycle. We have previously recorded from both types of neurons in vivo and characterized distinct responses in histologically identified loci during naturally evoked chirps (Dye & Heiligenberg, 1987, J Comp Physiol, in press).

This presentation reports the reproduction of this behavior in an in vitro pacemaker preparation. The pacemaker nucleus fires at its characteristic frequency after removal from the brain and placement in a slice chamber. By retaining in the brain slice a large extent of tissue rostral to the nucleus, one which includes the sole afferent pathway to the pacemaker from the prepacemaker nucleus, characteristic chirps can be elicited with bipolar stimulation of this tract. These chirps, while briefer and somewhat stronger than those occurring in the animal, display the same distinctions between cell types and loci as those described in experiments in vivo. Moreover, a phase-dependence of the efficacy of afferent stimulation has been found, identical to that which has been seen in response to single prepacemaker cell spikes in vivo. The suggestion of chemical transmission from prepacemaker afferents, based thus far on anatomical studies, is further supported by the demonstration of frequency-dependent facilitation, blockage by manganese, and transient changes in input impedance during the chirp acceleration. Finally, brief trains of stimuli cause longer-lasting accelerations as well, which may be caused by excitation of those fibers responsible for the JAR.

114.12 CENTRAL INTEGRATION OF MECHANORECEPTIVE LATERAL LINE INFORMATION IN THE THORNBACK GUITARFISH, PLATYRHINOIDIS TRISERIATA (ELASMOBRANCHII). H. Bleckmann*, O. Weiss* and T.H. Bullock. Neurobiology Unit, Scripps Inst. of Oceanography & Dept. of Neurosciences, Sch. of Medicine, Univ. of California, San Diego, La Jolla, CA 92093.

The mechanoreceptive lateral line of P. triseriata is represented at all levels of the neuraxis, from medulla to telencephalon (Bleckmann, Bullock & Jørgensen, J. Comp. Physiol., in press). In the present study we have investigated in the same species the physiological properties of mechanoreceptive lateral line areas, from medulla to telencephalon, using averaged evoked potentials (AEPs), few unit and single unit responses as windows to brain functions. Recordings were analyzed with respect to frequency sensitivity, dynamic response properties, response latency, receptive field (RF) organization, and interaction with light flash, electric field and vibration. All lateral line areas encountered were sensitive to water movement. Best frequencies (BF) in terms of displacement vary between 75 and 250 Hz with threshold values as low as 0.02 um peak-to-peak, calculated at the skin, in the most sensitive units. For the frequencies 25, 50, and 100 Hz, the dynamic range of each lateral line area was tested and found to vary from 30 to at least 65 dB. Response latencies increase with increasing stimulus rise time and decreasing stimulus frequency below BF. Responses recorded in the medullary medial octavolateralis nucleus are phasic-tonic, i.e. the responses are strongest at stimulus onset but last for the whole stimulus duration in the form of a frequency following response (FFR) similar to that known for acoustic stimuli. The lateral line FFR persists after bilateral section of n. VIII. In contrast, unit responses recorded in the midbrain and the diencephalon are in most cases phasic. Responses recorded in the medulla are phase coupled to the stimulus; those recorded in the midbrain or forebrain are usually not. Units tested in several lateral line areas showed small well defined RFs. RFs are mostly contralateral to the recording side and usually in the anterior third of the fish. No obvious interaction of modalities was seen except in posterior central thalamic and anterior midbrain nuclei. AEPs in the telencephalic hot spot show positive peaks at 65, 200, and 360 ms with optimal stimuli and require stimulus intervals of 20 s or more.

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114.13 ISOLATION - DEPENDENT ACTIVATION OF FOUR FOREBRAIN AREAS IN THE ZEBRA FINCH.
H.-J. Bischof* and K. Herrmann* (SPON: European Neuroscience Association). University of Bielefeld, Dept. of Ethology, POB 8640, D-4800 Bielefeld 1, F.R.G.

In a previous study (Bischof, H.-J. and Herrmann,K., Beh.Brain Res.,21:215,1986) we demonstrated that four forebrain areas of zebra finch males are activated in situations which arouse the animal, for example when chasing the birds around the cage, or when exposing them to a female. These areas, the Hyperstriatum accessorium (HA), a part of the medial Neostriatum (Nint), the lateral Neostriatum (Nlat), and a portion of the caudal Archi-Neostriatum (A+Ncaud) show an enhanced 14C-2-Deoxglucose (2-DG) uptake according to the experimental situation. On the basis of these experiments, we examined whether the activation of these areas is correlated with motor activity and is influenced by different isolation times prior to the 2-DG experiment, where courtship of the male birds was elicited by exposing them to a female zebra finch. For this purpose, we isolated male zebra finches for 1 day, 1 week, or 8 weeks, respectively, before we injected the 2-DG and exposed the birds to a female. During the experiment, besides other activities the number of song motifs performed by the bird and the frequency of changing the perches was recorded. Our experiments demonstrate that there is a negative correlation between motor activity and 2-DG uptake, and a positive correlation between isolation time and 2-DG uptake. We suggest that long isolation blocks courtship behavior by some unknown mechanism, and that the "internal drive" of the animal, which possibly corresponds with the activity of the four forebrain areas, is enhanced by isolation and by the fact that the birds do not perform the consummatory behavior, which would lead to a reduction of the "internal drive level". Our results also demonstrate that the 2-DG method can show up small differences in the internal state of an animal which cannot be easily detected by behavioral measurements.

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114.14 PERIORAL STIMULATION FROM PUPS: ROLE IN NORWAY RAT MATERNAL RETRIEVAL, LICKING, CROUCHING AND AGGRESSION. J. M. Stern and J. M. Kolunie. Depts. of Psychology and Biological Sciences, Rutgers University, New Brunswick, NJ 08903.

The rat's snout is an obvious choice for reception of motivating and guiding cues from pups. Major deficits in pup retrieval were found following acute mystacial pad anaptia with lidocaine anesthesia (Kenyon et al., Physiol. Beh., 27:313, 1981) or infraorbital denervation (Kenyon, et al., Behav. Neurosci., 97:255, 1983). We now report the results of 3 studies in which primiparous postpartum (PP) rats were injected with mystacial pad lidocaine (LIDO) or saline (SAL) 30 min prior to testing.

Retrieval: On Days 2, 3, and 4 PP, retrieval of 5 pups in 5 min in the home cage increased from 5/8 to 8/8 in the SAL group, whereas 1/8 LIDO dam retrieved, on Day 4 only. In contrast, after a Day 2 practice session in the retrieval apparatus (light and dark compartments), 11/11 SAL and 8/10 LIDO dams retrieved on Day 3 PP, but the latency and duration were 3 and 2 times longer for LIDO dams. With no prior practice, 8/8 SAL vs. 1/8 LIDO dams retrieved in the test apparatus.

Licking (L), Handling (H), and Crouching (CR): After the home cage retrieval tests, onset of CR was 3-4 times longer for LIDO than SAL dams. LIDO dams also were greatly impaired in CR onset following a 6 hr mother-litter separation, even when retrieval was not required because pups were placed in the nest. The impairment was associated with markedly decreased L & H and was greater at 2 days than at 2 weeks PP, because older pups initiate nursing away from the nest. At 60 min post-reunion, pup weight gain was markedly reduced in the LIDO condition.

Materral Aggression (MA): Whereas all SAL dams attacked the strange male intruder (35-55 days old) at least once, MA was almost completely eliminated in LIDO dams. This effect was not altered by prior MA experience. Less MA was seen in the retrieval apparatus, but the group difference remained.

Comments & Conclusions: (1) In all these tests, initial sniffing (of pups, of male intruder) was not impaired, and duration often increased as dams failed to proceed in the usual sequence of behaviors. (2) Self-grooming was often increased, indicating that mouth opening and tongue protrusion are not impaired. (3) Whether or not retrieval occurs during acute perioral anesthesia is highly dependent upon an interaction of experiential and testing conditions, whereas MA seems to be disrupted more completely. (4) CR onset appears to require the dam's proximity to pups long enough for their initiation of suckling to take place.

115.1 PHENTOLAMINE SUPPRESSION OF IN VITRO POTASSIUM INDUCED LHRH RELEASE FROM THE BOVINE INFUNDIBULUM. G.A. Dissen*, P.G. Harms*, D.D. Zalesky* and N.H. McArthur. Departments of Animal Science and Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

The influence of phentolamine on infundibular LHRH release in response to a 60 mM Potassium (K) challenge in eight 2-year-old steers was examined. The steers were exsanguinated and decapitated under deep xylazine anesthesia. The infundibulum was split into right and left halves and then dissected from the hypothalamus at the tuberoinfundibular sulcus. The tissues were weighed and placed in 0.5 ml perifusion chambers. The tissue was perifused with Krebs-Ringer Bicarbonate media saturated with 95% O_2 and 5% CO_2 (with 5.5 mM glucose and 0.06 g/l bacitracin) at 100 µl/min. The perifusate was fractionated at 10 min intervals for 360 min. Each fraction was assayed for LHRH and expressed as pg/ml·mg of tissue weight. The left or right infundibular halves were randomly assigned to either media with phentolamine (0.5 mM) from 0 to 180 min or to control perifusion media (no phentolamine). Tissues were perifused for 120 min to establish basal LHRH levels. Tissues were exposed to 60 mM K in the perifusion media (60 mM KCl in equimolar substitution for NaCl) for 30 min at 120 min and 240 min. Area under the curve (AUC) was calculated using the trapezoidal rule. Response to 60 mM K perifusion media was calculated as the AUC above baseline. All comparisons were paired and tested with the Student's paired t test. All comparisons presented were different ($P<.05$). Response to the K challenge was less in the phentolamine treated halves, 9.4 ± 2.2 compared to control 59.0 ± 9.3 (AUC arbitrary units ± SE). There was a partial return of responsiveness by 240 min, 21.9 ± 5.5 for phentolamine treated and 44.3 ± 8.0 for control. While the responsiveness of phentolamine treated halves was less than controls at the second challenge (240 min), the phentolamine treated second K challenge was greater than the first (120 min) phentolamine treated K challenge (21.9 ± 5.5, and 9.4 ± 2.2, respectively). However, in control tissues the response to the first K challenge was greater than the second K challenge (59.0 ± 9.3 and 44.3 ± 8.0, respectively). In addition to changes in responsiveness, the baseline in phentolamine treated tissues was greater (21.4 ± 7.3 vs 6.8 ± 1.9) than control tissues, thus contributing to the decreased responsiveness. Phentolamine increased the basal in vitro release of LHRH while it reduced the response of infundibular tissues to K.

115.2 MEASUREMENT OF BASAL AND STIMULATED MONOAMINE RELEASE IN FEMALE RAT HYPOTHALAMUS USING IN VIVO DIALYSIS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. I. Vathy* and A.M. Etgen. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Med., Bronx, NY 10461.

The development of intracranial microdialysis methods has provided a valuable tool to study the release of endogenous neurotransmitters in living animals. In vivo microdialysis combined with high performance liquid chromatography (HPLC) and electrochemical detection (ECD) were used in the present experiments to measure extracellular monoamine concentrations in the ventromedial hypothalamus (VMH) of female rats. Urethane-anesthetized animals were implanted stereotaxically with a loop-type dialysis probe (Ungerstedt et al., 1982, Neurosci. Lett., Suppl. 10. 493). The probe was perfused at a flow rate of 2ul/min. with an artifical cerebrospinal fluid (CSF) composed of 147.2 mM NaCl, 2.0 mM $CaCl_2$, 2.76 mM KCl, 0.62 mM K_2HPO_4 with 0.113 mM ascorbic acid (to prevent oxidation of monoamines). In vitro calibration of the dialysis probe demonstrated a 5-10% recovery of monoamines. The collected dialysate (25 min. samples) was injected directly into a HPLC outfitted with ECD to evaluate the concentration of extracellular monoamines.

Under basal conditions detectable levels of norepinephrine (NE) 3,4-dihydroxyphenyl acetic acid (DOPAC) and 5-hydroxyindole acetic acid (5-HIAA) were found. Extracellular levels of all three monoamines remained stable for several hours. To monitor depolarization-induced monoamine release, the dialysis probe was switched for 5 min. to an artificial CSF containing elevated potassium levels. The potassium stimulus increased extracellular NE levels, decreased 5-HIAA and had no effect on levels of DOPAC. Since the VMH is a major site for ovarian steroid regulation of female reproductive physiology, in vivo dialysis combined with HPLC-ECD provides a direct method to evaluate the role of monoamine neurotransmitter systems in the hormonal regulation of neuroendocrine function.

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115.3 **PRAZOSIN BLOCKS α_1 ADRENERGIC STIMULATION OF LHRH RELEASE FROM SUPERFUSED RAT HYPOTHALAMUS.** J.F. Gitzen, and V.D. Ramirez, Neural and Behavioral Biology Program and Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL. 61801.

The in vitro pulsatile administration of the α_1 adrenergic agonist Methoxamine (MTX) stimulates the release of LHRH from superfused female rat medial basal hypothalami (MBH) (Endo. Soc. Abstracts, 1985, #945).

The present experiments examined the specificity of this presumed α_1 effect. The specific α_1 antagonist Prazosin was used. Five treatment groups were run: 1) Pulsatile MTX. 2) Pulsatile MTX + continuous Prazosin. 3) Continuous Prazosin only. 4) Continuous MTX only. 5) Untreated controls. Doses were 10^{-5}M MTX and 10^{-4}M Prazosin. OVX unprimed rat MBH were used.

MBH were first superfused with Krebs-Ringer Phosphate medium for one hour to establish baseline LHRH release. In groups 1&2, MTX was infused 3 times, 20 min each, one hour apart. In groups 2&3, the medium contained Prazosin during the baseline and all subsequent periods to blockade α_1 receptors. Effluents were collected at 10 min intervals and assayed for LHRH by RIA.

As previously shown, 10^{-5}M MTX (N=8) significantly stimulated LHRH release in each of the hours following the three MTX treatments, increasing from 11.6±4.1 pg/baseline hour to 17.6±2.6, 18.9±3.2, and 21.1±3.2 pg/hour during the three MTX treatment hours, respectively. In contrast, 10^{-4}M Prazosin (N=7) completely blocked the stimulatory effect of MTX: LHRH release fell from 11.7±3.2 pg/baseline hour to 10.5±3.0, 4.4±1.9, and 2.6±0.8 pg/treatment hour. Hypothalami treated only with Prazosin (N=7) showed a similar pattern, dropping from 13.1±2.3 pg/baseline hour to 11.3±2.8, 7.5±1.9, and 4.8±1.1 pg/treatment hour. Untreated controls were similar. Interestingly, constant MTX (N=8) did not stimulate LHRH release, with LHRH release of 9.33±2.8 pg during the baseline hour and 11.6±3.6, 9.6±3.9, and 7.1±2.7 during subsequent hours.

Prazosin's ability to completely block Methoxamine's stimulation of LHRH release demonstrates the α_1 specificity of this MTX effect. Also, in contrast to pulsatile MTX treatment, but like exogenous constantly applied norepinephrine's effects on rat LH levels (Gallo, 1982) and progesterone's effect on in vitro and in vivo LHRH release (Kim & Ramirez, 1982; 1984), administration of continuous MTX does not stimulate LHRH release. This implies that the adrenergic mechanisms involved in the release of LHRH in vitro are analogous to those in vivo.

115.4 INTERMITTENT INFUSION OF PREGNANOLONE INTO THE HYPOTHALAMUS OF CONSCIOUS UNRESTRAINED FEMALE RABBITS STIMULATES IN VIVO LHRH RELEASE. W.W. Lin* and V.D. Ramirez,(SPON: C.L. Prosser) Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Previously, we reported that intermittent infusion of progesterone (P_4) into the hypothalamus of conscious unrestrained female rabbits stimulates in vivo LHRH release. New Zealand female rabbits were implanted with push-pull cannulae (PPC) aimed at the tuberal region of the hypothalamus and perfused with modified KRP at 10-14 µl/min. In six animals perfused with KRP, pH 7.4, for 200 min followed by six pulses of P_4 (10-min on, 30-min off, 10 ng/ml) delivered through the PPC during a 240 min period, the mean basal level of LHRH increased from 1.12±0.16 pg/10 min during the control period to 1.97±0.24 pg/10 min during the period of P_4 infusion. In order to determine whether progesterone is acting through a metabolite, pulses of pregnanolone were applied, at various doses, directly into the hypothalamus of female rabbits. Two of these animals were also perfused with 20- α-OH-progesterone and/or cholesterol.

Five female rabbits were used in nine perfusion experiments. In these experiments, animals were initially perfused with modified KRP for a control period of 100-150 min followed by six pulses of pregnanolone (n=4 at .001 ng/ml, n=1 at .01 ng/ml, n=1 at .1 ng/ml), cholesterol (n=2 at 10 ng/ml), or 20-α-progesterone (n=1 at .001 ng/ml) 10-min on, 30-min off. In two rabbits treated with pregnanolone at .1 ng/ml and .01 ng/ml, there was a decrease in mean LHRH levels during pregnanolone infusion. The rabbit treated with .01 ng/ml was also perfused with pregnanolone (.001ng/ml), 20- α-OH-progesterone (.001 ng/ml), and cholesterol (10 ng/ml) in three subsequent experiments. Only the experiment with .001 ng/ml pregnanolone showed an increase in mean LHRH release. A third rabbit was treated with pulses of cholesterol (10ng/ml) and pregnanolone (.001ng/ml) in two different experiments. Only the treatment with pregnanolone showed a marked increase in mean LHRH release levels. Two more animals, also treated with pregnanolone (.001ng/ml) responded with an increase in LHRH levels.

In the four animals treated with pregnanolone (.001ng/ml), there was a significant increase in mean LHRH release (.92±.31 to 1.80±.99 pg/10min $p<.035$) and amplitude of LHRH pulses (.86±.52 to 2.32±1.30 pg, $p<.035$) using the Wilcoxon's Match Paired test. Overall, these results clearly demonstrate that pregnanolone, at very low doses, stimulates the LHRH pulse generator, suggesting that this 3 ß-5 ß metabolite of progesterone may play a role in the control of LHRH release in rabbits.

115.5 THE ROLE OF PROTEIN KINASE C IN PROGESTERONE-INDUCED LHRH RELEASE IN VITRO. F.C. Ke and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

It has been reported that phorbol ester as well as other protein kinase C activators enhance in vitro LHRH release from incubated median eminence tissue derived from the hypothalamus of male or female rats.

This study was designed to clarify the role of protein kinase C in the LHRH releasing action of pulsatile progesterone (P_4) from hypothalamic fragments superfused in vitro. To this effect, preoptic-mediobasal hypothalamic units from ovariectomized (OVX) as well as ovariectomized estrogen primed (OVX + E_2) 30-day-old rats were used to test the effect of phorbol ester on in vitro LHRH release.

4 α-Phorbol- 12,13, dibutyrate (PDBU) markedly stimulated LHRH release from either OVX or OVX + E_2 rat hypothalami. The mean LHRH release from the hypothalamic units during the two hours period after the initiation of PDBU treatment (10^{-6}M) increased 5.4 and 4.0 folds over basal level (N=5) in OVX and OVX + E_2 groups, respectively. This result contrast with the P_4-stimulatory effect on in vitro LHRH release which is estrogen-dependent. To identify more precisely the role of protein kinase C in the P_4-stimulatory effect on LHRH release, a protein kinase C inhibitor was used. Sphingosine (a physiological inhibitor of proten kinase C) at 10^{-5} M but not at 10^{-6} blocked the 10^{-6} M PDBU-stimulating effect on in vitro LHRH release. P_4-(10 mins on, 20 mins off, 10 ng/ml pulses, N=5) stimulatory effect on in vitro LHRH release was also inhibited by sphingosine at 10^{-6} M.

These results demonstrated that protein kinase C is involved in the in vitro P_4-stimulatory effect on LHRH release from superfused hypothalamic units. Future work will test the hypothesis of the involvement of protein kinase C in the postulated P_4 activating sequence of the LHRH neural apparatus (P_4-NE-LHRH) that ultimately lead to LHRH release in vitro.

115.6 ELECTROPHYSIOLOGICAL ACTIONS OF HISTAMINE ON ARCUATE NUCLEUS NEURONS IN VITRO. K.L. Jorgenson, L.-M. Kow and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Histamine (HA) has been localized in cell bodies, fibers and terminals of the medial basal hypothalamus (MBH), an area of importance in the neuroendocrine regulation of the pituitary gland. Receptors for HA are also found in the MBH. Furthermore, HA alters luteinizing hormone and prolactin secretion in female rats. Thus, in the present study we investigated the electrophysiological effect of HA at the level of extracellular single-unit activity of the arcuate nucleus in an in vitro slice preparation of the MBH.

Coronal slices (400 uM thick) of hypothalamus containing the arcuate nucleus were prepared from female rats and kept in artificial cerebrospinal fluid (ACSF) gassed with 95% O_2 and 5% CO_2. Extracellular single-unit activity was recorded with a ACSF-filled micropipette from slices submerged in a perfusion chamber containing ACSF continuously flowing at 2ml/minute and warmed to 36°C. Histamine, and other agents, were injected directly into the chamber. Changes in activity were assessed from continuous polygraph records of a firing rate histogram.

From a total of 96 arcuate neurons, HA caused a dramatic increase in firing rate in 66 neurons. No effect was seen in the remaining cells. The excitatory response was typically a sharp increase of 3-fold or more from basal firing rate within a minute followed by a gradual return to baseline. The response was repeatable in a single neuron. Histamine was routinely administered in 50ul of a 0.5 mM solution which was effectively diluted to 12.5 uM in the chamber. Dose-response testing revealed a linear decline in maximal firing rate with decreasing concentrations of HA and a loss of excitation around 1.0 uM HA. Neurons excited by HA varied in their basal firing patterns and responsiveness to other transmitters. Use of ACSF with high [Mg++] and low [Ca++] verified that HA was acting directly on recorded neurons rather than through synaptic inputs.

Tests with HA receptor antagonists showed that the excitation was mediated through H_1 receptors. The H_1 blockers pyrilamine and chlorpheniramine inhibited excitation in all 34 units tested. Recovery from inhibition could be seen after an hour or more, and the antagonists did not interfere with excitatory responses to other transmitters such as serotonin, norepinephrine and acetylcholine. Cimetidine, an H_2 blocker, had no effect.

These results confirm the reports of abundant H_1 receptors in the MBH and suggest that the H_1-mediated effects of HA on luteinizing hormone secretion (Miyake et al., Neuroendocrinology 45:191, 1987) could take place at the arcuate nucleus.

115.7 ESTROUS CYCLE-CORRELATED ALTERATIONS IN SINGLE UNIT ACTIVITY RECORDED FROM MULTIPLE EXTRA HYPOTHALAMIC NEURONS DURING LOCOMOTION. Sheryl S. Smith and John K. Chapin, Dept. of Physiology, Hahnemann Univ., Philadelphia, PA 19102-1192.

Ongoing studies have shown that, in the urethane-anesthetized, ovariectomized rat, both systemic and local administration of estrogen augment, while progesterone attenuates, excitatory responses of cerebellar Purkinje cells to iontophoretically applied glutamate. Using a behavioral paradigm, we have also shown that estrogen (s.c.) increases cerebellar discharge during treadmill locomotion to a greater extent than it alters activity during stationary periods. In contrast, progesterone decreases discharge during locomotion. Both sets of results are compatible with the hypothesis that estrogen enhances and progesterone diminishes evoked neuronal excitation, whether elicited pharmacologically or behaviorally.

In the present study, a more physiological approach was undertaken in order to determine changes in locomotor-correlated discharge relative to spontaneous across the days of the estrous cycle. Towards this end, adult female rats were implanted with 7 stainless steel microwires (.001" dia) lowered 1-4 mm into the cerebellum 1.5 mm lateral from midline. Inserted into Canon pins and a connector, which was cemented onto the skull, the wires transmitted neuronal activity from 5-7 individual units at one time. An additional wire from the connector grounded the plug to 5 skull screws. Neuronal discharge could then be monitored from the same neurons over a period of weeks. Implanted animals were trained to locomote on a computer-controlled treadmill device (alternately on 5s, off 5s) for a period of 1-3 hs daily. Preliminary data suggest that evoked discharge correlated with treadmill locomotion is increased on proestrus, when estrogen levels are elevated, relative to diestrus 1. This phenomenon also exhibited light-dark cycle variations, as peak levels of evoked discharge were seen on the night of behavioral estrus. Furthermore, these changes in discharge rate during treadmill locomotion were of significantly larger magnitude than corresponding cyclic alterations in discharge during stationary periods. These results are consistent with earlier reports suggesting that sex steroids may possess modulatory properties in the extrahypothalamic CNS. (Supported by NS25809 to SSS; BNS8519579, AA06965m abd AA00089 to JKC.)

115.8 GENOTYPE AND REPRODUCTIVE FUNCTIONS OF INBRED FEMALE MICE: EFFECT OF H-2 ALLELES. S.P. Lerner*, C.P. Anderson* and C.E. Finch (SPON: L. Weiner). Dept. of Neurobiology/Gerontology, University of Southern California, Los Angeles, CA 90089-0191.

The main histocompatibility complex (MHC; H-2 system in mice) is considered to be the "master genetic control" or regulatory system for immune function. It is possible to study links between alter- ations in physiologic functioning and specific genetic variants at the H-2 locus by utilizing highly-inbred strains of mice, bred to be genetically-identical except for the chromosomal region which carries the H-2 system. Thus far, H-2 variants are associated with differences in lifespan (Smith and Walford, Nature 270:727, 1977), fertility and other physiologic characteristics. The effect of the H-2 system on reproductive cycles, and the interaction of polymorphisms at this locus with other physiologic affecters of the reproductive system have yet to be examined. The purpose of this study was to determine the effect of H-2 variants female reproductive cycles and fecundity.

Beginning by 4.5 mo of age, estrous cycles of virgin mice of three congenic strains (C57BL/B10Sn, "B10"; B10.Br/Sg, "B10.Br"; and B10.RIII) were monitored for 3 mo. For each mouse, estrous cycles were categorized by length: 4, 5, 6, or 7-14 d. The effect of strain on estrous cycles was determined using a multivariate analysis of variance. For comparison we included C57BL/6J (B6) mice, whose cycling characteristics have been well documented (Nelson et al., Biol. Reprod. 27:327, 1982). Additionally, the number of litters produced, birthdate of last litter, and number of pups/litter were recorded for 18 age-matched pairs of mice (3-8 wks of age) of three strains (B10, B10.Br, and B10.RIII).

Estrous cyclicity differed with strain ($P<.0001$). B6 and B10.Br mice had predominantly 4-d cycles, whereas B10.RIII and B10 mice had more 5-d cycles than the other strains. F_1 hybrids from the reciprocal B10XB10.Br cross displayed a clear dominance of 5-d cycles, which indicates an autosomal allele on chromosome 17 in or near the H-2 complex. Moreover, F_1 hybrids from the reciprocal B6XB10 cross also display a dominance of 5-day cycles, which indicates a non-H-2 autosomal allele. The number of litters/pair, maternal age at last litter and total number of pups/pair differed with strain. In each case, B10.Br and B10.RIII had similar values and both had greater values than B10 mice. In conclusion, the present data that H-2 alleles influence female reproductive cycles and function. These data also raise the possibility that stable differences in menstrual cycle length of humans (Treloar et al., J. Fertil. 12:77, 1967) may also have a genotypic basis. (Supported by grant AG-004419)

115.9 A FAST, RELIABLE METHOD FOR REPEATED BLOOD SAMPLING FOR HORMONAL ASSAY IN RATS. L. O'Farrell, F. C. Martin, D. Novin and J.C. Liebeskind. Brain Research Institute and Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563

Chronic blood sampling is useful for hormonal and pharmacokinetic studies. Blood cannot be sampled past 2 weeks using most cannulation methods, however, and chronic stress as (reflected by changes in plasma hormones) often occurs. We therefore modified the jugular cannulation technique of F. J. Smith and L. A. Campfield (Am J Phys. 251, R70, 1986) to improve blood sampling reliability, decrease time required for surgery and reduce stress. Cannulae were made from 3 pieces of tubing: 2.3 cm of PE-100 tubing pulled over a flame at one end to an O.D. of .65 mm; 9.5 cm of .3 mm I.D. by .64 mm O.D. Dow Corning Silastic cut on a bevel at one end; and 1.5 cm of .76 mm I.D. by 1.65 mm O.D. Silastic. Four mm of the small end of the PE tubing was inserted into the lumen of the non-beveled end of the smaller diameter Silastic. After securing this junction with a piece of suture, the larger diameter Silastic was slid over the smaller and pushed over the junction. Under aseptic conditions, the beveled end of the smaller diameter Silastic was inserted 32 mm into a small incision in the right jugular vein made 5 mm from where the vein enters the chest muscle. The cannula was tied to the surrounding tissue with a suture secured to the cannula with Silastic Elastomer. The other end of the cannula was threaded s.c. to an incision between the scapulae. The cannula was tied to the underlying muscle using a suture secured to the larger diameter Silastic, and the skin was closed with a purse string suture. Cannulae were flushed daily with 50 U/ml heparinized saline mixed with 25 mg/ml cephalosporin. Twenty-one of 24 cannulae implanted in this way remained patent for 1.5 months. Four features of this method were shown to be critical to long-term sampling: the placement of the cannula tip at the junction of the superior vena cava and the atrium (which the method described insures for rats 250-400 g); the small size of the tubing in the vein; the aseptic surgical conditions; and the use of antibiotics. Lights-off plasma corticosterone and prolactin levels were 174 ng/ml and 23 ng/ml 1.5 weeks postoperatively, indicating this technique is minimally stressful and suitable for measurement of stress-related hormones. (Supported by the David Murdock Foundation for Advanced Brain Studies and NIH grant NS07628).

115.10 NEUROCHEMICAL MECHANISMS OF REPRODUCTION IN THE JAPANESE QUAIL : I. THE PREOPTIC AREA. J. Balthazart (1)*, G.F. Ball (2), M. Gahr (3)* and B.S. McEwen (2) (SPON : ENA). University of Liège, Belgium (1), The Rockefeller University, New York, NY 10021 (2) and University of Kaiserslautern, F.R.G. (3).

The activational effects of testosterone (T) on copulation are sexually differentiated in quail : T activates copulation in males but not in females. We previously identified several neurochemical correlates of this behavioral dimorphism. The inducibility of the preoptic aromatase by T is significantly higher in males than in females (Brain Res. 1986, 370, p285) and the concentration of norepinephrine in the preoptic area (POA) is higher in females than in males (Poultry Sci. 1986, 65, p1413).

To determine more precisely the neuroanatomical localizations of these sexually differentiated aspects of brain biochemistry, we have used immunocytochemistry and quantitative autoradiography to map estrogen receptors on one hand and adrenergic and muscarinic receptors on the other hand. Immunocytochemical maping using the antibody H222SPgamma raised against E2 receptors from mammary tumors demonstrated the presence of E2 receptors in the POA and especially in the medial preoptic nucleus (POM). Presence of E2 receptors in POM is especially significant as this nucleus is sexually differentiated (Volume larger in males than females; Neurosci. Ltrs. 1986, 64, p129), its volume and coloration by Nissl stains are testosterone-sensitive (decrease by castration and increase following T treatment; Brain Res. 1987 in press) and in addition, POM contains most of the preoptic aromatase as demonstrated by the Palkovits punch technique combined with in vitro radioenzymoassays (Brain Res. 1987, in press).

Quantitative autoradiography demonstrated the presence of specific and saturable binding for alpha-2-adrenergic and for muscarinic ligands (respectively tritiated para-amino-clonidine, PAC and n-methyl-scopolamine, NMS). PAC binding was very high in all POA, the highest concentrations of receptors being measured in the POM. Interestingly, PAC binding permitted to outline the area of the POM. This adrenergic receptor type is thus a specific marker for the sexually differentiated nucleus. By contrast, NMS binding was extremely low throughout the POA demonstrating the nearly total absence of muscarinic transmission in this area.

The coexistence in the sexually differentiated POM of E2 and alpha-2-adrenergic receptors suggests that this nucleus is the site of neurochemical interactions which are probably playing a key role in the control of copulation.

115.11 NEUROENDOCRINE MECHANISMS OF REPRODUCTION IN THE JAPANESE QUAIL II: HYPOTHALAMIC AND EXTRA-HYPOTHALAMIC AREAS. M. Gahr*, G.F. Ball, J. Balthazart*, and B.S. McEwen. (SPON: P. Marler) University of Kaiserslautern, Kaiserlautern, FRG. The Rockefeller Univ., N.Y, N.Y. 10021, and University of Liege, Liege, Belgium.

The neuroendocrine control of reproduction involves the interaction of steroids and a variety of neurochemical substances in discrete regions of the brain. In the Japanese Quail, the sexually dimorphic pre-optic medial nucleus (POM) has been the focus of recent investigations of the central action of steroids underlying reproductive behavior. In order to better specify other potentially significant sites of steroid-neurotransmitter interactions involved in the control of reproductive processes we have mapped using immunohistochemistry, the distribution of 17β-estradiol (E_2) receptors and using quantitative autoradiography the distribution of $alpha_1$, and $alpha_2$ adrenergic receptors, and muscarinic cholinergic receptors.

Subjects were male and female Japanese Quail. For autoradiography, frozen brain sections were taken on a cryostat and freeze-dried. $Alpha_1$ adrenergic receptors were labeled with (^3H) prazosin (PRA), $alpha_2$ adrenergic receptors were labeled with (^3H) p-aminoclonidine, and muscarinic cholinergic receptors were labeled with (^3H) N-methyl scopolamine. Autoradiograms were analyzed with an image analysis system that converted optical densities to fmoles/mg protein using a standard curve derived from co-exposed plastic standards. E_2 receptors were visualized using the PAP immunohistochemistry technique. A monoclonal antibody (H-222SPγ) raised against E_2 receptors was employed.

E_2 receptors were seen in the lateral septum (LS), the n. intercollicularis (ICo), and in sub-regions of both the hypothalamus and the archistriatum. Heavy PAC binding was often apparent in regions that contained E_2 receptors. This was particularly striking in certain areas that may play a role in reproduction such as the LS, and the tubero-infundibulum area of the hypothalamus and an area important in vocal behavior, ICo. The distribution of PRA binding was quite different from PAC binding in that the heaviest regions were the dorsolateral cortex (CDL), and portions of the thalamus. NMS binding was highest in the Lobus parolfactorius and area archistriatalis. Other areas showing specific NMS binding include steroid sensitive areas such as the ventro-medial nucleus of the hypothalamus (VMN) and the ICo as well as several non-steroid sensitive areas. The co-occurrence of apparent alpha-adrenergic binding, muscarinic cholinergic binding, and E_2 receptors in a variety of discrete areas implicated in reproduction suggests that steroid interactions with these neurotransmitters may play an important role in the regulation of reproductive processes, including behavior.

115.12 INCREASE OF CIRCULATING LEVELS OF GLUCOSE AFTER THE ELECTROLYTIC LESION OF THE ARCUATE HYPOTHALAMIC NUCLEUS. I. Zarco de Coronado, M.C. Yepez Chamorro. Dpto de Fisiología. Fac. de Medicina. Universidad Nacional Autónoma de México.

It is well known that the arcuate nucleus of the hypothalamus (ARH) shows specific chemical characteristics, and previous results obtained in our laboratory suggest a role for this nucleus in the regulation of the blood glucose. To further define this participation we examined the effects of electrolytic lesions of the ARH on the circulating levels of glucose.

Anesthetized male Wistar rats (250gr. body weight) 16 hrs. food deprived were stereotaxically implanted, bilaterally, in ARH with a bipolar concentric electrode. A 2 miliAmp. direct current was applied during 30 sec. in experimental animals. In control rats, no current was applied. Serum glucose was determined before and after lesioning or placing of the electrode.

Subsequent histological analysis revealed that the lesions were confined almost exclusively in the ARH nuclei. Circulating glucose was increases about 130 % after the lesions.

The results obtained suggest that the ARH nucleus participates in the regulation of the glucose. This effect could be mediated by central or peripheral neural connections or by endocrine mechanisms.

115.13 FURTHER EVIDENCE FOR EXCITATORY AMINO ACID NEUROTRANSMISSION IN THE SUPRAOPTIC NUCLEUS OF THE RAT HYPOTHALAMUS. V. K. Gribkoff and F. E. Dudek. Dept. of Physiology, Tulane Univ. Sch. of Med., 1430 Tulane Avenue, New Orleans, LA 7011.

The development of specific antagonists for the excitatory amino acids has produced a wealth of evidence demonstrating the widespread mediation of synaptic events in the CNS by this class of substances. In the magnocellular neuroendocrine system of the hypothalamus, which includes the oxytocinergic and vasopressinergic neurons of the supraoptic nucleus (SON), the degree of involvement of these compounds in synaptic excitation may be extensive, based on previous studies in our laboratory. In the present experiments, we have further characterized the actions of an excitatory amino acid antagonist, kynurenic acid (KYN), on synaptic transmission in the SON of rat hypothalamic slices.

Stimulation of a region dorsolateral to the SON produces excitation, postsynaptic potentials (p.s.p.'s), and increases in the frequency of "spontaneous" p.s.p.'s. Bath application of KYN produced a rapid, concentration-dependent, and reversible antagonism of both evoked and spontaneous p.s.p.'s in every cell in which it was tested. Direct comparisons were made between the effect of KYN in this system and at the Schaffer-collateral synapse in the hippocampus, which is thought to use an excitatory amino acid as a transmitter. In both systems, the time course of effect was similar, with onset at 3-5 min and maximal effect at 10-20 min. The degree of suppression at millimolar concentrations was also similar. In the SON, concentrations of KYN as low as 200 µM produced partial antagonism, while 1-3 mM produced dramatic suppression.

The cells were often hyperpolarized to near -80 mV during the experiments to prevent spike discharge. This membrane potential is below the reversal potential for GABA. Therefore bicuculline methiodide, a specific GABA antagonist, was applied to some cells to determine if these p.s.p.'s contain a GABA-ergic component. Bicuculline (30-100 µM) was minimally effective, antagonizing a portion of the p.s.p. in some cells. Likewise, d-tubocurarine chloride, a nicotinic cholinergic antagonist, had very little effect on these potentials at concentrations between 30 µM and 1 mM. KYN antagonized the bicuculline- and curare-resistant p.s.p.'s.

These results are consistent with the hypothesis that an excitatory amino acid mediates most fast excitatory synaptic events in SON neurons via the pathways and local circuits activated by local stimulation near the SON.

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115.14 ^{3}H-QUINUCLIDINYL BENZILATE BINDING IN THE BRAIN AND CAUDAL NEUROSECRETORY COMPLEX OF THE RAINBOW TROUT. D. Onstott and V. S. Seybold. Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

The caudal neurosecretory complex (CNC) of fishes provides a valuable, simple model for the study of vertebrate neurosecretion. As part of a series of investigations designed ultimately to determine the relationship between neurotransmitter binding and neurosecretory activity, we have undertaken experiments to examine the relationship between putative neurotransmitter binding sites and urotensin II (UII)-producing neurosecretory neurons in the CNC of the rainbow trout. Histochemical, electron microscopic, and in vitro urotensin release studies have suggested that acetylcholine plays a role in regulation of CNC activity and that its effect is mediated through muscarinic receptors. Therefore, the binding characteristics of tritiated quinuclidinyl benzilate (^{3}H-QNB), a muscarinic cholinergic antagonist, were determined in trout brain, and then utilized in combined autoradiographic-immunohistochemical experiments in the caudal spinal cord.

Optimum binding conditions for ^{3}H-QNB were determined using slide-mounted, 20 µm cryostat sections cut from unfixed, chopped trout whole brains, removed from animals immediately following stunning and decapitation. Sections were exposed to various rinse and incubation times and ligand concentrations. Nonspecific binding was determined by incubation of adjacent sections in ^{3}H-QNB plus 1 µM unlabeled atropine sulfate. Following incubation and wash, sections were wiped from slides into vials for assessment of binding by liquid scintillation counting. Scatchard analysis using nonlinear regression (McPherson version of LIGAND, Computer Prog. Biomed. 17:107,1983) indicated a K_d of 0.4 nM and a B_{max} of 4.4 nmol/g protein (r=0.97). Under optimum conditions, specific binding of 95% was obtained.

For autoradiography, the caudal spinal cord and urophysis were removed, embedded in gum tragacanth, and frozen. Ten µm cryostat sections were incubated according to conditions determined in the biochemical studies, dried and opposed to emulsion-coated coverslips according to the method of Young and Kuhar (Eur. J. Pharmacol. 59:317,1979). Urotensin II immunoreactivity was detected in adjacent sections using the peroxidase-antiperoxidase technique. Analysis of autoradiograms indicates the presence of specific ^{3}H-QNB binding sites in spinal cord areas dorsal to those containing UII-immunoreactive neurons. Image processing methods are currently being used to quantify binding sites and to determine their relationship to UII-immunoreactive structures.

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115.15 ULTRASTRUCTURE OF SEROTONIN AFFERENTS TO THE CAUDAL NEUROSECRETORY COMPLEX S.L. Cohen and R.M. Kriebel Dept. of Anatomy and Neurobiology, Univ. of Vermont Medical College, Burlington, VT 05405

The caudal neurosecretory complex (CNc) of fishes synthesizes and releases urotensins I and II. Biogenic amines have been associated with the regulation of the metabolism of these neuroendocrine cells. Serotonin (5HT) is one biogenic amine which has been identified in the CNc. A descending reticular projection and neurons intrinsic to the CNc are sources of serotonin inputs to this nucleus. This study examines the organization of these 5HT inputs at the ultrastructural level. Fish were anesthetized with MS-222, and perfused with 4% paraformaldehyde/0.1% glutaraldehyde in 0.06M phosphate buffer, pH 7.2. The unlabelled antibody method of peroxidase anti-peroxidase immunostaining was performed using anti-serotonin antisera (Immunonuclear). Serotonin processes in the CNc are of two types. Fibers with small (0.5-0.8um) varicosities travel in the central and dorsolateral aspects of the rostral CNc. This area contains the larger neuroendocrine cells and their processes, upon which many synaptic contacts are made from descending inputs. The density of these 5HT fibers is considerably reduced in the caudal portion of the CNc nucleus, which is characterized by tight clusters of smaller neuroendocrine cells that receive fewer synaptic contacts. Ultrastructural examination of these fibers reveals 1) varicosities filled with labelled vesicles (40-70nm). These varicosities were not found to make contact with neuroendocrine structures, and may be paracrine in nature. 2) 5HT terminals making contact onto clear vesicle filled terminals which in turn form synapses on neuroendocrine processes. Presynaptic modulation of this currently undetermined input is indicated for these 5HT afferents. 3) 5HT terminals on small (5-8um) cells that contain 70nm dense-core vesicles. These putative catecholamine cells are likely to be modulated by 5HT afferents.

The second type of fibers arise from intrinsic 5HT neurons. The cells and their initial segments are ensheathed by glial processes. Perikarya, axons, varicosities and terminals all contain 120-150nm vesicles. The axons course towards the ventrolateral edges of the spinal cord, and branch into fibers with many large (2-3um) varicosities. Some fibers terminate on the edges of the spinal cord in club-like extensions which increase in number and density as one proceeds caudally. These structures often lose their glial ensheathment to contact the basal lamina underlying the submeningeal space. Other fibers continue into the urophysis, which is the neurohemal contact site for the CNc. Varicosities in the urophysis are often situated near fenestrated capillaries. Secretion of 5HT into the CSF and blood is therefore possible. Intrinsic 5HT neurons may also modulate CNc function by paracrine secretion. PHS:RO119880

115.16 NOREPINEPHRINE AND SEROTONIN IN THE CAUDAL NEUROSECRETORY COMPLEX: A NEUROCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY. T.W. McKeon, S.L. Cohen, E.E. Black, R.M. Kriebel and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, Vermont 05405.

Intrinsic and supraspinal monoaminergic inputs have been identified previously in our studies on the poecilid caudal neurosecretory complex (CNc). The serotonin content and distribution in the CNc have been shown immunohistochemically (Cohen, S.L., Soc. Neurosci. Abst. 12:1487, 1986). The present study was done to determine biochemically the specific catecholamine(s) in the CNc and to show the relative contribution of indolamine and catecholamine to the CNc innervation.

Deafferentation of supraspinal monoaminergic projections was done by transecting the spinal cord at the level of the 5-6th preterminal vertebrae. Fish were killed 10 days after surgery; spinal cords were removed and processed along with control spinal cords for either HPLC or immunohistochemistry. HPLC analysis of monoamines was done on pooled samples (3-12) of the spinal cord from the terminal four vertebral segments. Dopamine and epinephrine generally were below the sensitivity limits of the HPLC methods. In one instance, a low level of dopamine, probably representing a precursor pool for norepinephrine (NE), was observed when 18 spinal cords were pooled. In control CNc, NE (35.4 $\pm$ 9.8 pg/µg protein) and serotonin (5-HT) (36.6 $\pm$ 6.9 pg/µg) protein) were detected. Following deafferentation, the content of the NE and 5-HT were significantly decreased. NE levels were generally below detectable limits. Serotonin was present in deafferented CNc (14.5 $\pm$ 7.5 pg/µg protein), with levels approximating 50% of control fish. For immunohistochemistry, terminal segments of the spinal cord were fixed with 4% paraformaldehyde. Thirty µm frozen sections were cut horizontally and stained using indirect immunofluorescent techniques with antisera for either serotonin (5-HT), tyrosine hydroxylase (TH), or dopamine β-hydroxylase (DBH). Patterns of innervation in the control spinal cords were consistent with previous reports using immunofluorescence and PAP methods. Similar patterns were observed using antisera to both TH and DBH. After deafferentation, the number of 5-HT labelled fibers was reduced, and numerous 5-HT labelled neurons were still observed. Occasional NE cell bodies and very fine fibers remained after spinal cord transection. The NE neurons were smaller and less numerous and their processes less extensive when compared to 5-HT labelled neurons.

This study confirmed biochemically the presence of serotonin and showed that NE is the catecholamine most prevalent in this neurosecretory nucleus. Both NE and 5-HT are associated with descending supraspinal projections, while 5-HT neurons predominate as the intrinsic monoaminergic innervation.

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115.17 MOLLUSCAN BRAIN HORMONE STIMULATES REPRODUCTIVE ORGAN GROWTH *IN VITRO*. P.G. Sokolove and G.R. Albert* (SPON: T.A. Viancour) Dept. of Biolog. Sciences, Univ. of Maryland Baltimore Co. (UMBC), Catonsville, MD 21228

Reproductive tract development in the hermaphrodite slug, *Limax maximus*, is environmentally regulated. Long day lightcycles (LD16:8) stimulate growth and maturation of the gonad and of male and female accessory sex organs (ASO). Previous work has indicated that long days trigger the release from cerebral ganglia of a neurohormone which acts indirectly (via the gonad) to stimulate ASO development. Recent results suggest, however, that long-day cerebral ganglia (CG) also produce a substance that can directly stimulate growth of at least one ASO -- the so-called sperm-oviduct or "common duct" (CD). CG from long-day-stimulated female-phase slugs were homogenized in culture medium and added initially, and again after 3 days, to culture wells containing immature common ducts. After 6 days, ^{3}H-thymidine (1.0 Ci/ml) was added along with CG homogenate, and ducts were incubated for an additional 3 days. CDs incubated with CG homogenate incorporated from 8- to over 20-fold more label than CDs cultured alone. Additional experiments showed that considerably less incorporation occurred when CDs were incubated with homogenates of either sub-esophogeal ganglia or CG from short-day slugs. The stimulatory factor in long-day CG homogenate was found to be protease sensitive and did not pass through a 50 kdalton cut-off ultrafilter. About 75% of activity was retained after heating the homogenate to 95°C for 30 min. It appears based on these results that the CG of slugs exposed to long days produce a polypeptide factor which stimulates ASO cell proliferation *in vitro*. This factor probably plays an important role in normal, long-day-induced reproductive maturation, but its exact function and coordination with gonadally secreted sex hormone are presently not known.

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115.18 ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL EVIDENCES OF AN UTERUS PROJECTION TO NUCLEUS OF THE TRACT. M. Ortega-Villalobos*, G. Ninomiya-Alarcón*, C. de los Santos-Toledo* and R. Guevara-Aguilar. Dept. of Physiology, Fac. of Medicine. U.N.A.M. Apdo. Postal 70250, 04510 México, D.F.

The nucleus of the solitary tract (NTS) is a visceropective cell group located in the dorsal medulla. The NTS receives primary afferent terminations from the vagus nerve, which convey visceral information. Several studies confer to the vagus nerve, a role in reproductive functions. It is well known that the vagus nerve innervates the ovary. The aim of this work was to search for a possible pathway from uterus to NTS. The results were obtained from 11 Wistar female rats with a 188-307 g weight. The rats were anesthetized with chloral hydrate (400 mg/Kg). The firing rates of the NTS neurons were recorded, using a micropipette 3 um diameter tip, during cervix distention (0.4-0.6 ml. volume) infused by means of a pediatric urinary latex catheter. Only those animals that showed estrous vaginal smear, were recorded. Ten cells located in the dorsal part of the NTS were sensitive to cervix distention, all of them showed a stop of their firing rate with a latency of 39.63 sec after the begining of the distention and only in one case, it was observed an increase of firing rate during the distention. In five additional rats, horseradish peroxidase (Sigma type VI) was dissolved in isotonic saline solution; using a microsyringe fitted with a 26-30 gauge needle, 20 ul of a 40% horseradish peroxidase solution was injected along the cervix, the body of the uterus and the ducts of Falopio. After a survival time of 24-48 hs, the rats were reanesthetized with chloral hydrate and perfused intracardially with glutaraldehyde and paraformaldehyde fixative, the whole brain was removed and sectioned. The 50 um thick sections were processed with the TMB method and counterstained with thionin. Labelled neurons were identified in the NTS utilizing bright field microscopy.

These results, in terms of neurophysiological and neuroanatomical data, demonstrate a direct input to NTS from the uterus.

MONOAMINES AND BEHAVIOR III

116.1 FRACTIONATION OF THE SUBCOMPONENTS AND TRANSMITTER CONTROLS OF CONTACT-RIGHTING IN LABYRINTHECTOMIZED LATERAL HYPOTHALAMIC-DAMAGED RATS. S.M. Pellis, T.K. Morrissey, V.C. Pellis and P. Teitelbaum. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Contact righting, that is, turning to prone from a recumbent lying position on the ground, is abolished from 2-4 days after large electrolytic lesions of the lateral hypothalamus. With recovery contact righting reappears, but does so in a distinct manner. At first the body is righted by backleg movements, in the absence of any active axial rotation. Later, a transitional form appears, in which tactile contact of the legs triggers axial rotation of the pelvis. Eventually, cephalic dominance is re-established, in which righting switches from back to front, so that righting begins in the shoulders and then proceeds to the pelvis. Such righting is achieved by pure axial rotation of the body, in which the limbs are carried by the torso. In shoulder girdle-led axial rotation, the head and neck do not turn, but are merely carried passively along by the body. Labyrinthectomy, when combined with the lateral hypothalamic (LH) damage, slows this recovery (now taking as long as three weeks), and reveals several intermediate stages of contact-righting more clearly.

The absence of axial rotation in the early stages of recovery from combined LH damage and labyrinthectomy is similar to the 'axial apraxia' seen in some Parkinsonian patients (Lakke, J.P.W.F. J. Neurol. Sci., 69: 37, 1985). In such Parkinsonian patients, axial rotation is not reinstated by L-dopa therapy, and thus has not been thought to involve the dopaminergic system. In the LH-damaged labyrinthectomized rat systemically injected 10 mg/kg quipazine maleate (a post-synaptic serotonergic agonist) reinstates shoulder girdle-led axial rotation. In contrast, systemically injected 2 mg/kg apomorphine hydrochloride (a post-synaptic catecholaminergic agonist) reinstates a different form of axial rotation, which involves tactile-triggered head and neck rotation which then recruits shoulder girdle and pelvis rotation cephalocaudally. In recovered animals systemically injected 25 or 50 mg/kg of pilocarpine hydrochloride (a post-synaptic cholinergic agonist) abolishes cephalic dominance and axial rotation reinstating backleg pushing of the body to prone. Thus, in the rat, several separate subcomponent reflexes, previously undescribed, are under separate transmitter controls, and act in an allied fashion to produce a composite reflex act of righting. These subcomponents, when acting in various degrees of isolation, may be a useful animal model of the abnormalities seen in Parkinsonian righting. Finally, the above results show that LH-damage produces major deficits in the proprioceptive/tactile controls over righting which are compensated for early in recovery by the vestibular system. One of these controls involves the rostro-caudal integration of righting.

116.2 ISOLATION-REARING IMPAIRS THE ACQUISITION OF SCHEDULE-INDUCED POLYDIPSIA. G.H. Jones*, T.D. Hernandez and T.W. Robbins* (SPON: S. Pellow) University of Cambridge, Department of Experimental Psychology, Cambridge, U.K. CB2 3EB

Isolation-reared rats develop an enduring spontaneous locomotor hyperactivity, are slower to habituate and are more aggressive than animals raised in social groups. Isolates also show enhanced oral behaviours in response to tail-pinch stimulation, are more responsive to stimulant drugs such as amphetamine and are less susceptible to CNS depressants. It has been proposed that isolation-reared animals exhibit higher levels of arousal than those reared in groups. In this experiment, the acquisition of schedule-induced polydipsia (SIP) was measured in rats reared in isolation and group-reared controls. The development of excessive drinking in response to an intermittent schedule of food presentation has been suggested to be a coping response which acts to reduce heightened arousal produced by the schedule (Brett, L.P. and Levine, S., *J. Comp. & Physiol. Psych.*, 93: 946-956, 1979).

Female Lister-hooded rats were weaned at 20 days and housed either individually or in groups of 4-5 per cage. At 14 weeks both groups of animals were food-deprived to 85% of their free-feeding weight and given 14 daily 30 minute exposures to a fixed-time food presentation schedule. Two 45 mg pellets were delivered every 60 seconds of the test and water consumption and activity were recorded. Rearing in isolation significantly impaired the acquisition of polydipsia. In contrast, mature animals housed in isolation for the same length of time did not differ from their control group. In confirmation of earlier studies, the isolation-reared rats weighed more and were hyperactive, not only in photocell cages, but also during SIP testing.

Plasma corticosterone (CCS) levels, a neuroendocrine index of stress, were determined following acquisition of schedule-induced polydipsia. The inverse relationship between CCS and drinking found in group-reared rats was not present in the isolates. Presynaptic dopaminergic function was also assessed, in vivo, using intracerebral dialysis in separate groups of isolates and socially-reared animals. Preliminary results indicate that there are enhanced extracellular dopamine levels in the striatum of isolation-reared rats after amphetamine treatment, when compared to group-reared controls. These results are discussed in terms of the hypothesis that isolation-rearing acts as a chronic stressor and enhances central dopaminergic activity.

116.3 EVIDENCE OF A FUNCTIONAL ROLE FOR EXCITATORY AMINO ACIDS IN THE MEDIAN RAPHE NUCLEUS OF THE RAT. D. Wirtshafter & R. Trifunovich*, Dept. Psychology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Several recent anatomical studies have provided evidence for the existence of excitatory amino acid containing afferents to the median raphe nucleus (MR). We have reported that microinjections of nanogram quantities of the glutamate agonist kainic acid into the MR result in a marked reduction of spontaneous and ritalin-induced locomotor activity (Wirtshafter & McWilliams, 1987). In the current experiments we sought to further examine the role of excitatory amino acids in the MR by studying the behavioral and biochemical effects of injections of several excitatory amino acid antagonists into the MR or adjacent structures.

Intra-MR injections of the broad spectrum amino acid antagonist kynurenic acid (0, 2.5 or 5ug in 0.5 ul) produced a marked, dose-dependent increase in photocell cage locomotion in habituated rats. The effect was immediate in onset and its maximum amplitude was similar to that observed after intra-MR injections of muscimol. Similar dose-dependent hyperactivity was seen after intra-MR injections of the specific NMDA antagonist 2-amino-5-phosphonovaleric acid (2-APV, 0,1 or 2ug).

In order to determine whether the locomotor responses seen after injections into the MR could have been mediated by diffusion of these compounds to adjacent structures, we next compared the locomotor responses seen following injections of kynurenic acid, 2-APV or gamma-glutamylglycine into the MR, the dorsal raphe nucleus or the ventral tegmental area. For all of those drugs, the locomotor responses seen after injections in the MR were much larger than those seen after injections into either of the other two sites.

Finally, additional animals received intra-MR injections of 2-APV or its vehicle and were sacrificed 30 min. later to allow for regional examination of serotonin and dopamine metabolism by HPLC. Preliminary results indicate that animals who showed a pronounced locomotor response to the 2-APV injections displayed reduced levels of 5-HIAA in the hippocampus and increased levels of DOPAC and HVA in the nucleus accumbens.

The results of these studies suggest that a tonic excitation of MR cells is exerted via a stimulation of excitatory amino acid receptors within the nucleus. Blockade of these receptors results in an increase in locomotor activity and a decrease in hippocampal serotonin metabolism. Both of these effects are similar to those observed after intra-MR injections of the GABA agonist muscimol.

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116.4 NEONATAL FOREBRAIN NOREPINEPHRINE (NE) DEPLETION REDUCES THE INFLUENCE OF THE REARING ENVIRONMENT ON LEARNING AND SOCIAL BEHAVIOUR. M.J. Saari, J. Armstrong*, J. Nobrega, J. Chambers* and D. Coscina. Dept. of Psychol., Nipissing Univ. Coll., North Bay, Ontario, P1B 8L7 and Dept. of Biopsychol., Clarke Institute of Psychiatry, Toronto, Ontario, Canada. M5T 1R8.

We have previously shown, that rat pups injected with 6-hydroxydopamine (6-OHDA) are less sensitive to environmental rearing conditions as measured by maze running latencies and in the Colony Intruder test. These results support our suggestion that NE plays a role in environmentally induced behavioural and neural plasticity. Although Mohammed, Jonsson & Archer (Brain Research, 398, 1986) have successfully replicated our findings Whishaw, Sutherland & Kolb (Beh. & Neural Biol., 46, 1986) have not. The present experiments further support our hypothesis that neonatal NE depletion attenuates the consequences of enriched and isolated rearing. We also provide some new data on cerebral glucose utilization following neonatal NE depletion and manipulation of the rearing environment.

Newborn male Wistar rats were injected subcutaneously, either with 6-OHDA (50 mg/kg) or vehicle (VEH, 1.0 mg/ml ascorbic acid in saline) within the first 12 and the first 24 hours after birth (assay results will be available at the poster). At 25 days the subjects were weaned and reared for 30 days either in isolation (IC), or enrichment (EC). Rats in IC were housed singly in standard, wiremesh, hanging cages (20x24x18 cm). EC housing consisted of social housing (6 per group) and daily, 30 min exposures to an open field containing regularly changed objects such as tunnels, platforms and balls.

At 60 days of age, in separate experiments, the rats were tested in the place navigation task and in two tests of competition/dominance. The tests were videotaped and scored by blind methods. The results of both experiments support the suggestion that the effects of rearing environment are reduced in NE depleted rats. Glucose utilization in several brain areas was found to vary primarily as a function of environmental rearing conditions.

116.5 TWO DISTINCT ROLES FOR THE MEDIALIS DORSALIS NUCLEUS OF THE THALAMUS ON FOCALIZED ATTENTION IN CONSCIOUS CAT. JJ. Bouyer, M.F. Montaron*, A. Rougeul* and P. Buser*.Institut des Neurosciences, CNRS and Université Pierre & Marie Curie, Paris 75005)

In cats, immobile focalized attentive behavior towards a target is accompanied by the development of beta (14 Hz) electrocortical (ECoG) rhythms in the pericruciate motor cortex (areas 4 and 6a). Both the behavior and the accompanying ECoG pattern are under the control of a dopaminergic mechanism originating in the ventral tegmental area (VTA, Montaron et al., Behav. Brain Res., 6 : 129, 1982) and are impaired after lesion of this structure. On the other hand, bilateral lesions of n.accumbens (Bouyer et al., Exp. Neurol., 92 : 698, 1986) were followed by the opposite syndrome, i.e.perseveration of attentive fixation and reinforcement of accompanying beta. Finally, retrograde HRP has shown that the cortical beta focus in the motor cortex receives some projections from the thalamic nucleus medialis dorsalis (MD). With this background we have now performed restricted kainic MD lesions and controlled the post-lesional beta and behavior. The results show that anteromedial MD lesions were followed by a clearcut deficit of the attentive immobility and accompanying beta (" VTA like" deficit), while posterolateral lesions induced the opposite syndrome(" ACC like"). From what we know of the connections between VTA, ACC, prefrontal cortex (PF) and MD, we suggest that two different parts of MD integrate two distinct, opposite controls from the VTA over attentive behavior and beta, the "meso-frontal" branch through PF, and the "meso-limbic" branch, via ACC.

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116.6 DOES NICOTINE INCREASE LOCOMOTOR ACTIVITY BY STIMULATING MESOLIMBIC DOPAMINE ? P.B.S. Clarke, A. Jakubovic and H.C. Fibiger. Kinsmen Lab. for Neurological Research, Univ. of British Columbia, Vancouver, BC V6T 2A1.

Certain stimulant drugs appear to increase locomotion by increasing DA neurotransmission within the mesolimbic system. Given systemically to rats, (-)-nicotine stimulates locomotor activity (Clarke and Kumar 1983, Br J Pharmac 80:587-594). Nicotine receptors are associated with cell bodies and terminals of ascending dopaminergic (DA) pathways (Clarke and Pert 1985, Brain Res 348:355-358), and direct injection of a nicotinic agonist into the ventral tegmental area increases locomotion, apparently by a dopaminergic mechanism (Pert and Chieuh 1986, Soc Neurosci Abstr 12:250.4).

Rats received daily home cage injections of (-)-nicotine 0.4 mg/kg sc for two weeks, in order to enhance the drug's stimulant action (Clarke and Kumar 1983). A locomotor stimulant action was then verified by testing each subject with saline and with (-)-nicotine 0.4 mg/kg sc in photocell cages. Some days later, rats were injected with saline or nicotine, confined to photocell cages, and killed after 30 minutes. Major DA terminal regions (medial prefrontal cortex - mPFCX, olfactory tubercle - OT, nucleus accumbens - NACC, caudate-putamen - CP) were dissected on ice prior to HPLC/EC assay of catechols. DA utilisation was assessed by HVA/DA and DOPAC/DA ratios, or, in rats treated with the DOPA decarboxylase inhibitor NSD-1015, by DOPA/DA ratios.

The following effects were significant ($p<0.05$-0.005). Expt 1: (-)-Nicotine (0.2 - 0.4 mg/kg sc) increased locomotor activity and increased OT HVA/DA. Expt 2: (-)-Nicotine (0.1 - 0.4 mg/kg sc) stimulated locomotor activity in a dose-related way, and increased DOPA/DA ratios in a dose-related way in both OT and NACC, but not in CP. Expt 3: (-)-Nicotine (0.4 mg/kg sc) increased locomotor activity and elevated DOPA/DA ratios in OT and NACC, but again not in CP; in all these respects, the same dose of (+)-nicotine was ineffective.

These results suggest that systemic administration of nicotine at doses which increase locomotor activity may do so by selectively stimulating the mesolimbic DA system.

Supported by the Medical Research Council of Canada.

116.7 PHYSIOLOGY OF ABUSE POTENTIAL SUBSTANCES IN CENTRAL NEURONAL CIRCUITS: COCAINE EFFECTS ON SYNAPTIC TRANSMISSION OF AFFERENT SIGNALS TO RAT SOMATOSENSORY CORTICAL NEURONS. C.A. Jimenez-Rivera, E. Garcia* and B.D. Waterhouse. Dept. of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102.

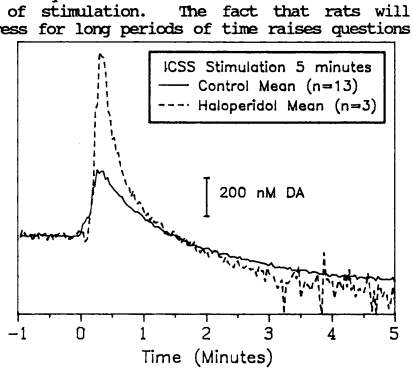
The psychostimulant effects of cocaine include increases in locomotion and enhanced sensitivity to tactile, visual and auditory stimuli. Biochemical studies suggests that such effects result from the ability of cocaine to elevate central synaptic levels of endogenous monoamines. The goal of the present study was to use electrophysiological assays developed in previous investigations of central monoamine function characterize the actions of cocaine on synaptic transmission of afferent signals through a primary sensory circuit in mammalian brain. Specifically, the responses of rat somatosensory cortical neurons to stimulation of thalamocortical afferent pathways were examined before and after parenteral (i.p.) administration of cocaine HCl. Extracellular unit activity from anesthetized rats was recorded using either glass or tungsten microelectrodes. Cortical afferents were activated via a bipolar stimulating electrode positioned in the VPL thalamus. Unit responses consisted of short latency excitation followed by a period of suppressed discharge or pure stimulus bound inhibition. Poststimulus time histograms of cell activity were collected every 3.5 min. for 15 min. before (control) and at least 40 min. after cocaine injection at 0.5, 1 or 2 mg/kg. Drug effects were quantitatively evaluated by comparing discharge rates during periods of stimulus bound and spontaneous activity in control and drug response histograms. In 6 of 11 (55%) neurons tested, cocaine suppressed spontaneous activity more than evoked discharges such that excitatory responses were enhanced relative to background firing. In one additional case, the magnitude and duration of a stimulus bound inhibitory response were augmented following cocaine injection. Facilitating effects of cocaine on evoked responses as well as suppression of background firing were observed within 3 min of drug administration, reached a peak around 7 min post-injection and began to return to pre-drug levels at 20-30 min post-injection. Enhancement of evoked responses was more consistently observed at lower doses (i.e. 0.5 mg/kg). Overall, these results indicate that cocaine can alter cerebrocortical neuronal responsiveness to synaptic inputs in a manner similar to that observed for norepinephrine. Thus, a physiological consequence of cocaine's biochemical action at central synapses and possibly a source of its stimulant properties may be the induction of noradrenergic-like modulatory actions in local brain circuits. (Supported by AFOSR-85-0155 and NS18081 to B.D.W.)

116.8 EFFECTS OF LY53857, A SELECTIVE 5-HT_2 RECEPTOR ANTAGONIST, ON COPULATORY BEHAVIOR OF MALE RATS. M. M. FOREMAN, R. L. LOVE*, J. L. HALL*, G. P. MARZONI* AND W. L. GARBRECHT*. The Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285.

Experimental manipulations that decrease serotonergic activity such as lesioning serotonergic pathways, inhibition of 5-HT synthesis with para-chlorophenylalanine or blockade of 5-HT receptor activity with antagonists such as methysergide, metergoline or mesorgydine, have been found to increase copulatory behavior in male rats. The conclusions that 5-HT has an inhibitory effect on sexual behavior are further supported by the observations of reduced sexual performance following administration of the 5-HTP, 5-HT reuptake inhibitors or the 5-HT receptor agonist, 5-MeO-DMT. However, reductions in sexual performance of male rats have also been observed following administration of 5-HT receptor antagonists, pirenperone and ketanserin (Mendelson and Gorzalka, Pharmacol. Bioch. & Behav. 22:565-571, 1985). These antagonists have a higher affinity for the 5-HT_2 receptor than the 5-HT_1 receptor, but also have high to moderate affinities for H_1-histaminergic, alpha$_1$-adrenergic and D_2-dopaminergic receptors (Janssen, T.I.P.S. 4:198-206, 1983). LY53857, a potent 5-HT_2 receptor antagonist that lacks prominent effects at other monoaminergic receptors (Cohen et al., J.P.E.T. 227:327-332, 1983; Drug Dev. Res. 5:313-321, 1985), was used in the present studies to evaluate the effects of selective 5-HT_2 receptor antagonism on male rat sexual behavior. The sexual behavior of each male, Sprague-Dawley rat was evaluated at two week intervals using a sexually receptive, female rat. Only rats with stable sexual performance in successive tests with vehicle administration were selected for these studies. These rats were given subcutaneous injections of either 0.01, 0.10 or 1.0 mg/kg 30 minutes prior to testing. No alterations in copulatory performance were observed at the 0.01 mg/kg. However, the administration of 0.1 or 1.0 mg/kg resulted in significant reductions in ejaculatory latency of 32.8 $\pm$ 8.8 and 40.6 $\pm$ 5.1 % compared to control responses, respectively. These latter doses also induced statistically significant increases in copulatory rate of 46.7 $\pm$ 18.1 and 110.7 $\pm$ 17.9 % compared to control responses. These data are supportive of the view that selective 5-HT_2 receptor blockade enhances copulatory performance of male rats. The previous findings of reduced sexual performance of male rats treated with pirenperone and ketanserin may be related to the alpha$_1$ antagonist activity of these compounds and not the 5-HT_2 antagonist properties. This conclusion is supported by the observations of suppressed sexual function in rats treated with the alpha$_1$ antagonist, prazosin (Clark et al, Neuroendocrinology 41:36-43, 1985).

116.9 **EFFECT OF STIMULATION CURRENT ON DOPAMINE RELEASE MEASURED BY IN VIVO VOLTAMMETRY: RELEVANCE TO INTRACRANIAL SELF STIMULATION.** **J. B. Justice, Jr., M. Ikeda*, L. C. Nicolaysen*, and D. B. Neill*. Departments of Chemistry and Psychology, Emory University, Atlanta, GA 30322.**

Electrically stimulated dopamine (DA) release is monitored voltammetrically in the rat striatum under varying stimulation conditions. At 200, 75 and 50 μA (80, 30 and 20 μA rms) stimulation, the maximum concentration of released DA was 29, 3.7 and 0.46 μM, respectively (100 Hz, 2 ms biphasic square waves). Computer modelling of the DA nerve terminal suggests that as lower stimulation currents are applied at constant frequency, fewer DA neurons are stimulated, rather than the same population being weakly stimulated uniformly. The data and model also indicate that the stimulated terminals become depleted of releasable DA during the stimulation of the MFB. From microdialysis studies, baseline extracellular DA is about 20 nM in the striatum of anesthetized rats. When typical intracranial self-stimulation (ICSS) conditions are applied for 5 minutes in anesthetized rats (bipolar electrode, 100 μA, 100 Hz, 0.5 ms biphasic square wave pulses in 150 ms trains at 2 Hz, overall 3 μA rms), DA reaches a maximum concentration of 342 nM (n=13) at 20 seconds, and then decreases within 1 minute to close to baseline values. After 0.05 mg/kg haloperidol, 5 minutes of ICSS stimulation produces a maximum DA concentration of 947 nM (n=3) at 20 seconds. Evidently the most intense release occurs within the first minute of stimulation. The fact that rats will continue to bar press for long periods of time raises questions about the role of DA in ICSS. The results also suggest that less than 10% of the DA nerve terminals in the striatum are stimulated during ICSS.



116.10 DOPAMINERGIC SUBSTRATES FOR CUES PRODUCED BY ELECTRICAL STIMULATION OF THE VENTRAL TEGMENTAL AREA. J.P. Druhan, H.C. Fibiger and A.G. Phillips. Dept. Psychol.; Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Previous studies have reported that the cue properties of amphetamine are mediated by mesocortical dopamine (DA) neurons originating in the ventral tegmental area (VTA). The present experiments investigated whether cues produced by electrical stimulation of the VTA might involve similar substrates. In the first experiment, rats were trained to discriminate between high and low intensities of VTA electrical brain stimulation (EBS). The discrimination task required that animals respond on one lever for food after being cued by prolonged, intermittent stimulation at a high intensity, or on an alternate lever after being cued by low intensity EBS. Rats that acquired this task were subsequently tested for generalization to 4 intermediate intensities after ip injections of amphetamine (0.5 & 1.0 mg/kg), haloperidol (0.1 & 0.125 mg/kg) and saline. Relative to saline tests, amphetamine caused the rats to respond to low-intensity cues as though they were higher, suggesting an enhancement of the EBS cues. In contrast, haloperidol resulted in the rats responding to high-intensity EBS cues as though they were of lower intensity, suggesting an attenuation of the perceived intensities of the EBS. The observed enhancement of the EBS cues by an indirect DA agonist (amphetamine) and their attenuation by a DA receptor antagonist (haloperidol) suggests a DA substrate for the VTA EBS cues measured in this experiment.

The second experiment assessed whether generalization could occur between amphetamine and VTA EBS cues. Eight rats were trained to discriminate 1.0 mg/kg amphetamine (ip) from saline, and then tested for generalization to VTA EBS. Each rat was given 6 generalization tests with VTA EBS presented intermittently at either 15 or 20 uA, and at a frequency of 1/10 s, 1/5 s or 1/2.5 s. Examination of the data for individual subjects revealed that three of the rats cosistently responded on the drug lever when VTA EBS was substituted for amphetamine, whereas the other five rats always responded on the saline lever. These results indicate that VTA EBS can mimick the amphetamine cue under certain conditions, but the generalization does not occur in all rats.

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116.11 UNIQUE EFFECTS OF ATYPICAL NEUROLEPTICS ON AMPHETAMINE-INDUCED BEHAVIORS. JoAnn Tschanz* and George V. Rebec (SPON: G.P. Frommer). Dept. Psychol., Indiana Univ., Bloomington, IN 47405

Although both atypical and classical neuroleptics are used in the treatment of schizophrenia, these drugs do not exert comparable effects. Certain atypicals, for example, are more effective in treating negative-symptom schizophrenics, and these drugs, unlike the classicals, fail to produce extrapyramidal side effects. These drugs also differ in animal tests. The classical neuroleptics elicit catalepsy in rats, whereas the atypicals are devoid of this effect, even at relatively high doses. There also is general agreement that although both types of drugs block the forward locomotion produced by amphetamine and other dopamine agonists, the classicals also block focused stereotyped behaviors. To the extent that different neuronal systems mediate the locomotion and focused stereotypy produced by dopamine agonists, these systems appear to respond differentially to atypical and classical neuroleptics. In fact, this interpretation has led to the proposal that a blockade of the mesolimbic dopamine system, which appears to mediate the blockade of amphetamine-induced locomotion, plays an important role in the antipsychotic efficacy of the neuroleptics, whereas blockade of the nigro-striatal dopamine pathway blocks drug-induced stereotypies and leads to extrapyramidal side effects. Recent evidence, however, indicates that the atypicals also attenuate at least some components of focused stereotypy and may even enhance others (Robertson, A. & MacDonald, C., Pharmacol. Biochem. Behav. 21:97, 1984). It is conceivable, therefore, that a simple blockade of the behavioral effects of amphetamine is not an adequate model for studying the antipsychotic efficacy of neuroleptic drugs. In order to study this issue further, we tested several different neuroleptics in rats that received either low (1.0 mg/kg) or high (5.0 mg/kg) doses of amphetamine, and we monitored individual items of the amphetamine behavioral response.

Consistent with previous reports, pretreatment with the classical neuroleptic, haloperidol (0.1 or 0.25 mg/kg), significantly reduced all components of the behavioral response to low as well as high doses of amphetamine. The atypicals, however, were more variable in their effects. Although both clozapine (1.0 or 5.0 mg/kg) and thioridazine (1.0 or 5.0 mg/kg) blocked either sniffing or head bobbing produced by a low dose of amphetamine, clozapine was more effective than thioridazine in blocking the oral behaviors produced by a high dose of amphetamine. Moreover, the high dose of clozapine blocked amphetamine-induced rearing, but a comparable dose of thioridazine enhanced this response. Thus, as a class, the atypical neuroleptics appear to block only some components of the amphetamine behavioral response, but when considered individually each drug appears to exert a unique dose-dependent effect.

116.12 ANTAGONISM OF TRIADIMEFON-INDUCED HYPERACTIVITY BY RESERPINE IN RATS. K. M. Crofton*, V. M. Boncek*, R. C. MacPhail, and L. W. Reiter, Neurotoxicology Division, U.S. EPA, RTP, NC 27711 and Northrop Services, Inc, Environmental Sciences, RTP, NC 27709

Triadimefon (TDF) is a substituted triazole fungicide that has been shown to produce hyperactivity in both mice and rats (Crofton et al., Toxicologist 7:254,1987). TDF-induced hyperactivity was characterized by alterations in temporal and spatial patterns of activity, similar to effects seen following administration of compounds with catecholaminergic activity (e.g., amphetamine). To determine whether the TDF-induced hyperactivity is due to an interaction of this fungicide with CNS catecholaminergic systems, we evaluated the effects of combined treatment of TDF with either d,l-α-methyl-p-tyrosine methyl ester HCl (AMT) or reserpine (RSP). Adult male Long-Evans hooded rats, approximately 70 days of age were used. Dosage-effect functions were determined for AMT (0-200 mg/kg in 1.0 ml/kg saline administered ip 3 hr prior to testing), and RSP (0-4.0 mg/kg in 2.0 ml/kg deionized water administered ip 18 hr prior to testing). Motor activity was measured as photocell interruptions for one hr in figure-eight mazes (Reiter et al., Environ. Hlth. Perspect. 12:119,1975). A non-effective dosage was selected for each compound (AMT = 100 mg/kg, RSP = 0.62 mg/kg). The interaction between TDF and AMT was determined with the following groups: 1) vehicle control; 2) 200 mg/kg TDF (po in 2.0 ml/kg corn oil 1 hr prior to testing); 3) 100 mg/kg AMT; and 4) both AMT and TDF (n=7-8/group). Similar groups were used to test for an interaction between TDF and RSP, with 0.62 mg/kg RSP substituted for the AMT (n=13-16/group). In the first experiment AMT did not block the increased motor activity produced by TDF (i.e., both TDF alone and AMT in combination with TDF produced significant increases in motor activity. Since AMT does not antagonize the effect of TDF, these data suggest that increased motor activity produced by TDF is not mediated through newly synthesized catecholamines. In contrast, pretreatment with RSP blocked the TDF-induced hyperactivity, suggesting that TDF-induced hyperactivity may be due to an interaction with CNS catecholamines stored in reserpine-sensitive pools. However, since RSP also depletes other CNS aminergic transmitters, an interaction with other amines cannot be ruled out.

116.13 THE TEMPERATURE OF THE REARING ENVIRONMENT DETERMINES SENSITIVITY TO STRESS POTENTIATION OF MORPHINE ANALGESIA AND BRAIN TRYPTOPHAN METABOLISM. [3]J.E. Jans*, [1]F.V.Abbott, [2]K.B.J. Franklin and [3]B.C. Woodside. [1]Dept. Psychiatry and Sch. Nursing, [2]Dept. Psychology, McGill University; [3]Dept. Psychology, Concordia University, Montreal, Canada.

Early environmental manipulations e.g handling (Levine 1957) permanently alter the rat's responsivity to stress. We now report that rats reared at room temperature show increased biochemical and behavioral responses to restraint stress compared to rats reared in a nest box warmed to 30-33°, the temperature of natural rodent nest chambers (Daly, 1973).

Restraint is known to potentiate morphine analgesia, an effect that is associated with a rise in brain tryptophan and brain 5HT synthesis (Kelly & Franklin, 1984). We examined the effect of rearing conditions on these phenomena. The subjects were adult male Sprague Dawley rats that had been handled and reared with their dams, in either warm (30-33°C) or cool (20-22°C) nest boxes. They were tested for their responsivity to thermal stimulation (55° water) applied to their tails. Rats were tested before and every 10 min after injection of 4 mg/kg morphine sulphate s.c. One hour after the injection all rats were killed and brainstem and spinal cord samples were assayed for tryptophan, 5HT and 5HIAA by HPLC. Half the rats in each group (n=6) were tested while restrained in wire mesh tubes, the others were gently hand held during each test. All rats had been habituated to the test environment and handling.

Baseline tail flick latencies were similar for all groups. Restraint stress significantly increased morphine analgesia in rats reared in a cool environment ($p<.01$) but had no effect in rats reared in warm conditions. In cool reared animals restraint also increased tryptophan levels in spinal cord ($p<.01$) and brain stem ($p=.05$) but warm reared rats did not show this effect. Curiously restraint increased spinal cord 5HIAA in cool reared rats and depressed 5HIAA in warm reared rats ($p<.05$). There were no differences between the groups in brainstem or spinal cord 5HT.

These results show that the environmental conditions under which rat pups are reared may profoundly influence their susceptibility to stress potentiation of opioid analgesia and the changes in brain trypophan uptake and metabolism associated with it. This effect is not attributable to an early handling effect as both groups received similar amounts of "handling" in infancy.

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116.14 MONOAMINE AND METABOLITE CONTENT IN THE RAT BRAIN DURING THE ESTROUS CYCLE, PREGNANCY, AND POSTPARTUM. W. Woodmansee*, P. Desan, S. Ryan, T. Smock and S. Maier (SPON: H. Alpern). Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

A wide variety of physiological and behavioral changes in the female rat have been associated with the estrous cycle, pregnancy, and postpartum and their accompanying hormonal fluctuations. The most notable changes demonstrated include changes in mating behavior, pain sensitivity, and overall activity of the animal. Previous research has focused on the investigation of monoamine metabolism in the hypothalamus. The present study examined norepinephrine (NE), dopamine (DA), serotonin (5HT) and their primary metabolites (DOPEG, MHPG, DOPAC, and 5HIAA) in the anterior cortex, hippocampus, and cerebellum during the estrous cycle, late pregnancy, and postpartum

Subjects were Sprague Dawley rats individually housed and maintained on a 12:12 light/dark cycle with lights on at 0700. Samples were obtained for each of the four days of the estrous cycle, the 19th day of pregnancy, and the 6th postpartum day. Maintenance of the estrous cycle was assessed by daily vaginal smears and pregnancy was determined by the first sperm-positive day following the introduction of males. The animals were rapidly decapitated between 1400 and 1600 hr and brain samples were dissected and frozen. All samples were sonicated in pH 5.0 0.1M acetate buffer containing the internal standard DHBA and centrifuged. High performance liquid chromatography coupled with electrochemical detection was used to examine the monoamine and metabolite content of the tissue samples. Measurements of all compounds, except NE metabolites DOPEG and MHPG, were obtained with oxidative electrochemical detection. DOPEG and MHPG were assayed by a serial oxidative-reductive detection system as described elsewhere at this meeting (Desan, Gerhardt, Woodmansee, and Smock, 1987).

Results of this study indicated that NE and its metabolites, DOPEG and MHPG, do not vary during the estrous cycle in any of the three brain regions examined. However, serotonergic systems do appear to change during the estrous cycle. Serotonin's principal metabolite, 5HIAA, was significantly higher in proestrus and estrus than on metestrus and diestrus in the anterior cortex and cerebellum ($n=8$, $p<0.05$). Additionally, both noradrenergic and serotonergic systems vary as a function of pregnancy. 5HIAA levels in all brain regions were lower in late pregnancy than in early postpartum, a difference significant in the hippocampus and cerebellum ($n=5$, $p<0.01$). Similarly, NE levels in all brain regions were elevated in early postpartum as compared to late pregnancy, a difference significant in cortex and cerebellum ($n=6$, $p < 0.01$).

Thus, differences in serotonergic function may be related to changes in behavior during the estrous cycle and fluctuation in both serotonergic and noradrenergic function may be related to behavioral changes during pregnancy and postpartum. Supported by a grant from the Colorado Heart Association and NSF grant BNS8520622.

116.15 EFFECTS OF ANOXIA ON SINGLE-UNIT ACTIVITY OF BRAIN STEM NEURONS IN FREELY MOVING CATS. W.J. Litto, C.A. Fornal and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Our laboratory has been engaged in a systematic analysis of the activity of brain stem monoaminergic neurons in behaving cats in response to various physiological and environmental challenges. A major aim of this research has been to define the role of these neurons in CNS mechanisms underlying the stress response. The present study examined the response of monoaminergic and non-monoaminergic neurons to acute histotoxic anoxia in freely moving cats. Extracellular single-unit activity was recorded in the region of the dorsal raphe nucleus (DRN) and locus coeruleus using movable nichrome wire electrodes. Serotonergic (5-HT) neurons were identified on the basis of previously described criteria (see Fornal et al., this meeting). Sodium cyanide (NaCN; 0.05-0.5 mg/kg) was administered remotely, via an indwelling jugular catheter, to produce an acute anoxic condition. Cortical EEG, nuchal EMG, EOG, ECG, respiratory rate (RR) and behavior were monitored continuously for 60-90 min following NaCN injection. In addition, serial blood samples were obtained before and after NaCN administration for the determination of blood glucose. High doses of NaCN (0.4 and 0.5 mg/kg) reliably produced a decrease in EEG amplitude within 10 sec and periods of isoelectric EEG during the first 3 min following injection. These effects were accompanied by polypnea, bradycardia and hyperglycemia, indicative of global anoxia and subsequent chemoreceptor activation. The effects of NaCN on RR and blood glucose lasted for 30-60 min following injection. The transient decrease in heart rate observed immediately after NaCN administration was followed by prolonged tachycardia which then paralleled the RR response. In addition, cats displayed mild seizure activity, salivation, loss of postural support and vocalization, and often, emesis, urination, and defecation during the first 10 min following NaCN injection. None of the above behavioral, autonomic or EEG responses were observed following lower doses of NaCN (0.05-0.25 mg/kg). Preliminary data indicate that the activity of DRN-5-HT neurons is markedly suppressed during the initial interval following high (but not low) doses of NaCN. Since the activity of non-monoaminergic brain stem neurons was either unaffected or increased following high doses of NaCN, this suggests that DRN-5-HT neurons may respond preferentially to anoxia. These experiments are presently being extended to include noradrenergic neurons in the locus coeruleus. Supported by NIMH grant MH23433 and NRSA MH09529 to W.J.L.

116.16 ALPHA-1 ADRENOCEPTORS AND JUVENILE PLAY IN THE RAT. S.M. Siviy, D.M. Atrens* and L.J. Holmes*. Dept. of Psychology, University of Sydney, Sydney, NSW 2006, Australia.

Rough-and-tumble play is common among the juveniles of most mammals. It has only been recently, however, that any experimental analysis has focused on delimiting those neurochemical systems which may mediate this behavior (see Panksepp et al., Neurosci Biobehav Rev 1984, 8:465-492). To further this aim, we evaluated the extent to which alpha-1 adrenoceptors may be involved in the elaboration of play among juvenile rats. Prazosin (PRZ), a selective alpha-1 antagonist, was injected (IP) 20 minutes before a 5 minute opportunity to play. Rats (25-45 days old) were housed individually to amplify the amount of play during the observation periods. At 0.1 and 1.0 mg/kg, PRZ reduced the frequency of pinning, an indicator variable of play, by 26% and 51%, respectively. Dorsal contacts, an index of play motivation, were affected only by the highest dose (1.0 mg/kg), being reduced by 29%. When 1 hour was allowed between injection and test, PRZ (0.5 mg/kg) still reduced pinning by 24%, but had no substantial effect on dorsal contacts. We next evaluated the effect of PRZ with lower baseline levels of play, to allow room for any putative increase in play. The rats were given a 1 hour opportunity to play, injected, and then observed 20 minutes later for 5 minutes. While baseline levels of play during the 5 minute observation period were substantially reduced by the prior opportunity to play, PRZ had no effect on frequency of either pins or dorsal contacts. In preliminary work, we also evaluated the effects of ST587, a selective alpha-1 agonist, on play. While the highest dose of ST587 used (1.0 mg/kg) reduced pinning and dorsal contacts by 19% and 14%, respectively, this dose also resulted in a net 24 hour weight loss when compared to rats treated with vehicle, casting doubt on the extent to which any play reduction could be considered behaviorally specific. Lower doses of ST587 (0.125 - 0.5 mg/kg) had no consistent effect on either pins or dorsal contacts.

Taken together, these data show that acute selective blockade of alpha-1 adrenoceptors results in a state which is not fully compatible with rough-and-tumble play in the rat. The extent to which the observed effects on play following PRZ are behaviorally specific and do not reflect a non-specific behavioral impairment is still unclear. However, given that play motivation was less affected than pinning, there does appear to be some degree of specificity associated with PRZ's action on play. The inability of ST587 to produce effects symmetrical to those of PRZ, on the other hand, would argue against behavioral specificity. Work designed to clarify these issues further is currently in progress.

Supported through an Australian Research Grants Scheme Programme Grant to D.M.A.

116.17 PERIPHERAL ADRENERGIC STIMULATION ATTENUATES PERFORMANCE DEFICITS IN RATS WITH HIPPOCAMPAL LESIONS. J. P. Ryan, D. L. Nock*, and R. L. Isaacson. Center for Neurobehavioral Sciences and Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13901.

Long-Evans hooded rats with bilateral hippocampal lesions, neocortical lesions or sham operations were trained in the Morris water maze. The time required to escape from the maze by climbing onto the stationary hidden platform was measured. For each trial, the place in the maze from which an animal started was determined quasi-randomly within and between training days. Six trials were given each day. After the first three trials, the animal was placed into its home cage for one min before starting the final three trials.

In half the animals, adrenergic supersensitivity was induced with bretylium (5mg/kg, i.p.) administered beginning 24 hr after surgery for 13 consecutive days. On the first 7 days, injections were given with no other handling, testing or training. On postoperative days 8 to 13, injections were given following initial pretraining and actual training (days 9-13). On these days, the animals that had received bretylium also received Norepinephrine (NE) (4μg/kg, i.p.) 30 min prior to maze pretraining and 30 min prior to training. Following this, the animals were neither trained nor tested for 12 days. Then the bretylium alone treatment was resumed for 7 days. After this, animals were again trained in the Morris maze with bretylium and NE as given earlier. When tested at 27 days postoperatively, rats with hippocampal lesions treated with bretylium and NE reached the platform more quickly than the untreated animals with similar lesions. By training day five, these animals were indistinguishable from untreated animals. A similar trend was found at the earlier test period but the lesioned animals behavior was inconsistent from trial to trial. As is known, there is a markedly enhanced adrenergic response to NE after 7 consecutive days of bretylium. However, the mechanism effecting drug-induced attenuation of the hippocampal lesion performance in the water maze is uncertain. The near normal escape time to the platform seen in bretylium and NE animals treated 27 days postoperative may reflect an interaction of the prior training and drug treatment or it may be due to the different neurological conditions found at this later time. These findings suggest that peripheral adrenergic stimulation attenuates performance in rats with hippocampal lesions.

116.18 ENVIRONMENT DEPENDENT SENSITIZATION TO AMPHETAMINE-INDUCED CIRCLING BEHAVIOR IN UNLESIONED FEMALE RATS. K.L. DREW and S.D. GLICK. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208

When paired repeatedly with amphetamine, stimuli such as the test apparatus have been shown to acquire conditioned stimulus properties; i.e., the test apparatus acquires the ability to produce amphetamine-like responses (e.g., Drew and Glick, 1986). Stereotypy, locomotion and circling behavior induced by amphetamine have been shown to increase over the course of repeated drug administration; the role of conditioning in the sensitization to behavioral responses induced by amphetamine has been debated (cf. Robinson, 1986). Here we report environment dependent sensitization to amphetamine-induced circling behavior in intact (unlesioned) female rats. No sensitization independent of the environment was observed regardless of the procedure used or the time interval between amphetamine injections.

All rats received two ip injections of 1.25 mg/kg d-amphetamine sulfate. One half of the rats experienced the effects of the first injection in the home cage and the effects of the second injection in the test apparatus. The other half experienced the effects of both injections in the test apparatus. Rats were further divided into subgroups such that for some the time interval between injections was 7 days and for others it was 24 hrs; for some rats the amphetamine was injected after a 15 min habituation period in the test apparatus and circling behavior was recorded for 60 min while for other rats the drug was injected 30 min prior to placement in the test apparatus and circling behavior was recorded for 30 min.

Regardless of the procedure used or the time interval between amphetamine injections, only those groups of rats that experienced the effects of the first amphetamine injection in the test apparatus displayed significant sensitization following the second amphetamine injection.

While other studies have shown (Browne and Segal, 1977; Robinson, 1984) and still others have suggested (Robinson et al., 1982) that conditioning is not necessary for behavioral sensitization, the present study as well as Tilson and Rech (1973) show that mechanisms independent of the environment are not necessary to produce behavioral sensitization to amphetamine. Both forms of sensitization may contribute to amphetamine-induced psychosis and both provide examples of neural plasticity. (Supported by NIDA grant DA 03817)

117.1 MONOCLONAL ANTI-CONJUGATED ACETYLCHOLINE ANTIBODY AS A RELIABLE MEANS TO STUDY THE TRIGGERING MECHANISMS OF MYASTHENIA GRAVIS. M.L. Souan, J.L. Chagnaud, A. Arné and M. Geffard. Lab. of Neuroimmunology, IBCN/CNRS - 33077 Bordeaux- France.

In addition to anti-acetylcholine receptor antibodies (ACh-R), we have found in the sera from patients suffering of myasthenia gravis (MG) other antibodies consisting of: (i) the antibodies directed against an epitope taking into account the structure of conjugated ACh called anti-conjugated ACh antibodies and (ii) the antibodies directed against the idiotypes of the above-mentioned ones and called anti-anti-conjugated ACh antibodies (Souan et al., 1986,1987; Souan and Geffard 1986). For a better evaluation and characterization of the human anti-anti-conjugated ACh antibodies, we have used a monoclonal anti-conjugated antibody raised from mouse. We have selected the AKR strain as the best responder to immunization with conjugated ACh. Spleen cells from one of this mouse were fused with cells of X63 mouse myeloma cells using polyethylene glycol 1000 as fusing agent. Using an ELISA method, specific antibodies present in cell medium from confluent cultures were tested. Selected hybridomas giving the best antibody affinity and specificity were then cloned twice. In order to determine monoclonal antibody affinity and specificity, competition experiments were performed by RIA. Using the lactoperoxydase method, monoclonal anti-conjugated ACh antibody was iodinated and then, G-25 chromatographed. From displacement curves we domonstrated that the most immunoreactive compound was thotin-glutaryl-protein (IC 50 = 5×10^{-10} M) The other related compounds were less recognized . Such a tool enabled us to confirm our previous results both in the experimental and clinical fields: (i) the administration of this monoclonal anti-conjugated ACh antibody to rabbits allows the raising of anti-idiotypic antibodies whose characterization, performed by RIA, shows that at the level of the antigen-combining site of the anti-conjugated ACh antibody, a popula tion competes with the conjugated ACh. Therefore these anti-idiotypic antibodies represent the internal image of ACh, and can bind to the ACh-R. After six injections, the immunized animals present signes of muscular weakness. The SFEMG recordings confirmed the symptoms suggestive of experimental autoimmune myasthenia gravis; (ii) we are able to evaluate the human anti-anti-conjugated ACh antibodies. The results obtained confirm that their titer in myasthe nic sera was higher than that in control sera. And what is more, when examining the sera from myasthenic patients we found a correlation between the anti-conjugated ACh antibody and the anti-anti-conjugated ACh antibody titers.These data confirm: on one hand the role of conjugated ACh, on the other hand that of the idiotypic/anti-idiotypic network in the triggering mechanisms of the MG autoimmune processes.

117.2 ALPHA-TOCOPHEROL PARTIALLY REVERSES THE IMINODIPROPRIONITRILE (IDPN)-INDUCED DYSKINETIC SYNDROME. J.B. Lohr, J.L. Cadet, T. Bryant*, R.J. Wyatt, W.J. Freed. Neuropsychiatry Branch, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Iminodiproprionitrile (IDPN) is a neurotoxin that causes a permanent motor disorder in rats and mice, consisting of excitement, dystonic or choreoathetoid neck movements, and circling behavior. The pathophysiological mechanism is unknown, but neuroaxonal changes following IDPN have been reported to resemble those seen in vitamin E-deficiency (Miyakawa et al., Acta Neuropath, 20:67, 1972). Moreover, since it is possible that the mechanism of IDPN-induced damage involves free radical production, and α-tocopherol (vitamin E) is a powerful free radical scavenger, we decided to test the hypothesis that treatment with α-tocopherol would decrease the movement disorder seen after IDPN administration.

Male Sprague-Dawley rats (500-600 g) were used in the experiments. Animals were injected daily with IDPN (100 mg/kg i.p.) until dyskinesia developed (14 days), following which they were tested using a modified version of a dyskinesia scale (Diamond, B.I. et al., Adv Neurol, 35:221, 1982). Animals were then assigned randomly to either daily injections of α-tocopherol (2 g/kg i.p., n=16) or of an equivalent volume of sesame oil placebo (n=18) for 7 days. After α-tocopherol, the animals were again evaluated on the dyskinesia scale.

Before treatment with α-tocopherol, there were no differences between the two groups. Following treatment, the α-tocopherol-treated rats demonstrated a significant reduction in vertical neck movements (10.0 ± 3.8 movements/min vs 5.9 ± 1.8 movements/min, t=3.9, $p<0.001$). In addition, there was a significant difference between the treated and untreated groups in the number of rats which demonstrated circling behavior (3/15 vs 10/16, $\chi^2=7.54$, $p<0.01$). There were no differences in hyperactivity.

These results suggest that α-tocopherol can partially reverse the movement disorder produced by the neurotoxin IDPN, and imply that IDPN may cause damage either through a free radical-based mechanism or through interference with the actions of tocopherols.

117.3 EFFECT OF MPTP AND ITS METABOLITES ON CATECHOLAMINES IN MOUSE BRAIN SLICES. J. A. Wilson, T. J. Doyle*, & Y. S. Lau. Depts. of Physiology and Pharmacology, Creighton Univ. Sch. of Med., Omaha, NE 68178.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is able to cause a Parkinson's disease like condition in man and several species of mammals. In idiopathic parkinsonism, symptoms begin to appear after a large percentage of the dopamine containing neurons in the substantia nigra have died. The death of these neurons causes a decrease in the amounts of dopamine available in the target nuclei, the caudate and putamen. Dopamine is thought to modulate corticostriate synaptic transmission; therefore alterations in the available pool of dopamine can be expected to alter synaptic transmission. Brain slices have been used to study synaptic transmission in a variety of brain regions. In the mouse, synaptic transmission between the cortical efferents and the neurons of the neo-striatum has been studied, and MPTP has been found to cause a non-reversible decrease in the amplitude of the synaptically evoked component of an extracellularly recorded field potential (Wilson et al. 1986, Brain Res. 368 357). The effect of MPTP on synaptic transmission in the mouse brain slice was blocked by using the same classes of agents which blocked the long term changes in vivo in mice as well as other species. In the slice one of the metabolites of MPTP, MPP^+, causes only a temporary and reversible decrease in the synaptic potential. This finding contradicts the hypothesis that MPP^+ may be the active toxin. To better understand the differences in the actions of these agents and that of $MPDP^+$, the other candidate for the active toxin, we have treated brain slices with MPTP, $MPDP^+$, and MPP^+ and assayed the tissue for catecholamines using HPLC.

Five hundred micron thick slices containing the neostriatum, substantia nigra, and nearby structures were made exactly as they had been made for physiological studies. They were incubated in an artificial CSF for an hour before drugs were tested. Each drug was then applied for 20 minutes and the tissue was washed. The tissue was fixed in 0.2N perchloric acid solution, sonicated, filtered, and analyzed. An overall decrease in the levels of catecholamines was seen in all the slices incubated in ACSF. MPTP and $MPDP^+$ caused decreases in levels of catecholamines which were different from those caused by MPP^+. Thus neurochemical changes are associated with the electrophysiological changes seen in the mouse brain slice preparation.

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117.4 **DOPAMINE TOXICITY TO PC-12 CELLS, PROTECTION BY SUPEROXIDE DISMUTASE AND CATALASE.** G.M. Alexander, J.R. Grothusen*, J. Joseph*, RL Knobler, and RJ Schwartzman. Department of Neurology, Jefferson Medical College, 1025 Walnut Street - Room 511, Philadelphia, PA 19107

PC-12 cells (rat pheochromocytoma) have been used to screen for compounds that are toxic to catecholaminergic cells. It has been shown by Denton and Howard, (Biochem. Biophys. Res. Commun. 119, 1984) that methylphenyltetrahydropyridine (MPTP) depletes the dopamine content of PC-12 cells and kills them in millimolar concentrations, and by Snyder and D'Amato (Neurology, 36, 1986) that methylphenylpyridine (MPP^+), the oxidation product of MPTP, is 20-50-times more toxic to PC-12 cells than MPTP. In this study, we found dopamine to be as potent as MPP^+ in killing PC-12 cells. This dopamine toxicity is due to the process or products of dopamine autooxidation; since dopamine that is allowed to autooxidize prior to addition to cells is as toxic as dopamine. It is known that oxidation of dopamine results in superoxides, hydrogen peroxide and aminochrome, a precursor of dopamine neuromelanin. We were able to show that addition of the two enzymes superoxide dismustase and catalase to the media completely protects PC-12 cells from lethal concentrations of dopamine. This study supports the hypothesis that dopamine autoxidation plays a key role in the fate of neurons in the pars compacta of the substantia nigra.

117.5

WITHDRAWN

117.6 PERIPHERAL NERVE REPAIR: RELATIONSHIP BETWEEN FUNCTIONAL RECOVERY AND THE IDENTITIES OF REGENERATED MOTONEURONS. J.E. Swett, R.P. Wikholm*, Y. Torigoe, R.H.I. Blanks. Dept. of Anatomy and Neurobiology and Dept. of Surgery, Div. of Otolaryngol.-Head & Neck Surgery, U.C.I., Irvine, CA 92717.

There are often serious problems with conventional repairs of severed peripheral nerves, including poor functional recovery, and/or painful neuroma formation. The amount of recovery depends on factors such as the type of injury (crush, tear, cut), the number of neurons that survived axotomy, the type of nerve repair used and the skill with which it is effected. We describe here an anatomical model for evaluating the consequences of nerve injury and the quality of nerve repair techniques in conjunction with measurement of behavioral recovery. The left sciatic nerve was crushed or cut at mid-thigh levels under deep anesthesia in 57 rats. Crush injuries were allowed to recover without intervention while the cut nerves were reconnected either by epineurial sutures or by a new freeze-trim technique (de Medinaceli et al., Exp. Neurol. 81:488, 1983). Behavioral recovery was measured from weekly records of the animals' walking tracks to obtain the sciatic functional index (SFI) (*ibid.* 77:634, 1982). When behavioral recovery reached a plateau, the common peroneal nerves on both sides were labeled with HRP in the popliteal fossa to obtain accurate numbers of regenerated motoneurons according to the methods of Swett et al. (Exp. Neurol. 93:227, 1986). Their identities were also determined from their topographic locations in the ventral horn. Crush injuries recovered to normal. Freeze-trim repairs were 300-400% better, functionally, than epineurial repairs. Functional recovery did not relate well to the total number of motoneurons that regenerated axons into the peroneal nerve distal to the site of injury. The correlation coefficient was minimal ($r = -0.04$). Conversely, when the amount of functional recovery was compared with the proportion of the regenerated population that was composed of original peroneal motoneurons, the correlation coefficient was very high ($r = 0.88$), producing a regression line intercepting the abscissa at a value of 50% correct proportion for 0% functional recovery. This lower intercept implies that more than half of the regenerating motoneurons contributing axons to a given tributary branch must be correct (original) occupants if there is to be any recovery at all. The other intercept is at 100% functional recovery when the regenerated population is composed entirely, or nearly so, of original peroneal motoneurons, the condition prevailing in crush injuries.

In summary, recovery of motor performance, following surgical nerve repair, was independent of the number of regenerating motoneurons, *per se*. It was best correlated with conditions that favored a return of greater proportions of motoneuronal axons into their original tributary branches. The technique used for surgical repair can have a profound effect on improving the probability that regenerating axons will return to their proper branches. This combined quantitative anatomical and behavioral model is effective for evaluating nerve repair techniques. (Supported by grants NS-17630 and NS-23707 from NIH)

117PO MISDIAGNOSIS OF SPINAL CORD INJURY IN HUMANS. J. Walker. 881 Alma Real Dr., Pacific Palisades, CA 90272,

A complete lesion of the spinal cord is defined by the absence of voluntary motor control, sensation, or proprioception below the lesion. Much of our knowledge of complete injury stems from the pioneering work of Riddoch (Riddoch, G. Brain, 40: 264, 1917) who studied spinal cord injuries caused by transection by machine gun fire. The term "transection" survives in the lexicon of rehabilitation settings to this day, although the overwhelming majority of cases with spinal cord injury are now due to motor vehicle accidents and sports injuries. I now report that the term "complete" spinal cord injury is greatly overused.

There is controversy about when the diagnosis of complete injury should be made. Some claim that the diagnosis should be given 24 to 48 hours after the injury. Other authorities prescribe serial neurological testing for up to two years after the injury (Guttmann, L.S., Spinal Cord Injuries, Blackwell, Great Britain, 1976).

In this study, 99 subjects with chronic traumatic spinal cord injury received a full day evaluation one to 16 years after the trauma. The evaluation consisted of a somatosensory evoked potential, video-taped examination of the motor system, and complete physical and neurological examination. Our diagnosis was then compared to the initial one obtained from medical records. Sixty of the 99 subjects were originally labelled as "complete". The error rate for "complete paraplegia" was 57 out of 60, or 95%.

There are 500,000 people with chronic SCI with an additional 10-15,000 occurrences each year. Even a diagnostic error of 50% would lead to a misdiagnosis of hundreds of thousands of patients. This misdiagnosis gives the patient false pessimism and hopelessness about the possibility of functional improvement and often discourages even minimal participation in pulmonary (Walker, J. and Cooney, M.M., New Eng J Med, 316: 486, 1987) and cardiovascular programs (Cowell, L.L., et al, Med Sci Sports Exercise 18: 501, 1986) which could benefit the patient.

118.1 AUTORADIOGRAPHIC LOCALIZATION OF SUBSTANCE P BINDING SITES IN THE GUINEA-PIG VAS DEFERENS. E. Burcher* and C.J. Mussap* (SPON: B.G. Livett). Biological & Health Sciences, Deakin University, Vic. 3217, Australia.

In this work, we have investigated the presence of binding sites for radiolabeled substance P (SP) in the guinea-pig vas deferens, using in vitro labeling and autoradiography. Slide mounted frozen sections were preincubated in 50 mM Tris HCl (pH 7.4) with 0.02% BSA, then incubated for 2 hours in the same solution with the addition of peptidase inhibitors, 3 mM $MnCl_2$ and 0.1 nM of [^{125}I]-Bolton-Hunter labeled SP (BHSP), prepared and purified as described previously (Burcher et al., *JPET* 326, 819, 1986). Slides were then rinsed in icecold Tris and deionized water, dried, fixed, defatted and coated with Kodak NTB-3 emulsion, exposed for up to 14 days before being developed, stained with pyronin Y and viewed using light and dark field microscopy. Nonspecific binding was defined using 1 μM unlabeled SP. Dense numbers of binding sites were seen in sections labeled with BHSP. Specific binding sites were found over all three smooth muscle layers of the vas deferens, especially the innermost longitudinal layer, with no specific binding seen over the mucosa.

In the guinea-pig vas deferens, SP enhances the twitch response to transmural stimulation, and also has a direct contractile effect on the smooth muscle (von Euler and Hedqvist, *Acta Physiol. Scand.* 90, 651, 1974). SP-like immunofluorescence is found in smooth muscle, adventitia and epithelia of the guinea-pig vas deferens, (Alm et al., *Neurosci.* 3, 419, 1978). The present data showing binding sites for BHSP on smooth muscle of the guinea-pig vas deferens are in accordance with previous findings suggesting a role for SP in this tissue.

Supported by the National Health & Medical Research Council of Australia.

118.2 ISOLATION, PURIFICATION AND IDENTIFICATION OF SUBSTANCE P MEMBRANE RECEPTORS. M. L. SWENBERG*.(SPON: D. M. Chuang). Hypertension and Endocrine Branch, NHLBI, NIH. Bethesda, MD 20892.

Considerable progress has been achieved in the last few years in localization of substance P (SP) receptors (Rc) and their binding properties. However there are no reports of isolation and purification of the SP receptors (SPRc). Therefore we searched various tissues enriched with SPRc as source for these sstudies.

Fresh frozen tissue sections (20 μ) of brain, olfactory bulb, intestine, stomach and uterus from decapitated Sprague Dawley rats were mounted on slides and dried under vaccum at 4°C overnight. SP receptors were localized with ^{125}I-SP labeled with Bolton-Hunter reagent(BHSP). Isolated membranes of olfactory bulb and intestinal mucosa were extracted with 1% Chapso(3[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propanesulfonate) and the extracts were centrifuged and supernatant was purified with G-25 column to remove surfactant, then with wheat germ lechtin affinity column and eluted with N-acetylglucosamine. Further purification and chemical identification were carried out by isoelectric focusing electrophoresis on either sucrose gradient or acrylamide gel.

The antiserum was obtained by immunizing New Zealand white rabbits with purified olfactory receptor protein and tested on guinea pig ileum for its biological activity. The receptor proteins were tested for the binding effect of BHSP to the Anti-SP antibodies and the receptor in localization on tissue sections.

SP receptors were found most densely distributed in olfactory bulb and in all the lining tissues of hollow organs studied. Anti-SP-receptor antibodies at extremely low concentration (100,000X) exhibited stimulatory effect on SP induced contractility on guinea pig ileum. At higher concentration the antiserum alone also induced contraction on the guinea pig ileum.

Receptors isolated from olfactory bulb and intestine are different in their chemical composition. There were at least 2 components in each receptor but with different isoelectric points (IP) 6.2±0.2 and 5.4±0.2 for the brain and 5.4±0.2 and 4.5±0.2 for the intestine. Brain receptors enhanced BHSP binding with anti-SP but the intestinal receptors showed very high inhibition at the same protein concentration. Since the bound and free BHSP were separated by dextran coated charcoal that bound the free, the inhibition shown by the intestinal receptor might be due to the inability of charcoal to distinguish between the BHSP-Rc(intestine) and BHSP.

The results shown above in addition to the specific binding of BHSP to receptor affinity gel(1 ml of Affi-Gel-10 coupled with SPRc of 100 ng/ml) strongly suggest that the proteins obtained in this study are indeed SP receptors.

There are different chemical properties between the receptors isolated from olfactory bulb and intestine.

118.3 A GTP-INSENSITIVE COMPONENT OF HIGH AFFINITY SUBSTANCE-P BINDING TO RECEPTORS FROM RAT SALIVARY GLAND. J.A. Luber-Narod, N.D. Boyd,* B. Oblas,* and S.E. Leeman. Dept. of Physiology, Univ. of Mass. Med. Ctr., Worcester, MA 01655

A component of ^{125}I-Bolton Hunter conjugated substance P (^{125}I-BHSP) binding to membrane-bound and 0.5% digitonin solubilized receptors from rat submaxillary gland is dependent on coupling to a GTP-binding protein. We report here that there is an additional GTP-insensitive component. 10-20% of the total equilibrium ^{125}I-BHSP binding to submaxillary gland membrane-bound receptors is uneffected by incubation with a maximal concentration of GppNHp (100 μM). 20-40% of the binding of ^{125}I-BHSP (assayed via filtration after precipitation with protamine sulfate and polyethylene glycol) remains bound to prelabeled digitonin-solubilized receptors after 30 min in the presence of GppNHp (100 μM). Using Scatchard analysis, the affinity of the residual ^{125}I-BHSP membrane-bound sites is similar to the affinity before GppNHp treatment. Kinetic analysis of ^{125}I-BHSP dissociation from membrane-bound and digitonin-solubilized receptors also indicates a GppNHp-insensitive component. Rates assessed at 4°C in the presence of 100 μM GppNHp are biphasic with an initial rapid phase ($T_{1/2}$ = 4 min) and a second slow phase ($T_{1/2}$ = 120 min). This second phase is similar to the rate seen in the absence of GppNHp indicating that a fraction of the sites are not effected by GppNHp. Alkaline (pH 11.5, 30 min) pretreatment of submaxillary gland membranes, a method known to inactivate guanine nucleotide binding proteins, partially inhibits ^{125}I-BHSP binding leaving a residual, insensitive component constituting 10% of total ^{125}I-BHSP binding. Likewise pretreatment of the membranes for 1/2 hr. with varying concentrations (0.01-10mM) of the sulfhydryl reagent N-ethylmaleimide prior to incubation with ^{125}I-BHSP decreases the total equilibrium binding with a maximal effect at 0.1mM leaving a residual insensitive component of ~20% which is not inhibited even at much higher concentrations.

We conclude that while a guanine nucleotide-binding protein is necessary for high affinity ^{125}I-BHSP binding to the majority of receptors in the rat submaxillary gland, 10-20% of the ^{125}I-BHSP binding sites are not coupled to such a protein and may have a different mode of action.

118.4 RECONSTITUTION OF HIGH AFFINITY SUBSTANCE P-BINDING BY PURIFIED GUANINE NUCLEOTIDE REGULATORY PROTEINS. N.D. Boyd,* J.A. Luber-Narod, B. Oblas* and S.E. Leeman. Dept. of Physiology, Univ. of Mass. Med. Ctr., Worcester, MA 01655

The binding of substance P (SP) to receptors in both neuronal and non-neuronal tissues is subject to regulation by guanine nucleotides suggesting the possibility of a functional interaction between SP receptors and guanine nucleotide-binding regulatory proteins (G-proteins). To provide more direct evidence for the role of a G-protein in the high affinity binding of SP to its receptor, a receptor-enriched membrane preparation from rat submaxillary gland membranes was pretreated with buffer at pH = 11.5, conditions that have been shown to inactivate both G_s and G_i and to decrease high affinity agonist binding to receptors that are regulated by these proteins. Scatchard analysis of the binding of ^{125}I-Bolton Hunter conjugated SP (^{125}I-BHSP) to alkaline treated membranes indicated a decrease in the B_{max} of about 90%. To confirm that this loss of high affinity binding was due to an effect of alkaline treatment on a G-protein rather than on the SP receptor, alkaline treated membranes were reconstituted with a purified preparation of G-proteins (G_i/G_o) from bovine brain. Reconstitution was achieved either by PEG-induced fusion with G-protein containing phospholipid vesicles or, more simply, by incubation of the treated membranes with the 0.3% cholate solubilized G-protein preparation and gradual 20-fold dilution of the detergent. Under both conditions, increasing the amount of G_i/G_o resulted in a concentration dependent restoration of high affinity ^{125}I-BHSP binding. A maximal effect (about 80% recovery of the GTP-sensitive high affinity binding, Kd = 1nM) was achieved at a 150-200 fold molar excess of G-proteins over SP receptors. These results indicate that a G-protein is the target of alkaline treatment and that reconstitution with G_i and/or G_o can restore the high affinity agonist binding. The relative ease and sensitivity of this reconstitution assay indicates that it can be used in the identification and isolation from rat salivary gland of specific G-proteins capable of functionally coupling to SP receptors.

118.5 **OPPOSITE EVOLUTION OF SUBSTANCE P AND ELEDOISIN BINDING SITES IN THE VERTEBRATE BRAIN: APPARENT ABSENCE OF THE NK3 RECEPTOR SUBTYPE IN THE HUMAN BRAIN.** M.M. Dietl* and J.M. Palacios (SPON. W. Sieghart). Preclinical Research, SANDOZ LTD., Basle, Switzerland, CH-4002.

Quantitative receptor autoradiography was used to examine the characteristics and distribution of tachykinin receptor subtypes in vertebrate brains. [^{125}I]Bolton-Hunter substance P (BHSP) was used to label the SP-P (NK1) subtype, [^{125}I]Bolton-Hunter substance K (BHSK) for the SP-K (NK2) and [^{125}I]Bolton-Hunter eledoisin (BHEle) for the SP-E (NK3) receptor subtypes. BHSP binding sites were present in low densities in the brains of lower vertebrates such as fish, frog and snake. An increase in BHSP binding was observed in pigeons and all mammalian species investigated, including man. In contrast, BHEle binding sites were rich in fish, frog, snake, pigeon and rat brains, but decreased in some mammals (mice, guinea-pig, cat) and were apparently absent in monkey and human brains. BHSK labeled sites with a distribution similar to that detected by BHEle. In species presenting both tachykinin receptors, the regional distribution of these two subtypes was different. Some species differences in the distribution of BHSP binding sites or BHEle binding sites were also observed. SP-E (NK3) receptors were enriched in the accessory olfactory bulb, the intermediate layers of the neocortex, portions of the hippocampus, hypothalamic supra-optic and paraventricular nuclei, central portions of the interpeduncular nucleus, substantia nigra, solitary tract and substantia gelatinosa of the spinal cord. SP-P (NK1) receptors were enriched in the olfactory bulb (plexiform layers), corpus striatum, hippocampus, septum, certain thalamic nuclei, superior colliculus and substantia gelatinosa of the spinal cord, the neocortex being moderately labeled and the substantia nigra showing no significant densities. We conclude that in the vertebrate brain an opposite evolutionary trend is observed for two of the subtypes of the tachykinin receptors. SP-P (NK1) receptors appear to be preserved during evolution and increase in density and regional distribution in higher vertebrates. The contrary occurs with the SP-E (NK3) receptors, which are more abundant in the lower vertebrates and apparently are absent from the human brain. These results suggest that the predominant tachykinin peptide in the human brain is probably SP.

118.6 CHARACTERIZATION OF SUBSTANCE P RECEPTOR ON CULTURED RAT ASTROCYTES. C.W. Shults,o+ P. Johnston,*o+ and J. Bressler++: oVA Med. Ctr. V-127, San Diego, CA 92161, +Dept. Neurosciences, Univ. of Calif., San Diego, La Jolla, CA, ++Lab. Neurobiology, NINCDS, Bethesda, MD.

In astrocytes cultured from the cortices of neonatal rat pups the stimulation of cAMP production by norepinephrine has been shown to be modified by substance P (Rougon, G. et al., Nature 305:715-717, 1983). We have characterized the substance P (SP) receptor in cultured rat astrocytes. Primary cultures of glial cells were prepared from the cortices of 1-2 day-old rat pups. After the cells had become confluent, purified cultures of astrocytes were prepared by shaking the cultures on an orbital shaker at 250 rpm overnight and then subculturing the astrocytes, which remain adherent to the flask (McCarthy K.D. and de Vellis, J., J. Cell Biology, 85:890-902, 1980). Subcultured astrocytes were plated at 100,000 cells/cm^2 and binding studies were performed 2-3 weeks later. Our radioligand was SP labelled with ^{125}I by the Bolton Hunter method (^{125}I-BH-SP). Pharmacological characterization of the ^{125}I-BH-SP binding site by use of competition studies with related tachykinins and fragments of SP demonstrated a pattern similar to that previously demonstrated in the rat brain using in vitro homogenate and section binding techniques and in cultured mouse neurons. The IC_{50}'s were:

SP	$7.2\pm2.5 \times 10^{-10}$M
physalaemin	$6.2\pm0.8 \times 10^{-9}$M
neurokinin A	$4.0\pm0.9 \times 10^{-8}$M
neurokinin B	$7.0\pm2.2 \times 10^{-8}$M
SP 2-11	$3.2\pm0.8 \times 10^{-9}$M
SP 4-11	$3.0\pm2.1 \times 10^{-8}$M
SP 6-11	$7.2\pm4.0 \times 10^{-7}$M
SP-COOH	$>1 \times 10^{-6}$M

The pharmacologic studies indicate that the receptor has relatively low affinity for neurokinin A and B, tachykinins also found in the rat brain which share a common carboxyl terminus with SP. Also, sequential elimination of the amino terminus amino acids causes a progressive reduction in affinity. Finally, the amide group on the carboxyl terminus appears to be crucial for binding as demonstrated by the weak displacement of SP-COOH. Saturation studies and Scatchard analysis indicated that SP bound to the receptor with high affinity K_D-$8.9\pm1.9 \times 10^{-10}$M, B_{max} - 11.8 ± 5.7 fentomoles/2 cm^2 well. The Hill coefficient was 0.94 ± 0.06.

These studies provide characterization of the SP receptor studied previously by Rougon and extend the work of Torrens and his coworkers (Torrens, Y. et al., Proc. Nat. Acad. Sci., U.S.A., 83:9216-9220, 1986) which characterized a SP receptor in cultured mouse astrocytes.

118.7 DEVELOPMENTAL CHANGES OF SUBSTANCE P RECEPTORS IN THE RAT BRAIN. C.G. Charlton and C.J. Helke, Meharry Medical College, Nashville, TN and USUHS, Bethesda, MD.

Studies of the physiology and the distribution of receptors and immunoreactivity for substance P (SP) show a role of SP in neuronal functions. These functions show ontogenic changes, which if mediated by SP, suggest that the peptide and its receptors may also undergo postnatal changes. Interestingly, ontogenic changes of SP-like cell bodies and SP receptors have been reported. However, receptor changes in specific brain nuclei, that may be related to the actions of SP, were not studied. In this study membrane homogenate binding and light microscopic autoradiography of binding of ^{125}I-Bolton-Hunter SP were used to characterize SP receptors and study their ontogeny in specific brain nuclei in rats.

The binding was saturable and reversible, with an IC50 of 0.1 nM. Two classes of receptors with Kds of 0.07 and 5.9 nM and Bmax of 23 and 130 fmol/mg protein, respectively, were identified in whole rat brain. The autoradiograms showed that binding in the motor (MC) and somatosensory (SSC) frontoparietial cortex of 1 and 3 day old pups was of low density and poorly laminated, but increased in density and lamination as the rats aged. The reverse occurred in the primary olfactory cortex (POC), where the density of binding was higher in the 1 and 3 day old pups than in older rats. SP binding in the caudate-putamen, septum and nucleus accumbens varies inversely to age. SP receptors in the hindbrain of young pups were diffusely distributed and of higher density than in older rats, in which the receptors became more defined and distinct in the nucleus ambiguus, nucleus of the solitary tract, hypoglossal nucleus and the dorsal motor nucleus of vagus. Membrane homogenate studies showed that the ratio for the concentration (CPM/mg protein) of specific binding was 9: 2.5:2:1 for rats 11, 38, 90, and 260 days, and the ratio for the total bound/brain was 2.5:2.1:2:1. These data suggest that the inverse relationship of the mean density of SP receptors to age was not due entirely to increases in brain mass.

The high density of SP receptors in the POC in rat pups may be correlated with the early maturation of olfactory processes. Conversely, the low density of receptors in the MC and SSC may be correlated with the undeveloped motor and somatosensory processes in young rats. Similarly, the ontogenic changes of receptors in the hindbrain nuclei may be related to developmental changes in the functions that these nuclei subserve.

118.8 BIOSYNTHESIS AND GLYCOSYLATION OF THE LYMPHOCYTE SUBSTANCE P RECEPTOR. M.L. Organist*, J.P. McGillis, D.G. Payan* (SPON: Z. Hall). Dept. of Medicine and Howard Hughes Medical Institute, University of California, San Francisco, San Francisco,CA 94143

Cellular membrane receptors for the neuropeptide Substance P (SP) have been identified in the CNS, on human peripheral blood T lymphocytes, and in the cultured lymphoblast cell line, IM-9. In addition to its neurotransmitter properties, SP exhibits immuno- stimulatory activities which include the enhancement of T cell proliferation. Results from the present studies suggest that the lymphocyte SP receptor is translated as a precursor protein that is glycosylated.

IM-9 lymphoblasts were incubated for 14 hrs in the presence of ^{35}S-met with or without 3 ug/ml of tunicamycin. Cells were lysed and the SP receptor proteins were immunoprecipitated with a monoclonal antibody directed against the receptor, αSPR. SDS-PAGE analysis of untreated cellular lysates revealed specifically precipitated proteins of 38 kD and 33 kD. In tunicamycin treated cells, whose SP binding was not effected, the major immunoprecipitated protein had an apparent mw of 29 kD. Further glycosylation studies were done on the immunoprecipitated 38 kD receptor protein which had been separated on SDS-gels. To characterize the types of N linked sugars present, the 38 kD protein was digested with the enzymes N-glycanase, endoglycosidase F, and neuraminidase which cleave all N linked sugars, high mannose and complex carbo- hydrates and sialic acid residues, respectively. N-glycanase and Endo F both cleave the 38 kD receptor to a protein of 30 kD and neuraminidase causes a shift of 2 kD to give a protein of 36 kD. These studies indicate that the mature SP receptor is a glycoprotein of 38 kD with complex N-linked carbohydrates present.

Post translational processing of the receptor was studied by pulse chase analysis with ^{35}S-met. SDS-PAGE analysis of immunoprecipitates of cells pulsed with ^{35}S-met and chased with an excess of cold met for time periods of 30 min to 18 hrs identified 3 proteins of molecular weights 38 kD, 36 kD and 33 kD. Pulse chase analysis of IM-9 receptor proteins on cells downregulated by preincubation with SP suggested that expression of the receptor is regulated in part by the ligand. Preincubation of IM-9 cells with 1 uM SP for an 18 hr period decreases the number of active SP receptors by 60%, which correlates well with the decrease in receptor biosynthesis seen in pulse chase analysis. Studies are being done to determine if this decrease is due to increased receptor degradation, decreased receptor synthesis and/or receptor mediated endocytosis. In summary, the SP receptor on lymphoblasts appears to be a 29 kD protein which is glycosylated with complex N-linked sugars to form a mature receptor protein of MW 38,000.

118.9 TACHYKININ NK-2 RECEPTORS IN HAMSTER URINARY BLADDER POSSESS A SIMILAR SENSITIVITY TO CATIONS AND GUANINE NUCLEOTIDES BUT A DIFFERENT PHARMACOLOGICAL BINDING PROFILE COMPARED TO NK-1 RECEPTORS IN RAT SALIVARY GLAND. S.H. Buck, M.M. Racke* and J.L. Krstenansky*. Merrell Dow Res. Institute, Cincinnati, OH 45215

Physiological investigations and ligand binding studies have led to the hypothesis that there are three mammalian tachykinin peptide receptors: NK-1 with preferential affinity for substance P (SP), NK-2 with preferential affinity for neurokinin A (NKA), and NK-3 with preferential affinity for neurokinin B (NKB). In the periphery, some tissues seem to contain NK-1 and NK-2 receptors (e.g., rat bladder), some seem to contain only NK-1 (e.g., rat salivary gland), and some seem to contain primarily NK-2 (e.g., hamster bladder). We have used the latter two tissues in standard filtration binding assays to compare the biochemical and pharmacological profiles of NK-1 and NK-2 binding sites. In hamster bladder membranes, ^{125}iodohistidyl^{1}-NKA (INKA) binding was linear with tissue concentration, 80-90% specific, and was saturated at 10-15 nM. Scatchard analysis revealed a K_D of 4 nM and B_{max} of 10 fmole/mg tissue. Specific binding was enhanced by $Mn^{++} > Mg^{++} > Ca^{++}$ with an optimal effect at 2 mM Mn^{++}. The Mn^{++} effect was attributable to a reduced K_D (1 nM) and an increased B_{max} (15 fmole/mg tissue). Mn^{++} reduced the INKA association rate and markedly increased the dissociation $T_{1/2}$. Monovalent cations inhibited binding only at concentrations well above 100 mM. GppNHp inhibited up to 60% of INKA binding with an IC_{50} of 30 nM in the presence of Mn^{++} and 3 µM in the absence of Mn^{++}. AppNHp was inactive up to 10 µM. INKA binding was antagonized by preincubation of bladder membranes with phenoxybenzamine (PBZ) concentrations as low as 30 µM whereas salivary binding of ^{125}I-Bolton-Hunter-SP (BHSP) was only slightly inhibited by 300 µM PBZ. In competition of bladder INKA binding, NKA (K_I = 1 nM) was the most potent followed by KAS>ELE>NKB>SP>PHYLLO>PHY>UPE. NKA(3-10) (K_I = 1 nM) was as potent as NKA whereas NKA(5-10) (K_I = 200 nM) was much less potent and NKA(1-5) and NKA (1-7) were inactive up to 10 µM. Phosphatidylinositol (PI) turnover was stimulated by NKA (EC_{50} = 10 nM) and other tachykinins in a similar rank order in bladder tissue. In competition of salivary BHSP binding, SP (K_I = 0.2 nM) was the most potent followed by PHYLLO>PHY>NKA>UPE>ELE>KAS>NKB. SP(2-11) (K_I = 0.3 nM) was as potent as SP whereas SP(3-11) and smaller fragments were substantially less potent. SP N-terminal fragments were inactive up to 10 µM. NKA(3-10) and NKA(5-10) were weak competitors (K_I = 100 nM) of BHSP binding. The dogfish, NKA-like cyclic tachykinin scyliorhinin II (SCY II) and SCY II(7-18) had poor affinity for NK-2 sites (K_I = 1000 nM each) whereas SCY II (K_I = 200 nM) had higher and SCY II(7-18) (K_I = 5000 nM) had lower affinity for NK-1 sites. Thus, NK-1 and NK-2 receptors possess pharmacologically distinct agonist affinity profiles but similar biochemical characteristics and linkage to the inositol phosphate second messenger system.

NEUROENDOCRINE CONTROLS: PITUITARY III

119.1 ONTOGENY OF PROOPIOMELANOCORTIN-DERIVED PEPTIDE HORMONE SECRETION IN THE RAT PITUITARY GLAND. D.I. Lugo and J.E. Pintar. Dept. of Anatomy and Cell Biology, Columbia Univesity P&S, New York, NY 10032.

Proopiomelanocortin (POMC)-producing cells are present in both the adult anterior lobe (corticotrophs, 5-10%) and intermediate lobe (melanotrophs 100%). Both cell types are derived from a single embryonic rudiment (Rathke's pouch) and synthesize the same precursor but differ in their adult pattern of precursor processing and regulation of hormone secretion. Anterior lobe (AL) secretion is primarily regulated by CRH(+) and glucocorticoids(-), while intermediate lobe secretion is regulated negatively by dopamine. No study thus far has assessed directly the secretory capabilities of POMC producing cells prior to birth. Here we have utilized the reverse hemoloytic plaque assay to study the ontogeny of basal and regulated secretion by POMC producing cells during rat pituitary development. Basal secretion of B-endorphin from trypan blue excluding cells was first observed at embryonic day 13.5 (e13.5), the first age when POMC-related peptides were first detected immunocytochemically, and decreased with developmental age. CRH (10^{-9}M)-stimulated secretion was not detectable until e15.5. Beginning at e17, the anterior and neurointermediate lobes were assayed separately. CRH (10^{-9}M) increased both the amount of peptide secreted by individual corticotrophs and the number of detectable secreting corticotrophs in the AL. The slides were subsequently processed for B-endorphin immunocytochemistry. It was observed that even upon addition of CRH (10^{-7}M) approximately 23% of the anterior lobe-derived cells that were immunostained did not secrete detectable levels of B-EP related peptides.

Inhibition by glucocorticoids (dexamethasome 10^{-6}M; 1 hr pretreatment) of CRH (10^{-7}M)-stimulated secretion was observed in corticotrophs at the earliest stages thus far examined (e17.5). The plaque size following glucocorticoid inhibition was similar to that from non-CRH stimulated cells. CRH (10^{-7}M) stimulation of IL melanotroph secretion was first observed at e19 and continued postnatally. Dopamine (10^{-6}M) inhibition of melanotroph secretion was observed at the earliest stages examined (e17.5).

These results demonstrate that the POMC cells of the rat pituitary gland are capable of hormone secretion as early as peptides are first detected immunocytochemically (Lugo and Pintar, in submission). Moreover these cells respond in-vitro to physiological regulators in an adult-like manner many days prior to birth. The latter results demonstrate that these cells possess functional regulatory receptor systems prior to maturation of the hypothalamic-hypophyseal portal system.

119.2 GONADAL STEROID TREATMENT DOES NOT CHANGE BRAIN POSTTRANSLATIONAL PROCESSING OF β-ENDORPHIN L.A. Berglund*, W.R. Millington, C. Manzini*, G.P. Mueller, J.W. Simpkins, Department of Pharmacodynamics, University of Florida, Gainesville, FL 32610 and Department of Physiology, USUHS, Bethesda, MD 20814

Our previous studies demonstrated reduced sensitivity to morphine in female rats treated with ovarian steroids. Morphine responses appeared to be inversely related to the magnitude of the steroid-induced LH surge, suggesting that the mechanisms which mediate morphine effects are rendered insensitive during the LH surge. The present study was undertaken to determine if a steroid-induced change in the posttranslational processing of β-endorphin (β END) may account for the observed desensitization to morphine. We examined the effects of ovarian steroid treatments on posttranslational processing of β END in the preoptic area (POA), medial basal hypothalamus (MBH), brainstem (BS) and neurointermediate lobe of the pituitary (NIL). Ovariectomized rats received no steroids; 7.5 ug estradiol benzoate at 1000 h 2 days before the experiment; or EB treatment at 1000 h and 5 mg progesterone 48 h later. At 1600-1700 h rats were decapitated and tissues were removed and frozen on dry ice until extraction by boiling in 1N acetic acid for 20 min and sonication. β END peptides were separated by ion-exchange chromatography using a SP-Sephadex C-25 column and eluted with 50% acetic acid solution containing a NaCl gradient (0.05 to 0.55M). Fractions (1 ml) were lyophilized and reconstituted in assay buffer, and β END was measured by RIA. Each treatment group (n = 10) was run twice with sample pools from 5 rats each. NIL samples demonstrated the highest degree of posttranslational β END processing with 93% of the β END activity eluting as N-acetyl- β END (1-26, 1-27, or 1-31) and 3% eluting as β END 1-31. MBH and POA areas contained β END activity eluting as β Lipotropin, β END 1-31, β END 1-27 and β END 1-26 in equal amounts and less than 1% eluting as N-acetylated peptides. BS tissues exhibited β END 1-31 as the primary product, with smaller quantities eluting as β END 1-26 and β END 1-27. Less than 2% eluted as N-acetylated peptides. None of the brain regions examined in this experiment demonstrated any differences in total β END or posttranslational processing patterns due to steroid treatment. These data indicate that the decreased responsiveness to morphine associated with the steroid-induced LH surge cannot be explained by total β END content changes or by β END biological activity changes due to differential posttranslational processing products. Supported by AG 02021 and USAMRDC 86PP6813.

119.3 ACUTE STRESS AND CHRONIC STRESS RELEASE DIFFERENT FORMS OF B-END-IR FROM ANTERIOR LOBE. E.A. Young and H. Akil (Spon: J.F. Greden). Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109.

We have previously reported that anterior lobe releases 2-fold more B-endorphin than B-LPH with acute stress in vivo or with oCRF stimulation of short-term anterior lobe suspension in vitro. This occurs despite the presence of B-LPH as the predominant form of B-endorphin-IR in anterior pituitary. The continued release of B-endorphin over B-LPH is also observed in short-term anterior lobe suspension from control and acutely stressed rats, despite the depletion of ACTH and B-endorphin content to 80% of control. This suggests an extremely rapid replenishment of the B-endorphin stores with acute stress. This is confirmed by pulse chase biosynthetic studies of POMC biosynthesis and processing by Shiomi, Kelsey and Akil in anterior lobe following acute stress.

Chronic footshock stress induces the anterior lobe leading to a doubling of the content of ACTH, B-END and B-LPH. It does not appear to induce changes in the ratio of B-LPH to B-END in the anterior lobe between control and chronically stressed animals. Although the anterior pituitary of chronically stressed rat stores normal ratios of B-LPH:BE (2:1), when the animals are restressed, B-LPH is the predominant form of B-endorphin-IR released with a ratio of B-END to B-LPH of 0.8:1. In short-term anterior lobe suspensions, we see this same change in oCRF stimulated release from predominantly B-END (2:1 ratio) to equimolar release of B-END to B-LPH (1.1:1). Again, this same stress paradigm has been demonstrated to decrease the rate of processing of POMC precursor to B-LPH and B-endorphin (Shiomi, Kelsey and Akil). Since this change in processing may be related to chronic CRF drive, we examined plasma from 7 day and 14 day adrenalectomized rats and see the same predominance of B-LPH over B-endorphin. This suggests that during times of high demand, the last step in the processing of B-endorphin is unable to keep pace with biosynthesis of POMC.

119.4 β-ENDORPHIN LEVELS IN TERM AND PREMATURE NEONATES FOLLOWING PERINATAL STRESS AND/OR ASPHYXIA

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Plasma β-endorphin levels were evaluated in neonates in the well baby nursery (n=26) and NICU (n=121) at University Hospital, Omaha, NE within 24 hours of birth. In twenty seven NICU infants additional samples were taken at 2 week intervals during NICU hospitalization. CSF samples also were evaluated in some infants. Data were evaluated with regard to general demographics (i.e. gestational age, birthweight, Apgar scores, and whether inborn or outborn); maternal history and mode of delivery; and the infants status at the time of sampling (general diagnosis codes, respiratory status and subjective evaluation of stress level). Mean plasma β-endorphin level for normal term newborns was 35.6 ± 12.4 pg/ml (4 infants were delivered by repeat C-section; all others were vaginal deliveries). Term NICU infants (n-28) had plasma β-endorphin levels that had a biphasic distribution. While one group fell within the normal range for term well babies, the second group had elevated levels. Forty-three percent of term NICU infants had levels at least two standard deviations above the mean for normal newborns. Premature NICU infants grouped by demographic factors had significantly elevated plasma β-endorphin levels. For example, 35% of infants 25-32 weeks gestation (n=26) and 53% of infants 33-39 weeks gestation had β-endorphin levels significantly higher (95% confidence level) than normal term infants. Stress (transport, major cardiovascular defects, mild/moderate birth asphyxia and/or meconium aspiration) were correlated with a significant elevation in plasma β-endorphin. In addition, infants under long-term treatment for apnea and similar problems in respiratory control continued to exhibit elevated β-endorphin levels beyond the perinatal period. Plasma β-endorphin levels can be one means for evaluating neonatal well being particularly with regard to an infant's ability to mount a neuroendocrine stress response.

119.5 ACTH, PROLACTIN, CORTICOSTERONE AND PITUITARY CYCLIC AMP RESPONSES TO REPEATED 15 MIN ACUTE STRESS EXPOSURES SEPARATED BY 45 MIN RECOVERY PERIODS. G. J. Kant, T.E. Eggleston*, G.C. Driver*, C.C. Kenion*, E. H. Mougey* AND J.L. Meyerhoff. Dept of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307-5100.

The stress-induced release of ACTH from the pituitary gland is regulated primarily via release of the hypothalamic peptide CRF. In vitro studies have shown that the release of ACTH by CRF is mediated via a cyclic AMP mechanism. We have reported that acute stress increases levels of pituitary cyclic AMP in vivo and that CRF is the most likely mediator of this in vivo response. Injection of exogenous CRF increases plasma levels of ACTH; however, plasma ACTH responses to repeated injections of exogenous CRF have been reported to be attenuated in animals receiving a second CRF injection within 2 hrs of the first. The present experiment was conducted to determine whether the responses of ACTH and pituitary cyclic AMP would attenuate upon repeated exposure to endogenous CRF generated via acute stressor exposure. Stress responses of plasma prolactin (a pituitary hormone not regulated via CRF) and corticosterone (an adrenal hormone regulated by ACTH) were also determined.

Control rats were sacrificed by decapitation immediately upon removal from their home cage. Stressed rats were exposed to one, two or three sessions of 15 min of forced running in a drum revolving at 6 rpm. Between sessions, rats were returned to their home cages for 45 min. Animals were either sacrificed immediately upon removal from their final stress session or after a 45 min recovery period. Trunk blood was collected with heparin and aprotinin (a protease inhibitor) and plasma subsequently frozen for later RIA of ACTH, corticosterone and prolactin. Pituitaries were rapidly weighed and heated in 90°C buffer to prevent post-mortem changes in cyclic AMP. Following sonication and centrifugation, supernatants were frozen until later RIA for cyclic AMP.

Acute stress quadrupled levels of plasma ACTH. In animals subjected to repeated stress exposures, the increase in plasma ACTH was similar following each stress exposure. Levels of ACTH returned to control levels after the 45 min recovery period. Pituitary cyclic AMP and other measured hormonal responses were similar to that seen for ACTH, i.e. similar responses to each stress period with return to control or near control levels between stress sessions. These results demonstrate that pituitary hormonal responses to brief stress exposures of moderate intensity do not attenuate upon repeated exposures, suggesting that the amount of CRF released by this stress is not sufficient in magnitude or duration to directly desensitize pituitary CRF receptors to a subsequent CRF stimulus 45 min later or to elevate corticosterone levels sufficiently to decrease CRF release via negative feedback.

119.6 CRF STIMULATION OF CYCLIC AMP ACCUMULATION, CYCLIC AMP DEPENDENT PROTEIN KINASE ACTIVITY AND ACTH RELEASE IN ANTERIOR PITUITARY CELLS PREPARED FROM CONTROL VERSUS STRESSED RATS. G.S. Dhillon*, C. Wormley*, C.P. D'Angelo* and G.J. Kant. (SPON= R.M Wylie) Dept of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307-5100.

Corticotropin releasing factor (CRF) is a 41-amino acid peptide that is thought to be the primary physiological regulator of pituitary ACTH release. It has been suggested that the action of CRF upon ACTH release is mediated by the stimulation of pituitary adenylate cyclase and subsequent activation of cyclic AMP dependent protein kinase (A-kinase). It has been recently reported that pituitary CRF receptors are down regulated after adrenalectomy or after 48 hours of immobilization stress. After adrenalectomy pituitary adenylate cyclase is less responsive to CRF stimulation. The present experiments were conducted to compare the effects of CRF on pituitary cyclic AMP response and ACTH release in control versus chronically stressed rats. The chronic stresser in the present study was 72 hours of food restriction.

Male Sprague Dawley rats (150-175g) were limited to one hour of feeding every day for three days. Water was freely available. Control animals had free access to food and water. The rats were then decapitated and anterior pituitary cells were prepared by collagenous dispersion of freshly dissected pituitaries. For A-kinase activity and cAMP determinations, 500ul aliquots containing 0.5×10^6 and 0.25×10^6 cells respectively were first preincubated for 40 minutes and then further incubated in the presence or absence of CRF for 10 minutes at 37° C. Cells were then either lysed and supernatants assayed for A-kinase activity or heat deactivated and assayed for cAMP by RIA. The standard A-kinase assay contained 20mM MOPS pH 7.0, 16mM magnesium acetate, 0.1mM ATP, 0.4 mg/ml histone H1, 4mM dithiothreitol and ^{32}P ATP (1uCi/assay tube). Corrections were made for non-cAMP dependent protein kinases. For ACTH determinations cells were preincubated for 120 minutes and then further incubated in aliquots of 500ul containing 0.2×10^6 cells in the presence or absence of CRF for 60 minutes. Supernatants were assayed for ACTH content by RIA using an ImmunoNuclear kit.

In anterior pituitary cells from control animals, CRF stimulated cyclic AMP accumulation, A-kinase activity and ACTH release in a dose dependent manner. Five micromolar CRF increased levels of pituitary cyclic AMP over 4 fold, while A-kinase activity was stimulated three fold and ACTH release was increased two fold. In stressed animals, CRF-stimulated A-kinase activity was similar to that of control animals while cyclic AMP response was slightly less than in controls. CRF-stimulated ACTH release appeared to be significantly diminished in stressed animals in one of two experiments. Further experiments are required to definitively determine the changes in ACTH response to CRF in stressed versus control rats.

119.7 ACUTE ETHANOL ADMINISTRATION ENHANCES ANTERIOR AND INTERMEDIATE LOBE PROOPIOMELANOCORTIN-DERIVED PEPTIDE SECRETION AND PLASMA CATECHOLAMINE LEVELS. A.B. Thiagarajan,* I.N. Mefford,* S. Daggett* and R.L. Eskay*. (SPON: S. Katz) Section of Neurochemistry, Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Blg 10 Rm3C218, Bethesda, MD 20892.

In order to explore certain of the apparent contradictions between the acute and chronic effects of ethanol on the hypothalamic-pituitary-adrenal axis (HPAA), adult male (8-10 animals per treatment group) rats (275-300gm) received a single infusion of ethanol via a chronic indwelling intragastric catheter followed by the determination of plasma ACTH, alpha-MSH, corticosterone (CS) and catecholamines (epinephrine (EPI), norepinephrine (NOREPI), and dopamine (DA)). Blood ethanol concentrations determined at 30,60,90 and 150 min post-ethanol infusion were similiar at all sample times and ranged from 240-280 mg%. In order to obtain stress-free plasma catecholamine levels, an indwelling jugular cannula was inserted 24 hrs prior to ethanol infusion and catecholamine levels were obtained by high performance liquid chromatography. Basal EPI, NOREPI and DA levels were 105 ± 18.7 (MEAN ± S.E.), 220 ± 22.4 and 254 ± 21.6 pg/ml plasma, respectively. Following ethanol infusion, plasma EPI levels increased 6 fold at 30 min and returned to basal levels by 90 min. NOREPI levels increased approximately 2 fold by 30 min post-ethanol infusion and remained elevated for up to 2 hrs, whereas, DA levels remained unchanged. In contrast to the jugular cannula studies for obtaining catecholamine levels, rats were handled twice-a-day for 5-7 days and sacrificed by decapitation for the peptide and steroid studies. Plasma ACTH, alpha-MSH and CS levels were determined by radioimmunoassay following appropriate extraction methods. Basal plasma ACTH and CS levels exhibited a diurnal rhythm and ethanol-induced increases in ACTH and CS release, as determined 1 hr post ethanol infusion, were maximal when basal ACTH and CS were at their nadir. The maximal observed increases in ACTH and CS levels were 10- and 4-fold, respectively. Although the primary regulation of intermediate-lobe-derived alpha-MSH is quite different from ACTH release, alpha-MSH levels were also elevated by acute ethanol administration. The probable mechanistic differences of our observed changes in the HPAA following acute exposure to ethanol, as compared to our previously observed ethanol-induced changes in the HPAA following chronic-ethanol exposure, will be discussed.

119.8 RELATIONSHIP BETWEEN THE DST AND THE 5-HTP-INDUCED CORTISOL RESPONSE IN PSYCHIATRIC PATIENTS. M.T. Lowy and H.Y. Meltzer, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

Failure to suppress serum cortisol following a low dose of dexamethasone (i.e. DEX suppression test; DST) and an enhanced cortisol response to the serotonin precursor, 5-hydroxytryptophan (5-HTP; 200 mg) are two abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis which have been reported in psychiatric patients. The present study was designed to evaluate the relationship between these two tests of HPA function in 26 drug-free psychiatric patients (16 depressed and 10 patients with other psychiatric diagnoses). Based on a standard DST (1 mg; 5.0 ug/dl cutoff) there were 13 suppressors(S) and 13 nonsuppressors (NS). A median split procedure was used to divide patients into those with a high cortisol response to 5-HTP (5-HTP-HC; N=13) and those with a low cortisol response (5-HTP-LC; N=13).

S and NS did not differ in their cortisol response to 5-HTP (1926 vs. 2392 µg/dl x min). There were more NS in the 5-HTP-HC group (8/13) than in the 5-HTP-LC group (5/13). There was a trend for the subjects in the 5-HTP-HC group to have elevated cortisol levels at 8 a.m. (19.9 vs. 16.5 µg/dl; p=0.1) and 4 p.m. (12.9 vs. 6.8 µg/dl; p=0.06) prior to DEX administration. The 4 p.m. post-DEX cortisol (8.9 vs. 2.6 µg/dl; $p < 0.01$) and the highest post-DEX cortisol levels (10.0 vs. 4.5 µg/dl; p=0.06) levels were greater in the 5-HTP-HC group compared to the 5-HTP-LC group. The differences in post-DEX cortisol levels between the 2 groups were not due to alterations in DEX bioavailability as there were no significant differences in DEX levels between the 2 groups at either 8 a.m. (165 vs. 205 ng/dl) or 4 p.m. (29.3 vs. 41.3 ng/dl). Regression analysis indicated there were significant positive correlations between the 5-HTP cortisol response and the 4 p.m. post-DEX cortisol level ($r= 0.72$; $p < 0.001$) as well as the highest post-DEX cortisol level ($r=0.59$; $p < 0.01$).

These results suggest that a common neurochemical abnormality, possibly a decrease in brain serotonin resulting in supersensitive serotonin receptors, may contribute to both of these HPA abnormalities present in psychiatric patients.

119.9 PLASMA CORTICOSTERONE RESPONSES TO ELECTRICAL STIMULATION OF THE MEDIAL FRONTAL CORTEX. J. Dunn and D. Miller*. Dept. of Anat., Sch. Med., Oral Roberts Univ., Tulsa, OK 74171.

Recent studies conducted in this laboratory have been directed towards identifying central neural sites involved in regulating plasma corticosterone (Cpd B) levels. To date differential changes in plasma Cpd B have been observed subsequent to electrical stimulation of several forebrain areas, i.e., amygdala, bed nucleus of the stria terminalis, hippocampus and septum. Absent from our studies, however, has been an assessment of a cortical influence on adrenocortical regulatory mechanisms. The latter, in light of recent identification of a visceral cortical region prompted the present study.

To pursue the possibility that the frontal cortex is involved in adrenocortical function, blood samples obtained prior to and following electrical stimulation of the medial prefrontal cortex of female rats were assessed for corticosterone (Cpd B) concentration.

Rats, anesthetized with urethane ($1.3g \cdot Kg^{-1}$), were tracheotomized, instrumented and positioned in a stereotaxic apparatus. ECG, heart rate, mean arterial pressure, respiration and hippocampal EEG were monitored and timed blood samples (0.2ml) were obtained from a catheterized tail artery. Blood samples were taken at 0.5 min. prior to and at 5, 10, 15 and 30 min. after initiation of stimulation (monophasic square waves, 100µA, 50HZ, 0.5 msec, 1 sec on/1 sec off for 30 min.). For purposes of plotting Cpd B responses to stimulation a change in plasma Cpd B was considered different from no change when the average of the 5, 10, 15 and 30 min. samples deviated by more than 10% from the pre-stimulus level. However, statistics were derived from Cpd B responses grouped according to stimulation site.

Whereas no change in Cpd B levels were observed following sham stimulation ($p>0.05$) or stimulation of the lateral or dorsal aspects of the frontal cortex ($p>0.05$), stimulation of the the pre-limbic area (PL) of the medial prefrontal cortex ($p<0.05$) produced significant changes in plasma Cpd B levels. Stimulation of other medial frontal cortical areas were not effective in inducing changes in adrenocortical activity. The overall change in plasma Cpd B following PL stimulation was 17%, the largest increase (22%) occurred at 30 min.

These data are consistent with our previous observations and offer additional support for the concept of a medial visceral cortex.

119.10 SOMATOSTATIN REGULATES THE BRAIN-PITUITARY-ADRENAL AXIS: IN VIVO EVIDENCE. J.M. Radke and S.R. Vincent. Div. of Neurological Sci., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Clinically, low CSF levels of somatostatin have been found to correlate with escape from dexamethasone-induced supression of the brain-pituitary-adrenal axis. There is also evidence that somatostatin can inhibit the evoked release of ACTH from anterior pituitary cells in vitro. In the present study the role of somatostatin in the supression of corticosterone secretion by dexamethasone was examined.

Male Sprague Dawley rats were group housed and handled for 1 week prior to receiving a standard dexamethasone supression test. Animals were injected at 9:00 a.m. with dexamethasone (10 µg/kg) subcutaneously and 5 hr later, at 1:00 p.m. blood samples were taken for the determination of plasma levels of corticosterone by radioimmunoassay. Immediately following the test, animals were killed and brain tissue was dissected and assayed for levels of somatostatin immunoreactivity.

Dexamethasone reduced plasma corticosterone from control values (174 ± 36 ng/ml) to undetectable levels (< 25 ng/ml). This dose of dexamethasone did not affect the levels of somatostatin in the hippocampus or hypothalamus. Subcutaneous injection of cysteamine (100 mg/kg) 5 min prior to dexamethasone, prevented the supression of plasma corticosterone. Levels of corticosterone 5 hr after injections of cysteamine alone did not differ significantly from control values. Cysteamine pretreatment was associated with a reduction in the levels of somatostatin immunoreactivity in the hypothalamus.

To block somatostatin receptors in the anterior pituitary and other peripheral sites, rats were given systemic injections of a somatostatin antagonist (cyclo [7-aminoheptanoyl-Phe-D-Trp-Lys-Thr(Bzl)]. Subcutaneous injections of this drug at 1.0 and 0.01 mg/kg had no effect on basal corticosterone levels and did not prevent the dexamethasone-induced supression of corticosterone levels. Pretreatment with this antagonist also had no effect on hypothalamic somatostatin levels.

These results suggest that a depletion of central somatostatin by cysteamine can prevent dexamethasone-induced supression of corticosterone secretion. Somatostatin neurons may therefore be involved in the feedback regulation of the brain-pituitary-adrenal axis by glucocorticoids.

Supported by the Medical Research Council of Canada.

119.11 ROLE OF CORTICOTROPIN RELEASING FACTOR (CRF) AND EPINEPHRINE ON PULSATILE ACTH SECRETION. F. J. López* and A. Negro-Vilar (SPON: M. Ching). LRDT, NIEHS, NIH, Research Triangle Park, NC 27709.

The importance of pulsatile hormone secretion for the modulation of target organ responsiveness is now clearly established. Regulation of pulsatile hormone secretion offers, perhaps, the best example of the complex neuroendocrine modulatory mechanisms that interplay to produce a coordinated pattern of episodic hormone secretion. The regulation of ACTH secretion is multifactorial, involving several secretagogues. Most of the experiments directed to analyze the physiological role of these factors involve situations of stimulated ACTH secretion. Until recently, no studies have been performed to provide a comprehensive characterization of the pulsatile pattern of ACTH release and of the factors that modulate that pattern. In the present study, we carried out experiments directed to analyze the role of a peptide, CRF, and of an amine, epinephrine, in pulsatile ACTH secretion. Intact Sprague-Dawley male rats were used. In one experiment, the animals were treated with 3 doses of α-helical-CRF (CRF antagonist). The first dose was given 5 min before the initiation of sampling (250 µg/rat) and the others (125 µg/rat), at 30 and 60 min after the bleeding started (10:00-11:00 am). Animals treated with the same volume of vehicle (PBS-BSA 1%) were used as controls. In another experiment, SKF 64139 (100 mg/kg BW i.p.), a PNMT inhibitor that crosses the brain blood barrier, was administered 12-13 h before sampling. Animals treated with saline were used as controls. In all cases, blood samples (0.15 ml) were taken through an indwelling atrial cannula during a 90 min period. A blood sample was taken every 3 min, and a replacement was given with a blood cell mixture obtained 15-20 h before from donor animals. Plasma aliquots (25 µl) were assayed for ACTH using a RIA protocol as previously reported (Nicholson et al., Clin. Chem. 30:259-265, 1984). The pulsatile ACTH pattern was analyzed using the "Cluster" analysis. α-Helical-CRF administration was not able to abolish the ACTH pulsatile pattern. We found no significant differences in the qualitative parameters of pulsatile ACTH secretion (pulse frequency, pulse duration and pulse interval). On the contrary, the treatment was able to decrease mean ACTH secretion, as well as the peak and trough values, without affecting pulse amplitude. On the other hand, blockade of epinephrine synthesis with SKF 64139 did not change any of the qualitative parameters. Nevertheless, peak values, trough values and the peak amplitude were significantly decreased. The data suggest that CRF and epinephrine affect the quantitative aspects of each ACTH pulse but not the frequency and other qualitative characteristics of the pulse. CRF, in addition, has a profound effect on maintaining mean ACTH levels probably by controlling the "gain" of the system.

119.12 INVOLVEMENT OF ALPHA-ADRENERGIC SYSTEM IN THE EFFECT OF MORPHINE ON HYPOTHALAMO-PITUITARY-ADRENOCORTICAL ACTIVITY. S.Suemaru*, M.F.Dallman*, D.N.Darlington, C.Cascio* and J.Shinsako* (SPON: M. Steele). Dept. of Physiology, University of California, San Francisco, CA 94143.

We examined the involvement of the α -adrenergic system in the effect of acutely administered morphine on the hypothalamo-pituitary-adrenocortical (HPA) activity in unstressed or stressed rats. The test substances were administered intraperitoneally (ip) or intravenously (iv) in non-cannulated or chronically cannulated (femoral artery and vein cannulas), conscious rats. In the ip experiment, prazosin (1 mg/kgBW) or vehicle was injected 45 min before morphine (2 mg/100 gBW) or saline injection. Some rats were exposed to ether vapor for 45 sec starting 15 min after the 2nd injection. The rats were decapitated 30 min after the 2nd injection and the plasma ACTH and corticosterone (B) were assayed. In the iv experiment, prazosin (0.5 mg/kgBW), yohimbine (0.5 mg/kg BW) or vehicle was injected 50 min before morphine (1 mg/100 gBW) or saline injection and 100 ul blood was taken through the cannula for plasma B assay before and 10, 20, 30, 45, 60 and 75 min after the 1st injection. Mean arterial blood pressure (MABP) and heart rate (HR) were measured throughout.

In unstressed rats, morphine markedly stimulated ACTH and B secretion. Prazosin increased plasma B levels and inhibited morphine-induced B secretion. Yohimbine also increased plasma B levels but potentiated morphine-induced B secretion additively. On the other hand, in ether-stressed rats, morphine inhibited stress-induced ACTH and B secretion and prazosin also inhibited stress-induced B secretion. Pretreatment with prazosin did not alter the inhibitory effect of morphine on stress-induced ACTH and B secretion. Prazosin caused a BP fall and tachycardia while yohimbine caused a BP rise and tachycardia. However, neither prazosin nor yohimbine affected morphine-induced enhancement of the vagal baroreflex, a pronounced bradycardia and short-lasting BP fall.

Taken together with the established concept that morphine acts in the HPA axis primarily at the level of the hypothalamus, these observations suggest that morphine acutely stimulates the secretion of corticotropin-releasing factor (CRF) or other ACTH secretagogues in the hypothalamus through a noradrenergic pathway via stimulation of α_1-adrenoceptors rather than α_2-adrenoceptors in unstressed rats and that, on the other hand, morphine may inhibit CRF secretion by attenuating the noradrenergic (α_1-adrenoceptor) activity in stressed rats. The stimulatory effect of morphine on the HPA system might be ascribed partly to the morphine-induced cardiovascular changes.

119.13 Adrenalectomy-Induced Changes in Hypophysial Portal Blood Concentrations of Corticotropin-Releasing Factor (CRF) in the Rat. J.I. Koenig, Neurology Service, Mass. General Hospital, Harvard Medical School, Boston, MA. 02114.

Several recent reports have indicated that metabolic and hemodynamic stressors change the secretion of corticotropin (ACTH) from the rat pituitary by altering the secretion of CRF and/or vasopressin (AVP) into the hypophysial portal circulation. Adrenalectomy (ADX) also is a potent stimulus for ACTH secretion. This effect is due to the removal of the negative feedback influence of the adrenal corticosteroids from the pituitary and the hypothalamus. Immunohistochemical studies have indicated that the paraventricular nucleus CRF-containing neurons produce more CRF but also begin to synthesize AVP after ADX. In fact, elevated concentrations of AVP and CRF have been reported in the median eminence after ADX. The concentration of AVP in the hypophysial portal blood of the rat also is elevated 6 days after the removal of the adrenal glands. Furthermore, the release of CRF from perifused median eminence fragments of ADX rats doubles, whereas the secretion of AVP from the same fragments increases 900%. These studies suggest that CRF may play a permissive role in controlling ACTH secretion after ADX. Therefore, the present study was undertaken to determine the effect of surgical removal of the adrenal glands on rat portal plasma concentrations of CRF. Male Sprague-Dawley rats (200-300 gm) were used in all studies. Bilateral ADX was performed under pentobarbital anesthesia. Hypophysial portal blood samples were obtained from urethane-anesthetized animals using the procedure outlined by Porter and Smith (Endocrinology 81:1182). Blood was collected in chilled plastic tubes containing aprotinin and EDTA. Plasma concentrations of CRF were determined by RIA using an antiserum purchased from IgG Corporation (Nashville, TN). Hypophysial portal plasma concentrations of CRF in urethane-anesthetized sham ADX animals were 680+170 pg/ml. One hour after ADX CRF concentrations in the portal plasma were unchanged. However, one day after ADX portal plasma concentrations of CRF were significantly lower (275+64 pg/ml). Portal plasma concentrations of CRF one or two weeks after ADX were no different than concentrations in control animals. These data suggest that after ADX, enhanced secretion of CRF may not be the primary driving force for the elevation of plasma ACTH concentration. However, CRF may play a permissive role in the activation of ACTH secretion after removal of the negative feedback influence of the adrenal steroids. This work was supported by NIH grant DK-39251.

119.14 EFFECT OF ALTERATIONS IN THE ACTIVITY OF TUBEROHYPOPHYSIAL DOPAMINERGIC NEURONS ON THE SECRETION OF ALPHA-MELANOCYTE STIMULATING HORMONE. S.E. Lindley*, J.W. Gunnet, K.J. Lookingland and K.E. Moore. Dept. Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Alpha-melanocyte stimulating hormone (αMSH) is secreted into the blood by melanocytes located in the intermediate lobe (IL) of the pituitary. This lobe is innervated by tuberohypophysial dopaminergic (THDA) neurons, whose perikarya are located in the medial basal hypothalamus (rostral arcuate nucleus). The objective of the present study was to examine the effects of stimulation of the rostral arcuate nucleus on the activity of the THDA neurons and on the secretion of αMSH from the IL.

The activity of the THDA neurons was estimated by measuring the concentrations of the DA metabolite dihydroxyphenylacetic acid (DOPAC) in the IL using HPLC coupled to an electrochemical detector. αMSH was measured in serum by radioimmunoassay.

Male Long-Evans rats were anesthetized with gamma-butyrolactone (GBL, 1000 mg/kg; i.p.), a drug which reduces dopaminergic neuronal activity. In the present study, GBL decreased the concentration of DOPAC in the IL and increased the concentration of serum αMSH. Bilateral electrical stimulation of the rostral arcuate nucleus in GBL-anesthetized animals increased the concentrations of DOPAC in the IL and decreased serum αMSH concentrations. Increases in the concentration of DOPAC in the IL and decreases in serum αMSH were related to the intensity and duration of stimulation of the THDA neurons. In complementary studies it was found that the administration of GBL decreased and electrical stimulation of the arcuate nucleus increased the activity of tuberoinfundibular dopaminergic neurons which project from the arcuate nucleus to the median eminence; this was evidenced by alterations in the concentrations of DOPAC in the median eminence and in circulating levels of prolactin. Administration of a DA antagonist (haloperidol, 1 mg/kg, i.p.) 30 min prior to starting electrical stimulation of the arcuate nucleus prevented the stimulation-induced decline in serum levels of αMSH and prolactin. These results indicate that stimulation-induced increases or GBL-induced decreases of THDA neuronal activity are reflected in changes in the metabolism of DA in the IL and in the secretion of αMSH. (Supported by NIH grant NS15911.)

119.15 EFFECTS OF ACUTE RESTRAINT STRESS ON THE ACTIVITIES OF TUBEROINFUNDIBULAR AND TUBEROHYPOPHYSIAL DOPAMINERGIC NEURONS AND ON THE SECRETION OF PROLACTIN AND ALPHA-MELANOCYTE STIMULATING HORMONE IN MALE AND FEMALE RATS. K.J. Lookingland, J.W. Gunnet and K.E. Moore. Dept. of Pharmacol./Toxicol., Michigan State Univ., E.Lansing, MI 48824.

Dopamine (DA) neurons comprising the tuberoinfundibular (TI) and tuberohypophysial (TH) DA systems have cell bodies located in the arcuate nucleus and axons that project to the median eminence (ME), and the intermediate (IL) and neural lobes (NL) of the posterior pituitary gland, respectively. Acute restraint stress increases prolactin (PRL) concentrations in the serum and this is associated in the female rat with a decrease in the activity of TIDA neurons (Demarest et al., 1985, Neuroendocrinol. 41: 437; Neuroendocrinol 41: 504). By contrast, little is known regarding the effects of stress on the secretion of hormones from the posterior pituitary and the activity of THDA neurons. In the present study the effects of acute restraint stress on plasma alpha-melanocyte stimulating hormone (αMSH) concentrations and the activity of THDA neurons were compared in male and diestrous female rats. The activity of THDA neurons was estimated by determining the rate of DA synthesis [accumulation of dihydroxyphenylalanine (DOPA) after the administration of a decarboxylase inhibitor] and DA metabolism [dihydroxyphenylacetic acid (DOPAC) concentrations] in the IL and NL of the posterior pituitary gland. By way of comparison, the effects of acute restraint stress on plasma PRL concentrations and the activity of TIDA neurons were also examined. TIDA neuronal activity was estimated by determining the rate of DA synthesis and DA metabolism in the median eminence (ME).

Ten, 20 and 30 min following placement of unanesthetized animals in restraining tubes (restraint stress) there was a marked increase in plasma PRL concentrations in both male and female rats. In female rats this increase in PRL secretion was accompanied by a decrease in DOPA accumulation and DOPAC concentrations in the ME. In contrast, restraint stress had no effect on ME DOPA or DOPAC concentrations in male rats. These results are consistent with previous reports of a sex difference in the response of TIDA neurons to restraint stress and indicate that the stress-induced increase in PRL secretion in female rats may be due, at least in part, to a decrease in activity of TIDA neurons.

Restraint stress produced a marked increase in αMSH concentrations in the plasma of both male and female rats, and in both sexes this was accompanied by a decrease in DOPA accumulation and DOPAC concentrations in the IL. By contrast, restraint stress failed to alter DOPA or DOPAC concentrations in the NL of male or female rats. Taken together these results indicate that restraint stress decreases the activity of THDA neurons terminating in the IL and that this effect may be responsible for the stress-induced increase in αMSH concentrations in the plasma. Furthermore, since restraint stress caused similar effects in male and female rats, there is no sex difference in the response of IL THDA neurons to this manipulation. (Supported by USPHS Grants NS09174 and NS15911.)

119.16 **Dopaminergic Projections to the Median Eminence.** M.D. Fitzsimmons, S.J. Wiegand, and G.E. Hoffman. Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester New York 14642.

Certain neurons regulate the function of the anterior pituitary by releasing trophic factors into the portal circulation at the median eminence, located on the ventral surface of the diencephalon. Use of retrograde tracers has allowed neuroanatomists to determine which cells project to the median eminence and therefore have the capacity to regulate directly anterior pituitary function. Dopamine is found in high concentrations in the median eminence and is thought to be the main physiological regulator of prolactin through a mechanism of tonic inhibition. By combining retrograde tract tracing and fluorescence immunocytochemistry, we have identified the population of dopamine neurons which projects to the median eminence.

Previous studies of afferents to the median eminence have included systemic injection of horseradish peroxidase (Broadwell and Brightman J Comp Neuro 166: 257-84, 1976). This technique takes advantage of the fact that the median eminence is a circumventricular organ (*i.e.*, has no blood-brain barrier). Hence, retrograde tracers in the blood can be taken up from the circulation by neurons whose projections end in the median eminence. We have taken a similar approach by injecting an autofluorescent retrograde tracer, fluorogold, systemically.

The fluorogold was introduced with a syringe into the jugular vein (0.5-5.0 mg/kg, 0.05-0.5% in saline). To date, the best results have been obtained with 5.0 mg/kg with a survival of 1to 5 days. The animals were perfused using a cannula inserted into the aorta with 0.9% saline followed by Zamboni's fixative. After the brains were removed, they were post-fixed, sunk in sucrose, and cut on a sliding microtome at 25 μm. The sections were incubated in 4% NGS and then rabbit-anti-TH (1:2000, Eugene Tech), diluted in 1% BSA and 0.4% Triton-X, overnight. After washing, the tissue was transfered to a solution containing biotinylated goat anti-rabbit (1:600-1:800) and then a solution of rhodamine coupled to avidin (10 μg/ml) for 1-3 hr. After thorough rinsing, the sections were mounted onto slides, cleared in alcohols and xylenes, and coverslipped with DPX mounting medium.

The distribution of fluorogold found in these animals closely approximates the distribution reported with systemic injection of HRP. Like HRP, fluorogold has a high affinity for endothelial cells, providing detailed information about neural vasculature. Components of the nervous system which have direct contact with the periphery, such as circumventricular organs and cranial nerve nuclei, also show fluorogold labeling. Included among the forebrain structures labeled with systemic fluorogold injections are neurons projecting to the posterior pituitary, subfornical organ, organum vasculosum of the lamina terminalis, and the median eminence.

Scattered TH-positive fluorogold cells were found as far rostral as the level of the anterior pole of the PVN, in the periventricular area. From the level of the rostral pole of the median eminence to the most posterior extent of the A12 group, all of the TH-positive cells were double labeled with fluorogold. None of the cells in the A13 (zona incerta) was double labeled. Thus, it appears that some TH-positive neurons in the more caudal A14 group and all of the TH-positive neurons in the arcuate A12 group project to the median eminence.

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119.17 IMMUNOREACTIVE TYROSINE HYDROXYLASE (TH) AND SEROTONIN (5-HT) IN THE RAT NEUROINTERMEDIATE LOBE: A COMPARISON OF FIBER STAINING PATTERNS. L. C. Saland, J. A. Wallace, A. Samora* and L. Gutierrez* Dept. of Anatomy, Univ. of New Mexico School of Medicine, Albuquerque, NM. 87131.

Several investigations have demonstrated the presence of serotonin (5-HT)-immunoreactive nerve fibers in the pituitary neurointermediate lobe. We have previously suggested that the 5-HT fiber immunostaining in the intermediate lobe may in part be the result of uptake of 5-HT (Saland et al, '85, Soc. Neurosci. Abst. 11: 360, and MS submitted), perhaps from the adjacent neural lobe. This suggestion was based upon our findings that 5-HT immunostaining in the intermediate, but not the neural lobe, was eliminated after the administration of either 6-hydroxydopamine or fluoxetine. Therefore, the possibility exists that 5-HT and catecholamines co-localize in the intermediate lobe innervation. Here, we compare the staining patterns for 5-HT and tyrosine hydroxylase (TH) in the rat neurointermediate lobe. Adult male Sprague-Dawley rats were ether anesthetized and perfused intracardially with ice-cold phosphate buffered saline (PBS) followed by 4% paraformaldehyde in 0.1M phosphate buffer. Pituitary glands were processed for paraffin embedding and sectioned at 6-8 μm. Sections were incubated with primary antisera to 5-HT or to TH (Eugene Tech International, Inc.), and visualized with the avidin-biotin-peroxidase technique. As shown in earlier studies, 5-HT staining in the pars intermedia was variable, with some sections having numerous fine varicose fibers surrounding and within tissue lobules, and others with very few fibers. In comparison, the neural lobe consistently contained large numbers of relatively coarse 5-HT-stained fibers, frequently along border areas. TH-immunostained fibers, in contrast, were observed in all areas of the intermedia and neural lobes, at edges and within central tissue locations. The staining patterns for 5-HT and TH differed sufficiently to suggest the existence of multiple fiber populations. In particular, TH staining in the neural lobe suggests that catecholamine synthesis takes place in fibers throughout the tissue, while fibers containing 5-HT appear more restricted to border areas. Studies to directly examine the potential co-existence of TH and 5-HT immunoreactivity in a subpopulation of neurointermediate lobe fibers are in progress. Supported by NIH NS-21256 and RR-08139 (LCS) and NSF-BNS-82-08433 (JAW).

119.18 INCUBATION OF RAT NEUROINTERMEDIATE LOBES WITH CORTICOTROPIN-RELEASING HORMONE (CRF) AND NEUROTRANSMITTERS: EFFECTS ON PEPTIDE IMMUNOREACTIVITY AND INTRACELLULAR STRUCTURE. L. Gutierrez*, A. Samora*, and L. C. Saland. (SPON: S. Rogers). Dept. of Anatomy, Univ. of New Mexico Sch. Medicine, Albuquerque, NM 87131.

Pro-opiomelanocortin peptide (POMC) secretion from the pituitary intermediate lobe is thought to be modulated by specific inhibitory and stimulating molecules, including dopamine (DA) and serotonin (5-HT), respectively. Corticotropin-releasing hormone (CRF) has been recently shown to induce release of POMC peptides from the intermedia, in addition to its effects on cells of the anterior pituitary. Direct interactions of hypothalamic peptide and neurotransmitters on pituitary tissue are likely to have important influences on regulation of POMC secretion. Here, we demonstrate alterations in POMC-immunostaining after in vitro incubation of neurointermediate lobes (NILS) with DA, 5-HT, CRF, or a combination of CRF and DA. Adult male Sprague-Dawley rats were ether anesthetized, rapidly decapitated, and the NILS separated from anterior lobes. Tissues (2 NILS/ well) were incubated in 6-well plates in Gibco medium containing glutamine, glucose, 0.1 mM bacitracin, 0.1mM ascorbic acid, and 10 μM pargyline. DA or 5-HT were added at concentrations of 10-6M, while CRF was added at 10-10M. Plates were incubated in a Dubnoff metabolic shaking incubator at 37 degrees C. with continuous 95% O2-5% CO2 gassing. After 90-120 minutes, tissue was fixed for light microscopic immunocytochemistry or for transmission electron microscopy. Immunoreactive sites for primary antibodies to beta-endorphin or alpha-melanocyte-stimulating hormone were visualized on paraffin sections using the avidin-biotin-peroxidase technique. Serotonin (5-HT) and CRF induced a diminution in immunoreactive POMC peptides in the pars intermedia as compared to controls, while DA-treated tissue was intensely stained. Combined CRF-DA treatment produced a "mixed" response, with some tissue lobules devoid of stain and others retaining intense immunoreactivity for peptides. TEM of cells treated with 5-HT or CRF contained secretory granules, with many oriented towards the plasmalemma, while those treated with CRF-DA varied, from granule-filled to partially depleted. The effects of CRF-DA combined on cellular peptide content may depend upon relative sensitivity of individual endocrine cells to stimulation or inhibition. Supported by NIH NS-21256, BRSG S-07-RR-05583-22 and RR-08139.

119.19 CIRCADIAN RHYTHMS OF ACTH AND GROWTH HORMONE AFTER INSULIN HYPOGLYCEMIA. J.F. Lopez*, R. G. Kathol, R.S. Jaeckle and W.H. Meller*. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109 and Dept. of Psychiatry and Internal Medicine, University of Iowa, Iowa City, IA 52242.

Insulin hypoglycemia is a known stimulant of pituitary ACTH and growth hormone (GH) secretion in experimental animals and humans. It is also known that both ACTH and GH show a circadian rhythm in normal humans. In this study we investigated the diurnal variation of ACTH and GH responses to insulin induced hypoglycemia in normal humans by administering insulin at three different times of the day: morning, afternoon and evening. To control for possible effects of repeated testing, we also performed the afternoon test three times.

Six healthy males (average age 31.6 ± 4 yrs) were given 0.1 units/kg IV of regular insulin, each on five separate occasions (three times at 16:00 hrs, once at 23:00 hrs and once at 8:00 hrs). Plasma ACTH and GH levels were assayed at 0,15,30,45,60,90 and 120 min after insulin administration. There was an interval of at least one week between each of the insulin infusions.

ACTH levels prior to insulin tended to be nonsignificantly higher at 8:00 hrs (30.7 ± 19.0 pg/ml) than at any other of the times (9.3 ± 8.3 pg/ml at 23:00 hrs; 10.6 ± 4.4, 10.8 ± 4.1 and 10.2 ± 3.2 pg/ml for each of the 16:00 hrs test). However, a repeated measure ANOVA as well as Kruskall-Wallis non-parametric testing failed to show any difference in peak ACTH levels ($F = .996$, $p > .25$), maximal ACTH increment ($F = .524$, $p > .25$) or at any other time points between the different times tested. Similarly, no differences were found in pre-insulin ($F = .546$, $p > .25$), peak ($F = 1.012$, $p > .25$) and maximal increment ($F = 1.003$, $p > .25$) GH levels between any of the insulin tests.

Our results suggest that the GH and ACTH response to insulin hypoglycemia is a stable response, not altered by repeated testing, and that similar results can be obtained with this stimuli regardless of the time of the day that it is performed.

119.20 THE EFFECT OF CHRONIC INTRACEREBROVENTRICULAR (ICV) INSULIN INFUSION ON FEEDING BEHAVIOR AND DIURNAL CORTICOSTERONE IN DIABETIC RATS. W. P. Meehan* and L. J. Leedom* (SPON: D. Lindsley). Dept. of Med., Sect. of Diabetes, Univ. So. Cal., Los Angeles, CA 90033.

Although hyperphagia is a cardinal symptom of diabetes, its etiology in diabetes is poorly understood. Chronic ICV insulin infusion inhibits feeding behavior in normal rats. Pancreatic insulin may be a satiety factor which, when missing in diabetes, results in hyperphagia. Another poorly understood concomitant of uncontrolled diabetes is elevated plasma corticosterone. It seems odd that a hormone which functions to increase glucose is elevated in diabetes under conditions of already profound hyperglycemia. It is our hypothesis that pancreatic insulin deficiency causes increased corticosterone by a central mechanism. To test this hypothesis, we chronically infused insulin into the ventricular system of diabetic rats. 40 male Sprague-Dawley rats had cannulae stereotaxically placed in the lateral ventricle. Rats were then made diabetic by IP injection (65 mg/kg) streptozotocin. 8 control rats received vehicle. 5 days post injection animals were bled at 08:00 and 17:00 hr for glucose and corticosterone determination, and implanted with 7 day osmotic minipumps. The following doses of insulin were given ICV and peripherally: 0, 0.5, 1.0 and 2.5 U/day. Food intake was measured daily. On day 7 (post pump) animals were bled at 08:00 and 17:00 for glucose and corticosterone.

During the 7 days of pump activity, nondiabetic rats consumed 23±4 sd g/day vs 36±6 g/day for vehicle treated diabetic rats ($p<0.001$). No insulin dose, central or peripheral, had a significant effect on feeding behavior in diabetic rats. Diabetes resulted in elevated AM (trough) corticosterone (diabetics: 31±13 vs 14±8, $p<0.01$). Peak corticosterone was not changed. Vehicle had no effect on corticosterone in diabetic rats. ICV insulin (0.5 U/day) normalized AM corticosterone in half the treated animals (to 9.0±5.6). Peripheral infusion of 0.5 U/day had no effect on plasma corticosterone. ICV infusion of 1.0 and 2.5 U/day normalized AM plasma corticosterone in all rats. Peripheral infusion of 1.0 and 2.5 U/day also lowered plasma corticosterone. No insulin treatment had a significant effect on plasma glucose.

In summary, ICV insulin infusion normalized trough plasma corticosterone but no effect on feeding or plasma glucose in diabetic rats.

120.1 SUPRACHIASMATIC KNIFE CUTS DISRUPT CIRCADIAN WHEELRUNNING IN HAMSTERS. R.F. Johnson, R.Y. Moore and L.P. Morin . Depts. Psychiatry and Neurology, Health Science Center, SUNY at Stony Brook, NY 11794.

The functional significance of suprachiasmatic nuclei (SCN) efferents in the control of circadian rhythms is not clear. The present study examined the effect of coronal cuts rostral or caudal to the SCN in hamsters.

Hamsters were entrained to LD 14:10. Cuts were made with a 1.5 mm L-knife lowered to the chiasm on both sides of midline. Another group received sham surgery (SHAM, N = 7). Wheelrunning was monitored for about a month in LD and then the cycle was switched to constant dark (DD) in the middle of the light phase.

Histology indicated three cut groups: Cuts rostral to the SCN with no SCN damage (ROST, N = 5) and cuts impinging on the Rostral SCN (ROST-SCN, N = 4) or on the caudal SCN (CAUD-SCN, N = 10).

Both the ROST-SCN and CAUD-SCN groups tended to show a loss of rhythmicity for about a week after surgery. The rhythmicity recovered and entrained in 1- 2 wks in most cases. Even when the LD rhythmicity recovered these animals showed a much higher tendency to run during the light phase than normal. Upon exposure to DD most of these hamsters showed dispersion of activity with many eventually losing clear circadian rhythmicity (3 of 4 ROST-SCN and 6 of 10 CAUD-SCN). In addition to the loss of rhythmicity these groups showed sharp persistent reductions in activity after surgery and several showed dramatic phase advances of several hours when switched to DD (not seen in SHAM or ROST Groups). The SHAM and ROST groups showed little effect on their entrainment, although the ROST group showed a slight dispersion of activity and reduced activity levels. All hamsters in the ROST and SHAM groups had clear freeruns proceeding from close to the previous phase of entrainment.

The effect of the CAUD-SCN and ROST cuts are consistent with previous work indicating the importance of caudo-lateral but not dorso-caudal or rostral (Brown, M. and Nunez, A., Brain Res. Bull., 16:705, 1986; Nunez, A. and Stephan, F., Behav. Biol., 20:224, 1977) efferents controlling circadian rhythmicity. The abolition of the circadian rhythmicity in the ROST-SCN may be due to destruction of a small portion of the rostral SCN, although very large portions of the SCN usually must be damaged to lose circadian rhythmicity (Rusak, B., J. Comp. Physiol., 118:145, 1977). The presence of rhythmicity under LD cycles in animals that gradually lose rhythmicity in DD suggests alternate photic pathways directly affecting wheelrunning activity or a driving effect of LD cycles on the SCN which increases the effectiveness of possible residual efferents controlling wheelrunning.

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120.2 LESIONS OF THE SUPRACHIASMATIC NUCLEI AND SURROUNDING BASAL HYPOTHALAMUS ABOLISH THE CIRCADIAN RHYTHMS OF ACTIVITY, DRINKING, AND BODY TEMPERATURE IN FEMALE RATS. E. Melanie W. Kittrell, University of Pittsburgh, Western Psychiatric Institute and Clinic, 3811 O'Hara St., Pittsburgh, PA 15213

While the suprachiasmatic nuclei (SCN) have been established as the primary neural substrate for the generation of rhythmicity, their role in the support of the circadian temperature rhythm (CTR) of body temperature is less clear. Studies concerning this particular function have been equally divided in supporting or refuting the claim that the SCN are responsible for the maintenance of the temperature rhythm.

In this study, we measured the CTR, activity, and drinking rhythms of 24 female hooded rats for 6 weeks under a 12/12 LD cycle. We then made bilateral electrolytic lesions of the SCN region in 20 of the rats and sham lesions in the remaining four. The survivors lived under 12/12 LD for 6 weeks and then under DD for an additional 6 weeks. The brains were then removed and alternate sections were reacted for cytochrome oxidase activity and stained with cresyl violet.

Results showed that all animals receiving complete SCN lesions (N=10) failed to recover rhythms in drinking, activity, and body temperature for the entire post-lesion period regardless of whether they were in entrained or free-running conditions. Three rats with some sparing of the SCN showed some return of rhythmicity in all three variables. All rats sustained varying degrees of damage to the optic chiasm and surrounding basal hypothalamus. Histological details will be presented in detail along with analyses of rhythmicity.

The fact that other areas of the basal hypothalamus were damaged in addition to the SCN means we cannot conclude definitely that the SCN are the sole support of the CTR. However, we found that the animals with the lowest amplitude ultradian fluctuations in their post-lesion measurements (the "flattest" looking results) had lesions that were primarily confined to the SCN and lateral SCN region. Animals with some SCN sparing but damage elsewhere in the hypothalamus did not lose their rhythms (as might be expected if the clock for temperature were located nearby the SCN) although some of their characteristics were altered.

These results suggest that the SCN are critically involved in the support of the CTR.

120.3 HAMSTER PHOTOPERIODISM AND THE PARAVENTRICULAR NUCLEUS: EFFECTS OF CORONAL, PARASAGITTAL OR ROOF CUTS. L.P. Morin, R.F. Johnson and R.Y. Moore. Depts. Psychiatry and Neurology, Health Science Center, SUNY, Stony Brook, NY 11794.

Lesions of the paraventricular nucleus (PVN) of the hamster hypothalamus block short photoperiod-induced gonadal regression (Lehman et al., Brain Res. 308:25, 1984; Pickard and Turek, Neurosci. Let. 43:67, 1983; Hastings et al., Neuroendocrinology 40:316, 1985). Horizontal knife cuts dorsal or ventral to the PVN or in the ventral preoptic area may also block the photoperiodic response (Nunez et al., Brain Res. Bull. 15:149, 1985; Eskes and Rusak, Neurosci. Let. 61:261, 1985; Inouye and Turek, Brain Res. 370:102, 1986). These studies have suggested that the PVN mediates photoperiodic information from the suprachiasmatic nuclei which is ultimately conveyed to the pineal gland. Two routes of PVN efferent fibers to the spinal cord have been identified which may be involved in the photoperiodic response (Luiten et al., Brain Res. 329:374, 1985; Swanson and Kuypers, J. Comp. Neurol. 194:555, 1980). The present experiments were designed to evaluate the importance of these two pathways to short photoperiod induced testicular regression.

Expt. 1 - Cuts were made with a retractable wire knife extended 1.5 mm except for the roof cuts which were made with a 1.5 mm L-shaped knife. Cuts were placed parasagittally on each side of the brain 0.7 mm lateral, in the coronal plane rostral or caudal to the PVN or horizontally above the PVN. After surgery, animals were kept in LD 14:10 for 10 wks then transferred to LD 8:16 for 19 wks. Testes and body weight were measured at weeks 0, 10, 20 and 29.

Expt. 2 - Roof cuts (ROOF) were made with a 2 mm L-shaped knife. Rostral (ROST) and caudal (CAUD) coronal plane cuts, near parasagittal (NPS) cuts made bi-laterally 0.6 mm and far parasagittal (FPS) cuts made 1.2 mm bi-laterally were made with a 3 mm L-knife. Rostral (ROST) and caudal (CAUD) coronal plane cuts were also made with the 3 mm L-knife. All groups were transferred to LD 8:16 after surgery as was a surgical control group. Another control group remained in LD 14:10.

In Expt. 1, there were small effects of surgery on body weight in LD 14:10 which disappeared in LD 8:16. None of the cuts blocked gonadal regression in short days. In Expt. 2, there was large variability in body weight associated with ROOF, ROST, NPS and FPS cuts. About 50% of these animals had elevated weights. Testis regression was blocked by most ROOF and FPS cuts. NPS cuts also blocked regression in a few animals while ROST cuts appeared to delay regression.

Information for photoperiodism may be transmitted from the SCN to the pineal by a dorso-laterally moving sub-PVN pathway (Watts et al., J. Comp. Neurol. 258:204, 1987), not usually reached by the NPS cuts, but usually destroyed by the FPS and wide ROOF cuts. Supported by NINCDS grant NS22168.

120.4 THE RETINOHYPOTHALAMIC TRACT (RHT) IN HAMSTER: NORMAL ORGANIZATION AND RESPONSE TO INJURY. L. Smale* R.F. Johnson, L.P. Morin and R.Y. Moore. (SPON: I. Feinberg) Depts. Psychiatry and Neurology, Health Science Center, SUNY, Stony Brook, NY 11794.

The suprachiasmatic nuclei (SCN) are important constituents of the circadian system in mammals. The RHT provides a direct pathway for photic information to reach the hypothalamus and, particularly, the SCN. Prior work (Pickard, G.E. and Silverman, A., J. Comp. Neurol., 196:155, 1983) indicates that the RHT in the hamster has a wider distribution than previously described for hamsters and other mammals. In this study, cholera toxin conjugated to HRP was used to re-examine the RHT in intact hamsters and hamsters with knife cuts designed to transect fibers of the RHT as they leave the optic chiasm. The cuts were made using an L-knife lowered to a position on the chiasm and rotating it to produce a linear cut between the chiasm and the SCN. RHT projections were analyzed by injection of cholera toxin-HRP (2 μl - 0.1%) into the vitreous and preparing sections for HRP histochemistry after a 24-hour survival period.

The RHT in normal animals first appears as scattered fibers in the rostral anterior hypothalamic area (AHA) above the optic chiasm. A moderate plexus is present in the rostral SCN with some fibers extending into adjacent AHA. In the caudal two-thirds of the SCN labeled fibers are present in a dense plexus in that portion of the nucleus containing vasoactive intestinal polypeptide-immunoreactive neurons and less dense in the area containing vasopressin-immunoreactive neurons. This projection to SCN extends significantly beyond the boundaries of the nucleus in several directions; laterally across the AHA into the perifornical region, dorsally in the periventricular zone to run along the ventral border of the paraventricular nucleus with a few fibers extending into it, and caudally into the retrochiasmatic area and in a periventricular zone, adjacent to the periventricular nucleus, to the level of the rostral ventromedial nucleus. In addition to these projections, there is an extensive innervation of the lateral hypothalamic area arising from fibers off the lateral chiasm and optic tracts with a few fibers extending into the supraoptic nucleus. Other fibers from the optic tracts run along the ventral surface into piriform cortex and the cortical amygdaloid nucleus.

In animals with cuts the RHT projection to SCN was markedly reduced, often with extensive anomalous growth of the RHT rostral to the cut in the AHA, medial preoptic area and extending into the septal nuclei. These observations indicate that the RHT has much more extensive projections than hitherto described and exhibits sigificant sprouting in response to injury with innervation of areas not normally receiving a projection. These findings should be important in the continuing functional analysis of the circadian system. Supported by NIH NS 22168 to LPM.

120.5 ENTRAINMENT OF ACTIVITY TO MULTIPLE FEEDING SCHEDULES IN RATS WITH SUPRACHIASMATIC LESIONS. F.K. Stephan. Dept. of Psychology, Florida State University, Tallahassee, FL 32306.

Wheel running activity of intact rats, as well as rats with suprachiasmatic (SCN) lesions, entrains to restricted food availability if the period of food access is between 23h and 31h and this effect is mediated by a circadian mechanism. Rats also are able to anticipate food access at 12h intervals, presumably by using a circadian clock.

Entrainment to multiple food availability per circadian cycle was further investigated in rats with SCN lesions. When two feeding times (1.5h each) were presented 8h apart, all rats (N=8) entrained to both schedules. Anticipatory activity (AA) to the second schedule was diminished when the interval was reduced to 5h. An 8h phase delay of one schedule resulted in delaying transients and a concomitant disappearance of AA to the unshifted schedule.

Seven rats were exposed to 3 feeding times (1h each) at 6h-6h-12h intervals, followed by 8h-8h-8h intervals. All rats entrained to two of the three schedules but no rat anticipated all 3 schedules simultaneously. However, AA occasionally shifted from one schedule to another so that entrainment was very unstable.

Five rats were maintained on 2 feeding times (1.5h each) 10h apart. The period of both schedules was 23:45h. The period of one schedule was then changed to 24:00h. Two rats entrained to both schedules for up to 40 days. The other rats entrained to the 24:00h schedule only. After the schedules intersected, 2 rats shifted from the 24:00h to the 23:45h schedule.

These results indicate that entrainment to food availability depends on phase angle differences and period differences of the feeding schedules and some of the observations suggest mediation by more than one pacemaker. Lack of AA (i.e., entrainment) was not related to food consumption in any of the conditions. It is unclear why activity failed to entrain to more than 2 feeding times per circadian cycle.

120.6 CONSTANT LIGHT SUPPRESSES VASOACTIVE INTESTINAL PEPTIDE (VIP)-LIKE IMMUNOREACTIVITY (LI) AND PEPTIDE HISTIDINE ISOLEUCINE (PHI)-LI, BUT NOT NEUROTENSIN (NT)-LI, WITHIN THE SUPRACHIASMATIC NUCLEUS (SCN). H.E. Albers, N. Minamitani*, E. Stopa* and C.F. Ferris. Lab. Neuroendocrinol. Behav., Depts. Biol. and Psych., Georgia State Univ., Atlanta, GA 30303; Dept. Endocrinol. & Pathology, Tufts Univ. Sch. Med., Boston, MA 02111 and Dept. Physiol., Univ. Mass. Med. Sch., Worcester, MA 01605.

Many neurotransmitter candidates have been localized within the SCN, however little is known about the involvement of these substances in the generation of circadian rhythms or the synchronization of circadian rhythms with the day-night cycle. Two substances of particular interest because of their co-localization within SCN neurons situated in a dense field of afferent terminals from the retina and lateral geniculate are VIP and PHI. The present studies examined whether the concentration of VIP, PHI and another peptide found within the SCN, NT, vary rhythmically within the SCN and whether their concentrations are altered by exposure to constant light(LL) or dark(DD). In experiment 1 adult male rats housed in LD 14:10 for at least three weeks were killed during the middle of the light(Day) or dark period(Night). Brains were removed and frozen on dry ice for subsequent removal of the SCN using a punch technique. SCN tissue was homogenized, extracted in 1 N acetic acid and boiled for 10 min prior to RIA using antisera generated to rat VIP, PHI and NT. The day-night concentrations of VIP-LI, PHI-LI and NT-LI were found to be similiar: Day: 6.91+0.87 pmol/mg protein (N=11), 6.69$\pm$1.0 (N=11) and 0.37$\pm$0.08 (N=7) and Night: 6.34$\pm$0.59 (N=9), 6.03$\pm$0.76 (N=9) and 0.28$\pm$0.07 (N=8), respectively. In a second experiment the effects of continuous lighting conditions on the concentration of each peptide was determined in rats exposed to LL or DD for 20 days. In rats exposed to LL VIP-LI and PHI-LI concentrations within the SCN were significantly ($P<0.01$) depressed when compared to the levels found in rats housed in DD or LD 14:10. In LL the VIP-LI concentrations of 2.16$\pm$0.37 pmol/mg protein (N=9) were 3-4 fold lower than seen in DD 8.18$\pm$1.65 or LD 14:10. Similiarly PHI-LI levels in LL (i.e. 3.12$\pm$0.39; N=9) were approximately 2 times lower than those seen in DD (i.e. 6.00$\pm$0.79; N=9) or LD 14:10. In contrast, NT-LI levels in LL (0.49$\pm$0.07; N=8) were not found to differ significantly from those observed in DD (0.54$\pm$0.14; N=10) or LD 14:10. In summary, no day-night differences were observed in the SCN concentrations of VIP-LI, PHI-LI or NT-LI, however VIP-LI and PHI-LI, but not NT-LI were significantly lower in rats housed in LL than in rats housed in either DD or LD 14:10. The selective effects of light on VIP-LI and PHI-LI levels within the SCN suggests that these peptide may be involved in the processing of photic information within the SCN. (Supported by NIH-384412 and ONR-4411013)

120.7 DEVELOPMENT OF THE SUPRACHIASMATIC NUCLEI (SCN) IN FETAL HYPOTHALAMIC TRANSPLANTS TO THE THIRD VENTRICLE OF ADULT RATS WITH SCN LESIONS. Raul Aquilar-Roblero* and Robert Y. Moore. (SPON: C. Welt). Department of Neurology and Neurobiology, SUNY @ Stony Brook, Stony Brook, NY 11794.

It has been shown that fetal grafts from the anterior hypothalamus, but not the occipital cortex, are able to restore the circadian rhythm of drinking behavior in adult rats with SCN lesions. In the present study we attempt to identify the anatomical subtrate for these behavioral results. In particular we address whether the SCN develops in the grafted tissue and whether connections between the graft and the host are established.

Male Sprague-Dawley rats sustained electrolytic lesions of the SCN and received grafts from E-17 embryos into the 3rd ventricle either from the anterior hypothalamus or the occipital cortex. After one month, the animals received intraocular injection of cholera toxin conjugated to HRP, were allowed to survive for 48 hrs, and were then perfused and tissue processed for HRP histochemistry and immunohistochemistry for vasopressin (VP), vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY).

Animals showed varying degrees of SCN destruction ranging from complete to partial, and unilateral lesions. Hypothalamic grafts routinely survived in the rostral aspect of the 3rd ventricle where they exhibited an SCN-like appearance with VP- and VIP-like immunoreactive neurons in adjacent parvocellular populations and extensive axonal plexuses with VP-, VIP- and NPY-like immunoreactivity. Projections to the host brain appear limited to tissue adjacent to the graft. All cortical grafts survived and showed neurons and fibers containing VIP, and NPY but not VP. HRP histochemistry showed retinal innervation of grafts, both hypothalamic and cortical. This was variable in extent but an anomalous retinal innervation of the medial anterior hypothalamus was present in all cases.

The present study shows that a structure with SCN-like appearance develops in grafts from the anterior hypothalamus, but not the cortex, of E-17 embryos. These findings provide an anatomical correlate which supports the suggestion that the SCN is responsible for the restoration of circadian rhythms by fetal implants in rats with SCN ablation. The anomalous retinal innervation of the hypothalamus and the grafts could explain the behavioral effects of both types of grafts on the drinking behavior seen under diurnal lighting conditions.

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120.8 THE AFFERENT AND EFFERENT ORGANIZATION OF THE CAT SUPRACHIASMATIC NUCLEUS. Dean M. Murakami* and Charles A. Fuller (Spon: Pamela A. Pappone). Department of Animal Physiology, University of California, Davis, CA 95616.

The suprachiasmatic nucleus (SCN) is critical for the control of circadian rhythms. In order to understand the neural organization of the circadian system, it is important to determine the afferent and efferent connections of the SCN. This study examined the neural interconnections of the cat SCN using horseradish peroxidase (HRP) as an anterograde and retrograde marker. A 30% HRP solution was electrophoretically injected into the SCN. Following a 3-day survival the animal was sacrificed, and brain prepared for histology. One series of coronal sections was reacted with tetramethyl benzidene, while the alternate series of sections was reacted with diamino benzidene. A second series of animals was injected with HRP into identified SCN efferent nuclei. The histological protocol was the same as above.

Injections of HRP into the SCN demonstrated that the region lateral to the SCN (LASCN), paraventricular nucleus, preoptic nuclei, ventromedial nucleus, septum, arcuate nucleus, retrochiasmatic region, anterior hypothalamus, diagonal band region and raphe nuclei send afferents to the SCN. The major nuclei that the SCN sends projections to include the: LASCN, paraventricular nucleus, preoptic nuclei, ventromedial nucleus, septum, arcuate nucleus, retrochiasmatic region and anterior hypothalamus.

The preoptic nucleus, anterior to the SCN, was injected with HRP. The SCN revealed labelled neurons in the dorsal and lateral portion of the nucleus. A few labelled SCN neurons were also found in the ventral portion of the SCN. The central region of the SCN appeared void of labelled neurons. The labelled neurons were primarily found in the SCN ipsilateral to the injection site, and concentrated from the middle to posterior SCN. The arcuate nucleus was also injected with HRP. Labelled SCN neurons were primarily located around the medial, dorsal and lateral borders of the SCN. The central and ventral regions of the SCN were essentially void of labelled neurons. The labelled neurons were found in highest concentrations from the anterior to middle sections of the SCN. In addition, the labelled neurons were nearly bilateral in distribution. Finally, the LASCN was injected with HRP. Labelled neurons were found primarily within the ventral SCN.

The afferent and efferent connections of the cat SCN were identified as being similar to those regions described by other investigators in other species. Further, this study identifies that the pattern of labelled SCN neurons following HRP injections was distinct for each of the nuclei examined. This suggests that the SCN neurons that project to different nuclei are organized into distinct but overlapping regions.

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120.9 FREE-RUNNING CIRCADIAN PERFORMANCE RHYTHMS IN MONKEYS. W.N. Tapp and B.H. Natelson. Primate Neurobehavioral Unit, VA Medical Center and Department of Neurosciences, New Jersey Medical School, East Orange, NJ 07019.

We have developed a monkey performance model for examining the effects of time-of-day, circadian rhythms, and work-rest schedules on the efficiency of the performance of learned tasks. In this vigilance-discrimination model, rhesus monkeys must perform a two-component task to obtain food. The first component is a vigilance task, where the monkey must detect the onset of a trial and respond with a lever press within a time limit. Success on the vigilance task initiates a discrimination task where the monkey must press either a right or left lever, depending upon whether a red or green cue light comes on. Choices and response latencies are recorded on computer. The monkey's temperature and activity rhythms are recorded every 10 min around the clock. In previous work, we found strong time of day effects that differed in their peak time for the two tasks, and we found that a 6 hr phase advance produced a jet-lag-like syndrome that included significant performance deficits. However, the monkeys were working 8 hr sessions in entrained conditions in those studies. The current study was designed to examine the circadian characterstics of performance by determining whether (1) periodic performance variations are found in constant conditions, (2) the effects of free-run on overall performance, and (3) its effects on task-dependent performance patterns.

We studied the performance of three adult, male rhesus monkeys in constant light with trials presented around the clock on a variable interval schedule averaging one trial every 2.4 min. All 3 monkeys exhibited free runs as long as they were maintained in these conditions (up to 180 days). The monkeys exhibited free-running performance periods of 23.4 hr, 23.7 hr and 24.6 hr. Performance differed significantly from the levels seen in the 8 hr task in entrained conditions. Vigilance performance declined by an average of 30.7% $\pm$ 8.1 SEM ($p < 0.01$). The decline in vigilance may be due to the fact that monkeys were able to eat significantly more food in the continuous task than in the 8 hr task ($p < 0.01$). However, discrimination performance improved an average of 16.5% $\pm$ 1.2 SEM ($p < 0.01$) in free-running conditions. It seems unlikely that increased performance in constant conditions is due to decreased food motivation. Instead, the data suggest that changes in the internal phase relationships of rhythms during free-run may improve performance on more complex tasks.

Supported by USARMDC

120.10 THE PATTERNS OF RETINAL PROJECTION AND OXIDATIVE METABOLISM IN THE SUPRACHIASMATIC NUCLEUS OF PRIMATES AND THE TREE SHREW. Charles A. Fuller and Dean M. Murakami*. Department of Animal Physiology and California Primate Research Center, University of California, Davis, CA 95616.

Previously, we reported the pattern of metabolic activity using the cytochrome oxidase (CyOX) stain in the hypothalamus of rodents. In order to understand the generality of this neural organization, we examined the pattern of CyOX staining in the hypothalamus of the tree shrew, a New World (squirrel monkey) and an Old World primate (bonnet macaque). The pattern of retinohypothalamic (RHT) innervation was examined by placing a 30% horseradish peroxidase (HRP) solution into the vitreous chamber of one eye in the tree shrew (15 µl), squirrel monkey (50 µl), and bonnet macaque (75 µl). Following a 3-day survival, the animal was sacrificed and prepared for histology. Alternate coronal sections (40 µm) through the hypothalamus were reacted with the TMB protocol in order to reveal the anterogradely transported HRP. Alternate sections were stained for CyOX.

The pattern of retinal projection to the hypothalamus in squirrel monkeys and macaque is very similar. The SCN is the primary nucleus to receive retinal afferents in agreement with Moore [1975]. However, in this study the distribution of HRP granules suggest that retinal terminals are present from the anterior to posterior pole. The retinal terminals appear restricted to the ventral portion of the SCN in the anterior and middle portions. The posterior SCN exhibits a relatively even distribution across the nucleus. The retinal projection to the hypothalamus in tree shrews is in good agreement with Conrad and Stumpf [1975]. The retinal projection to the hypothalamus in tree shrews exhibits several anatomical differences compared to primates. Dense retinal terminals project to a region of the hypothalamus comparable to the location of the SCN in rodents and primates, but there is little concentration of neurons suggesting the presence of a nucleus. In addition, there is a dense retinal projection to the nucleus hypothalamic anterior (NHA) located dorsal to the SCN along the third ventricle. This suggests that the tree shrew receives retinal input to two specific hypothalamic nuclei (SCN and NHA).

Primates exhibit a pattern of CyOX activity similar to that demonstrated in rodents and cat. The SCN exhibits intense CyOX activity in the anterior two-thirds of the nucleus that is primarily confined to the ventral portion. The posterior SCN also exhibits intense CyOX activity, but is evenly distributed across the nucleus. This coincides with the distribution of retinal terminals. However, intense CyOX activity also extends laterally from the SCN along the hypothalamo-optic chiasm border (LASCN). It will be important to determine what relationship the LASCN may have in circadian function.

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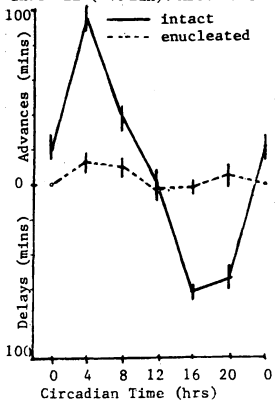
120.11 OPTIC ENUCLEATION ATTENUATES THE PHASE SHIFTING EFFECTS OF DIAZEPAM ON HAMSTER CIRCADIAN RHYTHMS. T.A. Houpt*, R.E. Mistlberger* and M.C. Moore-Ede. (SPON: Z. Boulos). Dept. Physiology & Biophysics, Harvard Medical School, Boston MA 02115.

Recently the benzodiazepines (BZDs), a class of GABA agonists, have been shown to cause both phase advances and delays in the circadian rhythms of hamsters. Phase response curves (PRCs) to triazolam (~25mg/kg) under constant light (LL) or constant darkness (DD) (Turek & Losee-Olsen, *Nature 321*:167-8, '86) and to diazepam (12.5 mg/kg) (Houpt *et al., Soc. Neurosci. Abs. 12*:1071, '86) under constant light have been obtained. A pharmacological agent that causes phase shifts might act either centrally on a circadian clock or peripherally on the pathways which mediate zeitgeber (e.g. light) input to the clock. Ralph & Menaker have reported that diazepam blocks light pulse-induced phase advances but not delays in hamsters kept in constant darkness, which suggests a modulation of photic input by BZDs (*Brain Res. 372*:405-8, '86). Also the PRCs to triazolam and diazepam are similar to a 2 hr dark pulse PRC (Boulos & Rusak, *J. Comp. Physiol. 146*:411-7, '82). We report here that removal of the retinal photoreceptors and cutting of the optic nerves by optic enucleation greatly attenuates or eliminates the phase shifting effects of diazepam. Male golden hamsters (90-100g) were enucleated under halothane and housed individually in cages equipped with running wheels and enclosed in light-tight ventilated chambers under LL (140 lux). After 2-3 wks recovery, 12.5mg/kg diazepam was given i.p. Animals received 2-3 injections at least 3 wks apart at one of the following circadian times: 0, 4,8,12,16,20h. While diazepam caused occasional phase shifts comparable in magnitude and direction to those in intact hamsters, the effects were not consistent and the average phase shifts were very small compared to the intact LL PRC. The attenuation of phase-shifting effects of diazepam in hamsters after enucleation strongly suggests that BZDs affect the circadian timing system by modulating photic input to the SCN. Future research producing a DD PRC to diazepam and in characterizing BZD receptors in the retina and SCN will further elucidate the site and mechanism of BZD action.



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120.12 PHOTIC SENSITIVITY OF A MAMMALIAN CIRCADIAN PACEMAKER D. E. Nelson and J. S. Takahashi, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201

For mammals the environmental cycle of light and dark is the primary agent responsible for the entrainment of endogenous circadian rhythms to the solar day. The physical characteristics of light necessary for entrainment, however, are poorly understood. To characterize the photoreceptive system which mediates photic information to a circadian pacemaker in hamsters we have used the phase-shifting response of the oscillator to discrete light pulses as an assay. We have previously demonstrated that this system appears equally responsive to light stimuli of equal energy over a range of durations from 300 msec to 2700 sec. In addition the sensitivity of the circadian system to light (total response/total photons/cm^2) is similar for stimuli of 300 and 900 sec in duration (Nelson and Takahashi, Soc. Neurosci. Abst., 12:1068, 1986).

To test for sensitivity changes in this photoreceptive system for light pulses of shorter duration, we have measured the sensitivity of the circadian system to pulses 3 sec in duration. After 7 days in constant darkness 3-sec monochromatic stimuli of several irradiance levels (503 nm, HW=20 nm) were administered to hamsters at circadian time 19. The steady-state phase advance of the locomotor activity rhythm was measured. Preliminary results suggest that the sensitivity to stimuli of 3 sec is different from the sensitivity measured for pulses of longer duration. These changes in sensitivity may be due to a saturation of the ocular photoreceptor which mediates photic information to a pacemaker perhaps due to the higher irradiance of the shorter stimuli. To determine the sensitivity of the pacemaker phase-shifting mechanism following stimulation we have also examined the effects of prior stimulation upon the phase-shifting sensitivity. A 300-sec monochromatic stimulus was administered to hamsters at circadian time 19. Immediately following or 1 hr following this stimulus test-stimuli of various strengths were administered to determine the sensitivity of the circadian system to additional stimulation. Results demonstrate that the sensitivity of the circadian system to these stimuli is much less than for stimuli presented alone. The decreased sensitivity, however, is due primarily to response saturation which would be expected if the circadian system was integrating the two light pulses and responding to the total number of photons delivered in both light pulses.

These experiments suggest that the mammalian circadian pacemaker and the photoreceptive system which subserves it may be less sensitive to light stimuli of short duration but at the same time capable of integrating light inputs over extremely long periods of time.

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120.13 PARAMETRIC ENTRAINMENT OF HAMSTER CIRCADIAN ACTIVITY RHYTHMS. G.E. Pickard. Dept. of Anatomy, West Virginia University, School of Medicine, Morgantown, WV 26506.

Circadian rhythms free-run in constant conditions and can be entrained by periodic environmental factors (zeitgebers). The light:dark (LD) cycle is the most powerful zeitgeber for mammals. Rodents entrain to a wide range of LD cycles, from 22:2 to 0.25:23.75. However, in all but a few cases, entrainment in mammals has been examined using square wave light cycles (non-parametric entrainment). To further explore the neural basis of photic entrainment, sine-wave light cycles (with no abrupt transitions in light intensity) were employed as zeitgebers to study parametric entrainment.

Male golden hamsters, housed individually in running-wheel cages, were maintained in light provided by electroluminescent panels (14cm x 30cm) positioned 28cm above the floor of each cage. Light intensity was varied by an electronic controller which produced a sine-wave light cycle with a period of 24 hr. The peak emission wavelength was approximately 510 nm and spectral shifts were not observed as panel voltage was varied.

In the initial experiment, 24 animals were maintained in a sine-wave light cycle for 65 days followed by 20 days of constant dark followed by 88 days in the same sine-wave light cycle. Between days 116-123, twelve animals received bilateral lesions aimed at the thalamic intergeniculate leaflet (IGL). The intensity of the light cycle varied from 10 lux to 0 lux with complete darkness lasting about 15 min. Using activity on-set as an index, all animals entrained to the sine-wave light cycle and many appeared to accomplish this by oscillating around a preferred phase; the mean phase angle of entrainment was 6.6 ±0.2 hr after peak intensity. The data also clearly indicated that many animals, if not all, were using the brief dark period as a daily cue. Under these conditions, ablation of the IGL had only minor effects on entrainment. Because the animals appeared to be entraining to the daily dark pulse, this light cycle did not represent a "pure" parametric signal.

In the second experiment, 24 animals were maintained in a low amplitude sine-wave light cycle (5-10 lux) which lacked a dark period. The effect of IGL ablation will be determined in these animals after they demonstrate parametric entrainment to the sine-wave light cycle. Supported by NIH grant NS-21165.

120.14 **THE EFFECTS OF ABERRANT LIGHTING ON THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN THE SYRIAN HAMSTER.** J.S. Ferraro[1], F.M. Sulzman*[2] and H.N. Krum*[1]. [1]Dept. of Biol. Sci., SUNY-Binghamton, Binghamton, NY 13901; [2]Div. of Life Sci., NASA, Washington, DC 20546.

Male Syrian hamsters (*Mesocricetus auratus*) were placed in Wahmann activity wheels contained in individual light-tight sound attenuated chambers and exposed to one of eight lighting conditions for a duration of 10 weeks; constant light (LL, 45-75 lux), constant dark (DD, 0 lux), feedback lighting (LD_{FB}, a condition which illuminates the cage in response to locomotor activity), a feedback lighting neighbor control ($LD_{FB\ NC}$, the animal receives the same light pattern as a paired animal in LD_{FB}, but has no control over it), reverse feedback lighting (rLD_{FB}, a condition that darkens an illuminated cage in response to locomotor activity), a light-dark cycle of 14 hours of light followed by 10 hours of dark (LD14:10), a short LD cycle of 1 hour of light and 1 hour of dark (LD1:1) and a high frequency or ultrashort LD cycle of 1 minute of light followed by 1 minute of dark (LD1m:1m). The circadian rhythm of locomotor activity, of hamsters exposed to LD14:10, entrained to the 24 hour photoperiod. Free-running periods were similarly and significantly lengthened by exposure to LD_{FB} (24.72±0.03), LL (24.56±0.06), rLD_{FB} (24.56±0.04) and LD1m:1m (24.61±0.06). Hamsters exposed to LD1:1 (24.50±0.07), despite substantial masking, had a significantly shorter period than hamsters exposed to LD_{FB} ($P<0.05$), but a similar period to those exposed to LD1m:1m, rLD_{FB} and LL. The mean free-running periods observed in LL, LD_{FB}, rLD_{FB}, LD1m:1m and LD1:1 were found to be significantly longer ($P<0.01$) than the period of hamsters exposed to DD (24.16±0.04). Animals exposed to $LD_{FB\ NC}$ did not free-run and were therefore not included in the statistical comparisons. Most animals exposed to the $LD_{FB\ NC}$ condition displayed "relative coordination" (i.e. the locomotor activity rhythm responded differentially to the light cycle, depending on the phase of the light pulse, but did not entrain to the stimulus). One animal appeared to be unaffected by the imposed light cycle and free-ran, four animals displayed modest changes in phase in response to the LD cycle, four animals displayed robust changes and one animal entrained to the period of the LD_{FB} controller for several weeks before breaking off and regressing into a condition of relative coordination. These results suggest that an animal assumes a specifically defined free-running period in any lighting condition that evenly illuminates the subjective night. It does not appear to matter whether the lighting condition contains transitions, is endogenously or exogenously derived or whether the subjective day is illuminated or not. The only determinants appear to be the pattern of photosensitivity, defined by the phase-response curve and its delay to advance ratio (D:A), and that the the D and A portions are relatively evenly illuminated. Supported by NIH grant 1 R01 NS23128-01 (JSF).

120.15 ENTRAINMENT OF THE PACEMAKER FOR HAMSTER ACTIVITY RHYTHMS BY DAILY HOARDING OPPORTUNITY. B. Rusak, R.E. Mistlberger, B. Losier* and C.H. Jones*. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

When food is available for only a brief period each day, rats exhibit an activity component that anticipates the feeding time. This component is driven by a circadian oscillator that is different from the pacemaker (identified with the suprachiasmatic nuclei, SCN) that normally drives the activity rhythm. Hamsters cannot tolerate similar restriction schedules, so we tested whether another food-related event, a daily hoarding opportunity, might generate similar anticipation by the circadian system.

Eight hamsters were studied for 4-13 months while housed in constant dim illumination (0.5 lux). Each cage had an activity wheel and a door, normally locked, that opened onto a plexiglas tunnel (10 cm diameter, 93 cm long) leading into an empty box (47 x 47 cm). During hoarding availability, hamsters were allowed access to the tunnel and box for 30 min every 24 h, at which time 15 g of sunflower seeds were available in the box. Hamsters usually retrieved some or all of the seeds to their cages. If hamsters were not in their cages after 30 min, they were escorted there, and sufficient laboratory chow was left in the home cage to ensure that animals were not food deprived.

Five of eight hamsters showed entrainment of the activity rhythm to a 24-h period. The rhythms of two others were clearly affected by the hoarding opportunity, but they could not be said to be stably entrained. Entrainment usually followed many weeks of freeruns (or transients). Activity onset typically followed hoarding immediately or anticipated it by a few hours, although one animal entrained with the end of activity near the hoarding time. An individual might show different phase relations to the hoarding time during two different tests. When hoarding ended, freeruns were always initiated from the entrained phase of activity onset. These findings indicate that stimuli associated with the hoarding opportunity were able to entrain the light-entrainable pacemaker that drives hamster activity rhythms.

Large, but subtotal, SCN lesions were made in two entrained animals. One responded with a ~10-h advance shift in the entrained rhythm, a finding compatible with a shortened period in the underlying oscillator. The other hamster eventually developed a rhythm with a very short period that failed to re-entrain. These results are consistent with the known effects of partial SCN lesions on pacemaker period. They reinforce the conclusion that, in contrast to the effects of food restriction on rats, hoarding-associated stimuli entrain the SCN-based pacemaker for the hamster activity rhythm.

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120.16 EFFECTS OF NUTRIENT RESTRICTION SCHEDULES ON CIRCADIAN RHYTHMS IN THE RAT. R.E. Mistlberger*, T.A. Houpt* and M.C. Moore-Ede. Dept. Physiology & Biophysics, Harvard Medical School, Boston MA 02115.

Schedules of restricted food availability can entrain behavioral and metabolic circadian rhythms in the rat. The clearest manifestation of entrainment is the development of intense locomotor activity anticipating the daily meal by several hours. Anticipatory rhythms do not occur if the food availability cycle is outside the circadian range (24±3h), suggesting that the rhythms are generated by food entrainable circadian oscillators. Physiological substrates of these oscillators are unknown, but are separate from suprachiasmatic nuclei-based oscillators critical for photically entrained circadian rhythms. Stimuli associated with daily feeding which are necessary for entrainment are also unknown, but identifying these stimuli could provide clues to entrainment pathways and the rhythm generating substrate. Restricted daily access to water, salt or palatable but non-nutritive foods does not entrain anticipatory rhythms, suggesting that ingestion of nutrients is critical for entrainment to occur. This study asks whether restricted access to specific nutrients (protein) can entrain anticipatory activity rhythms in rats with *ad lib.* access to other nutrients.

Eleven individually housed female rats (225g) were maintained on a protein free diet for 10 days and then provided with an additional 5g protein meal (33% casein in a saccharine-vanilla solution) for 2h once each day 7h after lights-on (14h light/10h dark). After 28-40 days of daily protein meals, the rats were transferred to tilt floor cages and maintained on the feeding and lighting schedules for a further 7-14 days. Locomotor activity (floor tilts) was monitored continuously by computer. None of the rats showed evidence of meal anticipation, although all rats began consuming the meal immediately on presentation and usually ate all of the protein within 30 min. The protein meal was then increased to 10g daily for a further 25 days for 6 rats but again none of these rats showed meal anticipation.

The results indicate that the motivation to consume protein and the physiological responses to protein ingestion are insufficient to entrain the circadian timing system responsible for food anticipatory rhythms. Further studies are planned to assess whether restricted access to other nutrients can entrain anticipatory rhythms in otherwise free-feeding rats. Intestinal digestive enzyme rhythms will also be measured in nutrient restricted rats.

120.17 URINARY BASAL BODY TEMPERATURE IN ANOREXIA NERVOSA: A MEASUREMENT OF CLINICAL AND THEORETICAL UTILITY. J.M. Jonas*, J. Ehrenkranz*, M. Helmicki*, M.S. Gold. Eating Disorders Program and Research Facilities, Fair Oaks Hospital, Summit, NJ 07901.

Hypothermia is a frequently reported feature of anorexia nervosa (ANN), a life-threatening disorder of unknown etiology. The mechanism of temperature reduction is unknown, and may relate to poor nutrition and reduced caloric thermogenesis, set point displacement (Luck P, Wakeling A, Clin Science, 62:677, 1982), or perhaps dysregulation of the opiate system (Clark WG, Clark YL, Neurosci Biobehav Rev, 4:175, 1980). No longitudinal studies of the effect of weight gain on basal body temperature (BBT) have been reported. Oral temperatures are subject to manipulation, and other methods are too invasive and not acceptable (e.g. rectal probes) for long-term clinical or research use. We studied 3 individuals with ANN who were inpatients at the Fair Oaks Eating Disorders Program, over a period of 1-3 months, using a new method for obtaining BBT via first morning voided urine (Ehrenkranz JR, Endocrinology, 116:312, 1985). Urine samples were obtained upon arising daily, before eating and before the patient had left the bed. Temperature was measured using a disposable Franklin urinary thermometer. The results are summarized in the table below. In all 3 cases, there was a close correlation between urinary BBT and weight gain ($r=.75$ - $.84$; $p < .001$ in all 3 cases). In addition, in each case this relationship was lost as the patient reached 85-90% of ideal body weight. These data suggest that urinary measurement of BBT may be a useful measure of clinical improvement in ANN, perhaps superior to simple weight measurement, which is subject to patient manipulation. This method may also prove a reliable and acceptable technique for investigating circadian rhythms and temperature dysregulation in ANN and affective disorders.

Table 1

#	Height (inches)	Initial Weight (lbs)	Initial BBT (°F)	End of Study Weight	End of Study BBT	r	p
1	68	91	95	129	98.6	0.75	<.001
2	68.5	102	96.6	127	98.6	0.84	<.001
3	65	100	96.4	112	98.4	0.79	<.001

121.1 EVIDENCE FOR ENKEPHALINERGIC MECHANISMS MEDIATING AFFECTIVE DEFENSE BEHAVIOR ELICITED FROM CAT MIDBRAIN PERIAQUEDUCTAL GRAY. M.B. Shaikh, A.B. Shaikh* and A. Siegel. Dept. of Neuroscience, N.J. Medical School, Newark, New Jersey 07103.

The midbrain periaqueductal gray (PAG) has been implicated in the initiation and regulation of aggressive behavior in the cat. Enkephalin - containing cells and axon terminals are known to be present in abundant quantities within the PAG. Recent studies conducted in our laboratory have shown that microinjections of the opioid antagonist naloxone placed into the PAG effectively modulated affective defense behavior (AD) elicited from the hypothalamus (Pott, et al., 1987). In the present study, enkephalin analog [D-Ala^2]Met-enkephalinamide (DAME) was microinjected into the PAG to further determine how the opioid peptide system could regulate AD elicited from the PAG of the cat.

Utilizing a cannula-electrode, AD was elicited by electrical stimulation of the dorsal PAG. AD is characterized principally by hissing, growling and paw striking. The experimental paradigm involved the establishment of stable baseline thresholds for AD over a period of 4 days. Then, DAME (1 ug in 0.5 ul saline, pH=7.4) or vehicle control (saline) was injected through the cannula-electrode at the PAG site from which AD was elicited. Response thresholds were obtained 5, 30, 60, 120 and 180 min. post injection.

It was observed that injections of DAME significantly altered AD response thresholds. Threshold elevations were observed 10-20 min. post injection and, surprisingly, were sustained for 180 min. following drug administration at which time thresholds returned to baseline levels. Suppression of AD was reversed by the opioid antagonist naloxone (1 ug/0.5 ul saline) when injected directly into the same PAG site 30 min. following DAME administration. In contrast, injections of vehicle alone into the PAG had no effect upon attack thresholds.

These results indicate that the metenkephalinergic system is involved in the regulation of affective defense behavior elicited from the feline PAG.

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121.2 NALOXONE INDUCED REVERSAL OF MIDBRAIN PERIAQUEDUCTAL GRAY MODULATION OF HYPOTHALAMICALLY ELICITED FELINE PREDATORY ATTACK. S. Weiner*, M. B. Shaikh, A.B. Shaikh* and A. Siegel. Depts. of Restorative Dentistry and Neuroscience, N.J. Dental and Medical Schools, Newark, New Jersey 07103.

It has recently been shown that electrical stimulation of the midbrain periaqueductal gray (PAG) can modulate quiet biting attack (QBA) and affective defense (AD) elicited from the lateral hypothalamus (LH) [Shaikh, et al., 1984]. Recently, our laboratory has demonstrated that endogenous opiates within the PAG can regulate AD [Pott, et al., 1987]. In the present study, we sought to determine the possible role of the PAG opioid peptide system in the modulation of QBA.

Initially, QBA, which involves the stalking and biting of the neck of an anesthetized rat, was elicited by electrical stimulation of LH. Cannula-electrodes were then implanted into PAG sites from which modulation of QBA could be identified by electrical stimulation. Here, a paradigm involving single stimulation of LH and dual stimulation of LH and PAG was employed to determine the presence and directionality of modulation. At this time, injections of 0.5 ul of naloxone (1 ug/0.5 ul of 0.9% saline, pH=7.4) were delivered through the cannula-electrodes into the modulatory sites.

Modulation of QBA was identified from 21 sites in the PAG. The attack response was inhibited at 16 sites and facilitated at five. While most modulatory sites tended to lie within the dorsal half of the PAG, sites which produced response facilitation were generally situated more rostrally than those which inhibited QBA. Following injections of naloxone, we observed a blockade of the facilitatory effect of PAG stimulation in four of the five cases examined. Concerning inhibitory sites, a blockade of modulation was noted in 10 of the 16 sites tested. In contrast, injections of vehicle alone (0.9% saline, 0.5 ul) did not alter the effects of modulation.

These data support the view that endogenous opiates are involved in the regulation of predatory aggression induced from the PAG. Further support for this notion was obtained in a separate experiment. Here, 0.5 ul of [D-Ala^2] Met^5 - Enkephalinamide (DAME) (1 ug/0.5 ul saline, pH=7.4) was injected into an inhibitory site within the PAG. This procedure reversed the naloxone effects generated upon the modulatory site.

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121.3 FOREBRAIN ENKEPHALINERGIC CONTROL OF FELINE AGGRESSION. M. Brutus and A. Siegel. Dept. of Neuroscience, N.J. Medical School, Newark, New Jersey 07103.

The bed nucleus of the stria terminalis (BNST) is known to facilitate affective defense behavior (AD) in the cat. These results can be understood in light of its connections with such structures as the corticomedial amygdala and pyriform cortex which also facilitate AD. The BNST is also known to be rich in enkephalin-containing cells and fibers. Therefore, the major goal of this study was to examine the effects of opioid administration upon hypothalamically elicited AD following injections placed into the BNST and adjacent forebrain regions.

Moveable stimulating electrodes were stereotaxically placed into the medial hypothalamus from which AD could be elicited and into those BNST, nucleus accumbens and caudate nucleus sites from which significant modulation of this response could be obtained when hypothalamic stimulation was paired with stimulation of a forebrain site. Within-day stable baseline latencies and thresholds were first established which were followed by intracerebral injections (IC) of D-Ala^2-Met^5-Enkephalinamide (DAME) [1 ug/0.5ul saline] or vehicle alone (0.9% saline, pH=7.4) delivered over 2 min. Response threshold and latency determinations were made 5, 30, 60 and 90 min. post injections. During experimental drug testing, only single stimulation of the hypothalamus was applied.

We observed a facilitation of attack after DAME was injected into BNST sites from which significant facilitation of AD had previously been obtained. The drug induced facilitation of AD was sustained up to 60 min. post injection. Injections of vehicle control into the BNST had no effect upon the attack response. In contrast, inhibition of AD was generally observed following DAME administration into nucleus accumbens sites from which dual stimulation had previously facilitated the attack response, while similar injections placed into the caudate nucleus were largely ineffective. Consistent with the findings obtained when DAME was injected into the nucleus accumbens, injections of the opiate antagonist naloxone (0.5 ug/ul or 5.0 ug/ul) into the region of the n. accumbens resulted in a facilitation of AD.

The results indicate that the enkephalinergic systems within the BNST and nucleus accumbens may differentially regulate affective attack behavior. The mechanisms underlying the expression of these enkephalinergic effects are generally unknown but are currently under examination in our laboratory.

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121.4 LESIONS OF THE NUCLEUS ACCUMBENS IN RATS REDUCE OPIATE REWARD, BUT NOT TOLERANCE. J. E. Kelsey, W. A. Carlezon, Jr.* and W. A. Falls*. Department of Psychology, Bates College, Lewiston, ME 04240.

Prior research has implicated the ventral tegmental area of the midbrain in the mediation of the rewarding effects of exogenous opiates such as morphine and heroin. One intent of this study was to determine if the nucleus accumbens (NACC) of the forebrain, which receives an extensive dopaminergic projection from the ventral tegmentum, is also involved in mediating the rewarding effects of morphine. Indicating that it is, lesions of the NACC were found to attenuate the rewarding effects of morphine as measured by a conditioned place preference test. A single i.p. injection of 10 mg/kg of morphine altered the place preference of rats with NACC lesions in a two chambered box less than it altered the place preference of the sham-operated control rats. The groups did not differ on initial place preference, and saline injections did not alter the place preference of either group.

Indicating that this effect did not reflect an inability of rats with NACC lesions to associate morphine with a distinct environment, a second experiment revealed that morphine produced as much conditioned tolerance to morphine in rats with NACC lesions as it did in control rats. Specifically, an i.p. injection of 5 mg/kg morphine in a distinct environment produced more tolerance, as measured by less analgesia, in rats that had previously received morphine injections paired with that environment than it did in animals that had previously received morphine injections unpaired with that environment. Moreover, the magnitude of the increased tolerance observed in the paired rats was identical for both rats with NACC lesions and control rats.

These findings that NACC lesions reduced the rewarding effects of morphine without altering morphine tolerance suggest that the neural systems mediating the initial rewarding effects of opiates are distinct from the neural systems mediating subsequent opiate tolerance and dependence. They further suggest that the NACC is only involved in the former process.

121.5 **PRO-LEU-GLY-NH2 INTERFERES WITH MORPHINE TOLERANCE AND ANALGESIA IN A MULTI-TRIAL TEST.** C. R. McLaughlin, A. H. Lichtman*, M. S. Fanselow* and C. P. Cramer. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Previous reports suggest that a single dose of Pro-Leu-Gly-NH2 (melanotropin-release inhibiting factor, MIF) will inhibit tolerance in rats that are chronically implanted with morphine pellets for up to three days without interfering with acute morphine induced analgesia (Bhargava et al, 1980, 1979, 1976). Differences in morphine administration may result in two different types of tolerance (Baker & Tiffany, 1985; Paletta & Wagner, 1986): short-term tolerance, which is facilitated by closely spaced and chronic administration of morphine (Tiffany & Baker, 1981), or long-term tolerance, which occurs with lower doses and widely spaced administration of morphine and appears to be mediated by Pavlovian conditioning (Siegel, 1977).

We tested MIF in both short- and long-term paradigms. In the short-term test, adult male rats were given either MIF (4 mg/kg, ip) or water on Day 1 only. Two hours later, they were exposed to either morphine or saline three times per day for three days with progressively higher doses (20, 30, 40 mg/kg, ip). Testing, begun approximately eight hours after the final morphine exposure, consisted of administering morphine (10 mg/kg, ip) 30 minutes prior to a hotplate (52°C) test of analgesia. Latency to lick the hindpaw, to a maximum of 90 seconds, was measured in two consecutive trials.

In the long-term test, the animals received either MIF (4 mg/kg, ip) or water each day two hours prior to either morphine (10 mg/kg, ip) or saline within a salient peppermint context to facilitate conditioned tolerance. The animals were tested, as described above, within the peppermint context approximately 48 hours after the final exposure to morphine.

In both experiments, animals treated with MIF prior to morphine exposure showed a longer latency to pawlick in the first trial, suggesting that the peptide had interfered with tolerance. In the second trial, however, both MIF-treated groups exhibited much shorter latencies, a surprising result given that the saline group had no prior experience with morphine. The animals treated with water did not show such a dramatic decrease across trials. These findings suggest a complex interaction between MIF exposure and the expression of morphine-induced tolerance in a multi-trial test.

121.6 DEVELOPMENT OF BEHAVIORAL SUPERSENSITIVITY TO APOMORPHINE DURING CHRONIC TREATMENT WITH CYCLO(HIS-PRO). S.A. Spahn* and C.Prasad (SPON: J.B. Green). Section of Endocrinology, Department of Medicine, Louisiana State University Medical Center, New Orleans, LA 70112.

Cyclo(His-Pro) (CHP) is an endogenous cyclic dipeptide that not only exists in the basal ganglia of rodents, monkeys, and human, but also exhibits a variety of central nervous system-related biologic activities. Some of these biologic activities (e.g. dopamine (DA)-antagonist reversible hypothermia, potentiation of amphetamine-induced stereotypic behavior (SB), in vivo inhibition of tyrosine hydroxylation, in vitro inhibition of DA uptake) suggest CHP to act like a presynaptic DA-agonist. Treatments that chronically attenuate DA synaptic transmission (e.g. DA antagonists, inhibitors of tyrosine hydoxylase, etc.) result in an enhancement of effects associated with DA synaptic transmission. This has been generally interpreted as evidence for the development of postsynaptic receptor supersensitivity. Therefore, we propose that chronic treatment with CHP will result into a postsynaptic supersensitivity. Experiments presented here are designed to test this hypothesis.

Male Sprague-Dawley rats were divided into two groups of 10 animals/group. The first group was allowed to drink water, while the second group received a solution of cyclo(His-Pro) in water (average daily CHP intake = 0.76 ± 0.07 mg/kg). Both groups were injected with apomorphine (0.5 mg/kg, i.p.) and SB scored (Brain Res. 84:1935, 1975) every 10 min. (90 sec. observation epoch) for a total of 90 min. APO-induced SB was measured every week for 8 weeks. The data show that median SB score of CHP-treated rats was significantly different than water-treated animals (ANOVA: $F_{1,14} = 16.84$, $p < 0.005$,). For example, there was a significant augmentation in median SB of group treated for 8 weeks with CHP (water group: control, 0.30 ± 0.19; treated, 0.29 ± 0.15; $p > 0.05$ and CHP group: control, 0.25 ± 0.11; treated, 0.90 ± 0.22; $p < 0.05$). Although these data clearly show a postsynaptic dopaminergic supersensitivity, the mechanism underlying such changes can only be speculated. CHP could act at a dopaminergic site, considered to be the primary mechanism in controlling SB, or at some modulating site involving cholinergic, noradrenergic, or serotoninergic mechanism. Alternatively CHP treatment could simply attenuate APO metabolism.

121.7 MODIFICATION OF NALOXONE-PRECIPITATED WITHDRAWAL SIGNS BY CAPTOPRIL AND CAPSAICIN IN THE MORPHINE-DEPENDENT RAT. L.G. Sharpe, J.H. Jaffe*, M.M.S. Lo and L.J. Porrino. Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224

The neurokinins, especially substance P (SP), appear to be among several endogenous substances that are released during withdrawal from chronic morphine. In the present study, we examined the possible role of SP in the opioid withdrawal syndrome with drugs that alter SP tissue levels. Captopril increases tissue levels of SP through enzyme inhibition whereas capsaicin produces a long-lived SP depletion in several tissues innervated by type c sensory fibers. Captopril in doses of 0.1, 0.3, 1 and 3 mg/kg was injected s.c. 15 min before naloxone (0.5 mg/kg, s.c.) in rats implanted (s.c.) with a 75 mg morphine pellet for 3 days (N=8-12 in each dose group). Only the 0.3 mg/kg dose increased naloxone-precipitated withdrawal, an effect observed in three strains of rats. Captopril alone had no effect in the morphine-dependent rat. In another experiment either saline or captopril (0.3 mg/kg) was injected (s.c.) immediately before naloxone in morphine-dependent rats that were pretreated (4-10 days before the morphine pellet implant) with either capsaicin (125 mg/kg, s.c.) or the capsaicin vehicle (N=8 for each of 4 groups). Capsaicin treatment inhibited withdrawal signs of rhinorrhea, lacrimation and salivation. Captopril increased the occurrence of the three secretory responses in the vehicle treated but not in the capsaicin treated animals. Other withdrawal signs (grooming, penile licking, activity, stretching, teeth chattering, chewing, body weight loss) were not altered by either captopril or capsaicin treatment. It was concluded that substance P and related neurokinins may be involved in the expression of some signs of opioid withdrawal.

121.8 EFFECTS OF MIF ON ROTATIONAL ACTIVITY. S. Davy*, J.W. Boja, B.J. Pomerants*, and H.K. Kulmala. Dept. of Pharmacology, NEOUCOM, Rootstown, Ohio 44272

The tripeptide, MSH Release Inhibitory Factor (MIF) or l-prolyl-l-leucyl-glycineamide (PLG), significantly potentiates the central effects of l-dopa. It has also been shown to dramatically improve the symptoms of Parkinson's Disease when administered intravenously. The present study was initiated to determine if peripherally administered MIF affects central dopamine neurotransmission.

A total of sixteen rats were tested using a hemiparkinsonian rat model and rotational activity. These were divided into four groups of four animals each. All groups were tested initially for rotational activity in a computerized rotometer (details of the apparatus and methods reported in Kulmala et al., Br. Res. Bull., 1987) in response to amphetamine and apomorphine. Two groups received unilateral nigrostriatal lesions and the other two groups received sham lesions. One of the lesioned and one control group were given daily i.p. injections of MIF (25 mg/kg) for four days. Rotational activity in response to apomorphine (0.5 and 1.0 mg/kg) was examined in all animals on days 5, 10 and 15 of the experiment (day 1 was considered the first day of MIF administration). The MIF treated animals all demonstrated a significant enhancement of rotational activity ($p < 0.05$) to the 1.0 mg/kg dose of apomorphine as compared to activity measured prior to MIF. Sham lesioned animals treated with MIF reversed the direction of their rotational activity. These changes in activity were still evident on day 10, but disappeared by day 15. In separate studies with lesioned and unlesioned animals, MIF was found to depress or have no effect on amphetamine-induced rotation. Further studies are planned to determine if the actions of MIF to enhance apomorphine-induced rotation are mediated through DA1 and/or DA2 receptors.

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121.9 THE ANTIOPIATE TYR-MIF-1 EXHIBITS GREATER EFFECTS AFTER MODERATE THAN HIGH STRESS. Z. Harry Galina & Abba J. Kastin. VA Medical Center and Tulane University School of Medicine. New Orleans, LA, 70146

We used the endogenous peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), a component of an endogenous antiopiate system, to test the concept that various environmental situations would alter the effectiveness of a peptide to antagonize stress-induced analgesia. Our observations demonstrate that Tyr-MIF-1, and by implication other putative antiopiates, functions better under conditions that induce less intense analgesia than under stronger analgesia.

Antinociception was measured in male CD-1 mice on a hot-plate (52.5°C). In each of the three studies, mice received a coded solution (10 ml/kg) of either diluent (0.9% NaCl, 0.01M acetic acid) or Tyr-MIF-1 (0.1 mg/kg) ip 20 min before exposure to stress.

Various levels of stress-analgesia were induced in mice by one of three methods which we or others have shown to be opiate mediated. We used 90 sec of 0, 0.25, 0.50, or 1.00 mA of constant current electric shock; forced swimming in warm-water (32°C) for 0, 3, 6, 12, 24, or 48 min; or 20 min of exposure to a novel environment. In the novelty experiment we manipulated the intensity of stress by using six different levels of temperature on the hot-plate varying from 47.5 to 60°C in increments of 2.5°C.

The results (ANOVA and Duncan's multiple range tests) indicated that shock effectively induced analgesia that was reduced by Tyr-MIF-1 at moderate but not high intensities of shock. Tyr-MIF-1 reduced the analgesia induced by warm-water swimming after moderate times of exposure. Novelty-induced analgesia was reduced by Tyr-MIF-1 measured at the lower temperatures but not at the higher ones.

These results indicate that the endogenous antiopiate system can discriminate levels of intensity of stress. One of the functions of the antiopiate system may be to curtail the suppression of pain, thus re-establishing antinociceptive homeostasis. Since the danger to the animal at any given moment may be related to the intensity of the stress-evoking situation, the balance of opiate and antiopiate action may be regulated by the intensity of stressor. This is supported by our findings that at least one system of endogenous antiopiates functions better at moderate than high levels of stress.

121.10 EFFECTS OF CYSTEAMINE-INDUCED MANIPULATIONS OF CENTRAL SOMATOSTATIN ON LOCOMOTION AND GENERAL ACTIVITY IN RATS. G.R. Sessions, C.L. Winstead*, K.H. Chantry* and G.F. Koob. U.S. Army Med Res & Dev Cmd, Ft. Detrick, MD 21701, U.S. Air Force Academy, Colorado Springs, CO 80840 and Scripps Clinic & Res Foundation, La Jolla, CA 92037.

The recently hypothesized role of somatostatin neurons in Alzheimer's Disease (Roberts, et al., Nature, 314:92, 1985) has greatly increased research interest in determining the functional role of this peptide in influencing behavior. Cysteamine HCl has been shown to produce initial release and temporary depletion of brain somatostatin (Bakhit, et al., Reg. Peptides, 6:169, 1983) without altering levels of other neuropeptides (Palkovits, et al., Bn. Res., 240:178, 1982) and has become a useful tool in exploring this problem. Cysteamine effects on activity have been very variable, with studies reporting increased activity (Bakit & Swerdlow, 1986), decreased activity (Vescie, et al., Pharm. Biochem. Beh., 21:833, 1984), or no effects at all (Sessions, et al., Neurosci. Abs., 11:1113, 1985). The current study was designed to investigate the effects of centrally administered cysteamine on locomotor and general activity in rats following acute administration, when somatostatin would presumedly be released in brain, and after chronic treatment, when somatostatin levels should be depleted. Locomotor activity was recorded continuously in automated chambers in three groups of rats (N = 5 ea.) for 150 min following 4 daily intracerebroventricular (ICV) infusions of buffered saline vehicle, 250 or 350 ug of cysteamine in volumns of 2 ul. Frequency and durations of individual behaviors were recorded during ten-min observational tests conducted 30 and 90 min following drug administration. Results showed that acute administration of cysteamine produced initial suppression of locomotor activity lasting approximately 5-15 min, followed by sustained increases 90 min following injections in behaviors characteristic of stereotypy: sniffing, rearing, or standing immobile, and concomitant decreases in resting behaviors, ie., lying down. The cysteamine-induced effects on activity were significantly attenuated following the fourth daily treatment of the drug, when no differences in locomotor activity or general activity levels of individual behaviors was observed. With the exception of first injection effects on rearing, no significant differences were observed between the effects of the two drug doses. The results suggest that the initial activation of stereotypical behaviors was produced by cysteamine-induced release of endogenous somatostatin in brain, and was attenuated following chronic treatments due to the depletion of neuronal stores of the peptide.

121.11 INTRA-MEDIAN RAPHE INFUSIONS OF TACHYKININS PRODUCE LOCOMOTOR HYPERACTIVITY. J.M. Paris and S.A. Lorens. Behavioral Pharmacology Laboratory (Bldg. 135), Stritch School of Medicine, Loyola Univ. of Chicago, Maywood, IL 60153

We have demonstrated previously that intra-median raphe (MR) infusions of the metabolically stable neurokinin analogue, [$pGlu^5$, $MePhe^8$, Sar^9] substance P(5-11), abbreviated DiMe-C7, produce dose-dependent increases in locomotor activity as measured in photocell chambers. This hyperactivity was found to be dependent upon intact intra-MR serotonin neurons, and is blocked by administration of the dopamine antagonist, haloperidol (200 µg/kg) (Behav. Brain Res., 1987). The present series of experiments were undertaken in order to determine the relative rank-order of potency of the various tachykinins for eliciting hyperactivity following intra-MR infusion. The non-mammalian tachykinins: eledoisin (ELE), kassinin (KAS), and physaelamin (PHY); and the mammalian neurokinins: substance P (SP), neurokinin A (NKA), and Senktide (a neurokinin B agonist) were tested.

In the first experiment, separate groups of rats, chronically implanted with intra-MR cannulae, were tested with vehicle and three doses of either ELE, KAS, or PHY. The doses used (0.03 nmoles/µl, 0.23 nmoles/µl, and 1.14 nmoles/µl) were equimolar with the approximate ED_{25}, ED_{50}, and ED_{100} doses, respectively, of DiMe-C7. The rank order of potency of the tachykinins for eliciting hyperactivity was: ELE = KAS = DiMe-C7 >> PHY.

In the second experiment, separate groups of rats, implanted with chronic intra-MR cannulae, were tested with the identical equimolar doses of either SP, NKA, or Senktide. The rank order of potency for eliciting hyperactivity was: Senktide > NKA = DiMe-C7 >> SP.

The results suggest that the hyperactivity produced by intra-midbrain raphe neurokinin injections is mediated by stimulation of NK-2 and NK-3 receptors located, most likely, on serotonin neurons which in turn regulate the activity or targets of ascending dopamine projections.

121.12 MODULATION OF CARDIAC ORIENTING REFLEXES BY MULTIPLE OPIOID SUBSYSTEMS. L.L. Hernández. Neuroscience Lab., Dorn Veterans' Hospital, Columbia, SC 29201.

Albino rabbits were tested for heart rate orienting reflexes (HR ORs) to novel tones following intravenous treatments with opioid peptide analogs and opiate antagonists. DADL (d-ala^2-d-leu^5-enkephalin; 10 µg/kg) or the antagonist naloxone-HCl (0.5 mg/kg) attenuated bradycardiac HR ORs to initial tone presentations compared to saline controls, but did not affect habituation during later tone presentations or retention of habituation 24 hours later. DALA (d-ala^2-met-enkephalinamide; 10 µg/kg) induced a much smaller decrease in initial HR OR magnitude but appeared to speed habituation, although it did not affect retention. The quaternary analog naloxone methobromide (0.5 or 5.0 mg/kg) failed to affect HR ORs, habituation or retention. Combined treatments with DALA plus naloxone-HCl or DADL plus naloxone-HCl did not affect HR ORs, i.e. the effects of naloxone and the peptides were antagonized by combined treatments. However, DADL plus naloxone did decrease HR OR magnitude during the retention test. These data suggest that multiple opioid subsystems in the central nervous system interact to modulate orienting and habituation. Specifically, preferential δ-opioid receptor stimulation by DADL, or preferential µ-opioid receptor blockade by this low dose of naloxone, attenuated HR ORs and therefore may influence attention and sensory processing of stimuli. Conversely, stimulation of both δ- and µ-opioid receptors by DALA had little effect on initial HR ORs but may have influenced later habituation. These data concur with other evidence indicating that opioid peptides modulate attention and sensory processing, and further suggest that an imbalance among multiple opioid receptor subsystems disrupts these perceptual functions.

The opioids also influenced baseline (pre-tone) HR following intravenous injection. DALA, DADL and naloxone-HCl generally increased HR, whereas naloxone methobromide and the combined treatments generally decreased HR. These data concur with other evidence suggesting that cardiovascular activity is influenced by complex interactions among central and peripheral opioid subsystems. However, these data also suggest that changes in tonic HR following intravenous opioid treatments cannot, by themselves, account for the observed changes in HR OR magnitude.

121.13 EFFECTS OF A NEW PEPTIDASE INHIBITOR ON MEMORY PROCESSES AND CENTRAL CHOLINERGIC METABOLISM IN MICE AND RATS. G. Wiemer*, P. Usinger*, F.J. Hock, H.J. Gerhards*, B.A. Schölkens*, R.H.A. Becker*, R. Henning* and H. Urbach*. Hoechst AG, 6230 Frankfurt/M. 80, FRG.

In the last years there are a large body of hints that several neuropeptides (e.g. enkephalines, substance P and somatostatin) are involved in memory and learning processes. For that reason, there is a suggestion that peptidase inhibitors are able to modulate cognitive functions by blocking the degradation of peptides.

A new heterocyclic peptidase inhibitor was found to be highly effective in inhibitory (passive) avoidance as well as eight-arm-radial maze. In the 2 tests an amnesia induced by scopolamine was antagonized by a pretreatment in mice and rats respectively on 3 consecutive days with the peptidase inhibitor (0.1 - 30 mg/kg p.o. and i.p. respectively). The resulting U-shaped dose-response-curves most likely reflect an interaction of two or more peptides influencing the acquisition and retention of information.

How these peptides interact with one another and other neurotransmitters is till now a matter of speculation. From our investigations it becomes obvious that acute or repeated administration of the peptidase inhibitor (0.03 - 3 mg/kg i.p.) induces a pronounced decrease of acetylcholine (ACh) in different brain areas of the rat. The fall in brain ACh-content is most likely due to an enhanced release of ACh. This interpretation is established by experiments carried out with the choline uptake inhibitor hemicholinium-3 (HC-3). Co-administration of HC-3 (20 µg i.c.v.) and the peptidase inhibitor (0.3 mg/kg i.p.) potentiates the decrease of ACh-content induced by HC-3. In parallel the peptidase inhibitor enhances in the same dose range the activity of the enzyme choline acetyltransferase. Probably this enzyme activation is the consequence of the augmented ACh-release. The dose-response-curves of the biochemical data are also U-shaped.

The results indicate, that the new peptidase inhibitor enhances cholinergic metabolism in rat brain which is accompanied with a nootropic effect.

121.14 CENTRAL ADMINISTRATION OF ALPHA-HELICAL CORTICOTROPIN-RELEASING FACTOR ATTENUATES THE ACQUISITION OF A CONDITIONED EMOTIONAL RESPONSE. B.J. Cole.* K.T. Britton and G.F. Koob. (SPON: C. Rivier). Div. Preclinical Neuroscience and Endocrinology. Scripps Clinic and Research Foundation. La Jolla. CA 92037. Dept. Psychiatry. San Diego Veterans Administration Medical Center. La Jolla. CA 92161.

Central administration of corticotropin-releasing factor (CRF) in the rat reliably induces behaviors normally exhibited during conditions of high stress. These findings have led to the hypothesis that this neuropeptide is important not only for the neuroendocrine, but also the behavioral response to stress. To examine the behavioral significance of endogenous CRF in fear motivated behavior, we used the specific, competitive antagonist of CRF, alpha-helical CRF (9-41). This CRF-antagonist has been shown to reverse the 'anxiogenic' effects of CRF in a conflict test, but does not have any effect itself in this behavioral paradigm (Brain Res.,369:303, 1986).

Food deprived rats were trained to respond on a RI-90 schedule for food reinforcement. After a stable level of responding had been attained, all the subjects were implanted with a guide cannula aimed at the lateral ventricle, and assigned to one of 4 experimental groups, equated for baseline response rates. After a post-operative recovery period, the effects of alpha-helical CRF on the acquisition of a conditioned emotional response were examined, by infusing the antagonist (0, 1, 5, 25ug / 5ul) into the lateral ventricle, 30 min before each test session. During these test sessions, 4 pairings of a light conditioned stimulus (CS) and a 0.5 sec, 2.1 mA (biphasic, direct, constant current) footshock were presented, while the animals were responding for food reinforcement.

The control animals showed a significant decrease in response rate during the presence of the CS in the 9 test sessions, demonstrating their acquisition of a conditioned emotional response. However, this response suppression was significantly attenuated by alpha-helical CRF, at all of the doses studied.

These results provide further evidence for the importance of CRF in the behavioral response to stress. However, further experiments are necessary to determine the nature of the behavioral impairment. One possibility is that alpha-helical CRF produces an 'anxiolytic-like' effect, qualitatively similar to benzodiazepines. Alternatively, endogenous CRF may be specifically involved in the acquisition of the conditioned fear.

121.15 FOOTSHOCK-ELICITED FREEZING IN THE RAT IS ENHANCED BY INTRACEREBROVENTRICULAR ADMINISTRATION OF CORTICOTROPIN-RELEASING HORMONE AND IS ATTENUATED BY ITS ANTAGONIST. J.E. Sherman,* C.M. Barksdale,* L.K. Takahashi,* and N.H. Kalin* (SPON: L. Hegstrand). Dept. of Psychiatry, Univ. of Wisconsin-Madison 53792, and William S. Middleton Veterans Hospital, Madison, WI 53705.

Freezing is a defensive behavior in rodents displayed in response to fear-eliciting stimuli including predators and aggressive conspecifics. In an initial study we found that footshock significantly ($p < .05$) increased freezing and plasma levels of ACTH in a manner directly related to shock intensity. To assess whether corticotropin-releasing hormone (CRH) plays an endogenous role in shock-elicited freezing, we administered synthetic CRH or its antagonist α-helical CRH[9-41] intracerebroventricularly to rats and observed their behavioral response to mild footshock. In Experiment 1, rats were given 0 (vehicle), 100, or 300 ng of CRH. Twenty-four minutes after drug administration the test rats received three brief footshocks (0.79 mA, 1.0 s each, 20 s apart); controls were not shocked. CRH significantly enhanced shock-elicited freezing in a dose-dependent fashion but had no effect on freezing in nonshocked controls. In Experiment 2, rats given vehicle or CRH (300 ng) displayed comparable response latencies on the hot-plate test of pain sensitivity, ruling out the possibility that altered sensitivity to pain could account for CRH's effect on freezing. In Experiment 3, rats were given 0, 5, or 25 µg of the CRH antagonist 24 min before footshock (1.0 mA, 1.0 s each, 20 s apart). The 25-µg dose significantly attenuated shock-elicited freezing but the 5-µg dose had no effect. Hot-plate tests of pain sensitivity showed that the CRH antagonist did not significantly modulate nociceptive processing. These findings suggest that in the rat, endogenous CRH systems play a role in shock-elicited freezing and do so via processes unrelated to nociception.

121.16 CORTICOTROPIN-RELEASING FACTOR (CRF) AND NOREPINEPHRINE INVOLVEMENT IN THE REGULATION OF EXPLORATORY BEHAVIOR. C.W. Berridge and A.J. Dunn.
Department of Neuroscience, University of Florida, Gainesville, FL 32610.

Corticotropin-releasing factor (CRF) within the brain has been postulated to participate in the regulation of both behavioral and physiological responding in stress. Using the multicompartment chamber (MCC) described by Arnsten & Segal (Life Sci. 25: 1035-1042, 1979) to study exploratory behavior in a novel environment, we have observed similar effects of restraint stress and ICV CRF on investigatory behavior in mice. Both restraint and ICV CRF (10-150 ng) decreased the mean time per contact with environmental stimuli in the absence of significant effects on locomotor activity as measured by rears or compartment entries. This effect of restraint was observed in hypophysectomized animals, indicating an action independent of the pituitary-adrenal axis. The CRF-antagonist, alpha-helical CRF_{9-41}, injected ICV 10 min prior to restraint reversed the effect of restraint in a dose-dependent manner with significant effects at 10, 20 and 50 µg. This effect of the antagonist was observed in the absence of consistent effects on locomotor activity. These results support the hypothesis that endogenous CRF participates in the regulation of behavioral responding in stress.

Both CRF and stress activate cerebral noradrenergic systems, and norepinephrine (NE) has been implicated in behavioral responding in stress. Therefore, we examined the effect of clonidine, an alpha-2 agonist, on the response of mice in the MCC. Clonidine increased the mean time per contact with the environmental stimuli in a dose-dependent fashion with significant effects observed at 25 and 50 µg/kg. This effect of clonidine was prevented by the alpha-2 antagonist, yohimbine (1 mg/kg). Our results suggest the possibility that one mechanism by which restraint and CRF act to decrease investigatory behavior in the MCC involves an activation of noradrenergic sytems.

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121.17 ENVIRONMENTALLY-DETERMINED BEHAVIORAL EFFECTS OF ICV ADMINISTERED CRF: DIFFERENTIAL INHIBITION BY CHLORISONDAMINE. D.R. Britton and E. Indyk*. Department of Pharmacology University of the Health Sciences / Chicago Medical School and Neurosciences Research, Abbott Laboratories, North Chicago, Ill. 60064

Rat/human corticotropin-releasing factor (CRF) (kindly provided by J. Rivier of the Salk Institute) was injected icv into rats prior to testing in either the home cage or a modified open field test (Britton and Britton, 1981). As previously reported, CRF produces an increase in grooming, a decrease in food consumption and an alteration in locomotor activity. The nature of the altered locomotion is dependent upon the environment in which the animal is tested. Twenty-four hr. fasted rats tested in a familiar environment with CRF (30 or 60 pmol, icv) show increased rearing and horizontal locomotion while in the modified open field test, both measures of locomotion are decreased.

In an attempt to address the question of the possible contribution of sympathetic activation to the observed behavioral effects, some animals were pretreated with the ganglionic blocking agent chlorisondamine which has previously been shown to prevent the sympathetic activation produced by centrally administered CRF (Fisher et al., 1982). In the home cage test, chlorisondamine reduced locomotor activation following 60 or 120 pmol CRF and also reduced the suppression of eating. However, in the open field test where the effect of CRF is to reduce locomotor activation, chlorisondamine failed to reverse the CRF effect.

These data demonstrate that the CRF-induced activation of locomotor activity in the home cage is either a) mediated by, or b) dependent upon, different processes than the locomotor suppressive effects of CRF observed in the open field test. The chlorisondamine-sensitive component of activation could be mediated in part by elevated catecholamine levels which could serve as stimuli to arousal. Alternatively, an intact autonomic system may be required to support the metabolic demands of heightened activity. Since the locomotor inhibition seen in the novel open field was unaltered by chlorisondamine it appears unlikely that sympathetic activation is critical to that effect of CRF. The data fail to support the hypothesis that the behavioral effects of CRF are directly mediated by activation of the sympathetic nervous system.

121.18 INTRA-ACCUMBENS INJECTIONS OF CCK-8 ATTENUATE THE LOCOMOTOR RESPONSE TO APOMORPHINE IN 6-OHDA LESIONED AND CHRONIC-NEUROLEPTIC TREATED RATS. F.Weiss, A. Ettenberg and G.F. Koob. Div. of Neuroscience and Endocrinology, Scripps Clinic and Res. Fnd. La Jolla, CA 92037, Dept. of Psychology, University of California, Santa Barbara, CA 93106

Substantial anatomical data show that CCK-8 coexists with dopamine (DA) in midbrain DA neurons innervating the basal forebrain. Several lines of evidence (including electrophysiological, biochemical and behavioral data) indicate that, in this coexistence system, DA and CCK may have functionally opposing roles. For example, in behavioral tests CCK-8 has been shown to antagonize the locomotor effects of psychostimulants. There is, however, some evidence to suggest that CCK is more effective at reversing the behavioral effects of indirectily, rather than directly acting DA agonists. In fact, the peptide has been reported to enhance DA-mediated behaviors following joint injection of DA and CCK-8 into the nucleus accumbens (N.Acc.) Thus, it remains unclear whether the DA antagonist-like action of CCK is pre-or postsynaptic in nature. We have, therefore, studied the postsynaptic effects of intra-N.Acc. administered CCK-8 on APO-stimulated hyperlocomotion using two rat behavioral preparations of DA receptor supersensitivity.

The locomotor response to SC injections of APO (0.15 mg/kg) was markedly enhanced following 12 days of cis-[z]-flupenthixol treatment (4 mg/kg; IP). Bilateral intra-N.Acc. injections of CCK-8 (2 μg) reliably reduced the supersensitive locomotor response to APO challenge and also attenuated APO-stimulated locomotor activity in chronic-vehicle treated rats. The magnitude of CCK-8 effects was comparable to those of intra-N.Acc. injected haloperidol (5 μg). A lower dose of CCK-8 (20 ng) was without effect in both supersensitive and intact rats.

Intra-N.Acc. injections of CCK-8 also antagonized APO-stimulated (0.1 mg/kg;SC) hyperlocomotion in rats with bilateral 6-OHDA (8 μg/side) lesions of the N.Acc. In contrast to its actions in chronic-neuroleptic treated rats, however, CCK-8 reversed APO effects in the animals with lesions in a dose-dependent manner, and at much lower concentrations (1-100ng).

This attenuation of APO-induced locomotor activity by intra-N.Acc. injected CCK-8 in both DA supersensitivity paradigms strongly suggests that the peptide has potent antagonist effects on mesolimbic DA transmission, and supports the view that DA and CCK are functionally opposed to each other at the postsynaptic level.

SUBCORTICAL VISUAL PATHWAYS II

122.1 FUNCTIONAL PROPERTIES OF THE RETINOTECTAL CONDUCTION GROUPS IN THE OPTIC TRACT AND BRACHIUM OF THE SUPERIOR COLLICULUS IN HOODED RAT. D. Impelman and D. A. Fox. College of Optometry, University of Houston, Houston, TX 77004.

The electrophysiological characteristics and photic responses of the three retinal ganglion cell (RGC) conduction groups recorded in the optic tract (OT) differ from their properties in the brachium of the superior colliculus (SC). Previous electrophysiological studies have shown that the fast conducting t1/Y-like (12.5 m/sec) and the middle conducting t2/X-like (6.0 m/sec) axons branch to innervate both the superior colliculus and the dLGN (Sefton, Vis.Res.8: 867, 1968). In addition, HRP retinotectal studies have demonstrated that the largest percentage axonal projection to SC (63%) is comprised of the slow conducting t3/W-like (3.5 m/sec) axons (Schober et al., 1977, Z. Mikrosk. Anat. Forsch. 92: 283, 1977). We are interested in the presynaptic transfer properties of retinotectal axons and therefore studied their functional characteristics in OT and SC brachium recordings of field potential responses to electrical and photic (flicker and spatial frequency) stimulation. Our orthodromic response amplitude measurements of t1, t2 and t3 correlate better with the SC HRP estimates of the RGC conduction group projections than with SC unit latency histograms. Conduction velocity slowing predicted by changes in fiber diameter was measured in optic chiasm responses to antidromic OT/SC stimulation. Onset conduction velocities for t2 and t3 are decreased 3-9% and 15-20%, respectively, while only t3 peak velocities are decreased. Waveform rise times, durations and chronaxie increases are inversely proportional to SC brachium conduction velocities. Orthodromic OT/SC amplitude intensity functions are linear and show a sensitivity decrease correlated with threshold excitability. Recovery of excitability in SC brachium axons is characterized by increased refractoriness and a subnormal period in t2 fibers which is probably mediated by an increased intracellular potassium concentration. The ionic sensitivity of the t3 conduction group to the 4-AP K+ channel blocker (5 mM) suggests that the t3/W-cell activity produces the depression period characteristic of SC recovery functions. The functional differences in the photic properites of OT and SC brachium axons show a decreased acuity correlated with their receptive field properties. Flicker and frequency following properties of the t1 conduction group are unattenuated in the SC brachium. The slowing of impulse transmission and the delayed recovery, which is selectively amplified in the t3 conduction group, may act to enhance the response stationarity characterisitic of the W-cell population. Supported by NIEHS Grant ES 03183 (DAF).

122.2 FURTHER OBSERVATIONS ON TOPOGRAPHY AND COMPARTMENTALIZATION OF THE CAT'S SUPERIOR COLLICULUS. R.-B. Illing* (SPON: European Neuroscience Assoc.), Unit for Morphol. Brain Res., HNO-Klinik, D-7800 Freiburg, Fed. Rep. Germany.

The pattern of the retinal innervation of the superior colliculus (SC) has been reexamined and correlated to the mosaic of terminal zones of those afferent systems represented by the distribution of acetylcholinesterase (AChE) activity in the intermediate tectal layers of the cat.

In the colliculi ipsilateral to the eyes injected with horseradish peroxidase, a tripartite organization of the visual field representation was apparent. The rostral quater is supplied only by a crossed pathway and thus remained largely unlabeled except for fibers on the level of stratum opticum, which bridge this region to reach their terminal areas further back. The middle part of the SC was sharply demarcated against the rostral zone by receiving a substantial uncrossed innervation. The terminals of this pathway form clusters which appeared in tangential sections as a rather regular mosaic of islands with an average spacing of about 500 μm. The caudal collicular quater was void of labeling, indicating a monocular innervation from the nasalmost contralateral retina. The SC contralateral to the injection was labeled throughout the caudorostral extent, but included "holes" of weaker labeling corresponding in depth and distribution to the clusters seen in the uncrossed path. Thus only the middle two collicular quaters harbor a binocular representation. Depth and extent of the total retinal innervation is closely matched by the area of high AChE-activity in the superficial layers.

AChE-activity in the intermediate layers of the SC has been reported to be distributed in a complex gridwork. While this grid is prominent at caudal levels, it is largely absent from rostral regions. Serial reconstructions of longitudinal and tangential sections revealed that this change in architecture corresponds to the retinotectal system as the non-compartmentalized rostral part lines up with the rostral quater of the superficial layers characterized by the lack of an uncrossed retinal input. The topographic landmarks supplied by the retinal innervation helped to project the AChE-rich gridwork, and thus the mosaic of afferents related to it, back into the visual field. The periodicity of this pattern agrees well with the receptive field sizes determined in the intermediate collicular layers.

It is concluded that the structural changes across the cat's SC correlate to its topographic organization. Those tectal parts representing ipsilateral stimuli lack a compartmentalization, while intricate mosaic arrays of functionally distinct domains apparently prevail in tectal zones that fit the classical concept of contralateral representation.

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122.3 DISTRIBUTION, MORPHOLOGY AND SOURCES OF CHOLINERGIC AXON TERMINALS IN THE SUPERIOR COLLICULUS OF THE CAT. W.C. Hall, D. Fitzpatrick, D. Raczkowski and L.L. Klatt* Dept. Anatomy, Duke Univ. Med. School, Durham N.C. 27710

We examined the cholinergic innervation of the superior colliculus (SC) in the cat using a monoclonal antibody for choline acetyltransferase (ChAT) and tract tracing methods. ChAT immunoreactive fibers with small en passant swellings are found throughout the SC, but the highest concentrations are in the superficial and intermediate layers. In the superficial layers, the distribution is homogeneous, while in the intermediate layers ChAT immunoreactive terminals are distributed in a patchy pattern similar to the pattern of acetylcholinesterase staining in this layer (Graybiel, A.M. Nature 272, 1978). Electron microscopic analysis confirms the presence of cholinergic axon terminals within the SC and indicates that, in both the superficial and intermediate layers, the terminals form synaptic contacts on dendrites with small but distinct postsynaptic densities.

Potential sources of the ChAT immunoreactive fibers were identified by making injections of WGA-HRP into the SC and processing the sections for the demonstration of both retrograde transport and ChAT immunocytochemistry. Double labeled neurons are present in the parabigeminal nucleus (Pbg) and in a band of cells that stretches across the midbrain and pontine reticular formation and includes nucleus tegmenti pedunculopontis and the lateral dorsal tegmental nucleus. Since the Pbg projects to the superficial layers (Graybiel, A.M. Brain Res. 145, 1978), it is probably the major contributor of cholinergic terminals in these layers. Anterograde transport of WGA-HRP suggests that ChAT positive neurons in the reticular formation are the major contributors to the terminals in the intermediate layers. These two sources of cholinergic innervation may have distinct functions. The pathway from Pbg may be involved primarily with influencing the relay of visual information through the superficial layers. In contrast, the cholinergic pathway from the reticular formation has close associations with the substantia nigra and may play a role in the premotor functions of the intermediate grey layer.

A third potential source of ChAT innervation in the SC is a population of neurons scattered throughout the superficial grey and stratum opticum. However, it remains to be determined whether the axons of these ChAT positive neurons have local arborizations and thus contribute to the innervation within the SC, or whether they terminate in distant targets with few or no local terminations (supported by NSF grants # BNS-86-07060, BNS-84-11964 and BNS-85-19709).

122.4 Origins of visual input to the deep layers of the superior colliculus in rabbits. R. W. Sikes and C. R. Payette*. Dept. of Physical Therapy, Northeastern Univ., Boston, MA 02115.

The deep layers of the rabbit superior colliculus (SC) contain neurons which respond especially well to visual stimuli moving in a particular direction. The receptive fields of these units are large but topographically organized. While visual afferents from the retina and primary visual cortex terminate in the superficial layers of SC, no direct projection from the superficial to deep layers has been demonstrated. Thus, the pathways which provide visual information to the deep layer neurons are unknown.

An indirect trans-striatal projection from the visual cortex to SC via the substantia nigra, pars reticulata (SNr) has been proposed as a source of visual input to the deep layers. The projection from SNr to SC may be topographically organized. To test this suggestion directly, multiple injections of Fluoro-Gold and rhodamine-labeled latex microspheres (0.05 to 0.1 µl) were made into physiologically identified regions of the SC in Dutch rabbits. Spread into the superficial layers of the SC and the overlying cortex was controlled by injecting through permanently implanted guide cannulas. After survival times from 3 to 7 days, the rabbits were perfused with 10% phosphate buffered formalin. The brains were then removed and sectioned at 30 µm. The sections were viewed with fluorescent microscopy and the location of single or double labeled neurons was plotted.

Injections placed into SC regions which corresponded to each visual quadrant labeled neurons throughout the substantia nigra. Labeled neurons were observed in both pars reticulata and lateralis but not in pars compacta. Both large and medium sized cells were seen. Medium sized neurons were more frequently located ventromedially both above and within the fibers of the cerebral peduncles. In the latter case, the cells were frequently spindle shaped. Although the projection was primarily ipsilateral, a considerable number of labeled neurons were observed in the contralateral SNr.

Little evidence for a clear topographical organization of the SNr projection to SC was obtained. Rostrally and caudally placed injections labeled neurons at both rostral and caudal levels of the SNr. Multiple injections into distinct quadrants of the SC produced double-labeled neurons at most rostrocaudal levels. Although clusters of singly labeled neurons were observed, these clusters were intermixed and had little or no topographical relation with the injection sites. Thus, the SNr projection appears to be quite diffuse and would be expected to have global effects on SC neuronal activity. Other labeled regions which might bring topographically organized visual information to SC will be described.

122.5 LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS ON THE DEVELOPMENT OF THE CORTICOTECTAL PROJECTION IN CATS. K. LORD*, AND M. BEHAN. (SPON: L. STANFORD). Neurosciences Training Program, Univ. of Wisconsin, Madison, WI 53706.

A robust projection from primary visual cortex to the superficial layers of the superior colliculus (SC), as demonstrated following injections of tritiated leucine, is present at birth in the cat. It has been shown, however, that the adult physiological properties of collicular neurons in the superficial layers do not appear until several weeks after birth (Stein, 1973). It has been suggested that this immaturity in the physiological properties of collicular cells may reflect the developmental stage of corticotectal neurons, or the lack of well developed synaptic organization in the SC. A further possibility is that collicular neurons are still maturing postnatally.

Adult cats, and kittens aged 1 day, 1 week, and 2 weeks were used to examine the corticotectal projection, and the progression of neuronal development in the superficial layers of the SC. The corticotectal projection was studied at the light microscopic level using the anterograde tracer, Phaseolus vulgaris-leucoagglutinin (PHA-L), and at the electron microscopic level using wheat germ agglutinin conjugated with HRP (WGA-HRP). The rapid Golgi method was used to examine the morphology of cells in the superficial layers of the SC.

Following injections of PHA-L in area 17, preliminary results indicate that the morphological appearance of the corticotectal projection at 1 and 2 weeks of age is similar to that in adults. However, the distribution of axonal arborizations in the superficial layers of the kittens appears to differ from that in adults. In the young animals the density of labeled axons and terminals is higher in the stratum zonale and the upper part of stratum griseum superficiale (SGS) than in the adult where the density appears slightly higher in the lower part of SGS.

WGA-HRP labeled axon terminals are present at all ages examined. Corticotectal terminals make synaptic connections at 7 days of age, but it is not yet clear how extensive synapse formation is before this time.

Light microscopic observations of Golgi-impregnated neurons have revealed that while all the major types are present at birth, they are morphologically immature. Based on comparsions with adult tissue, many vertical field and horizontal cells are well developed at birth, while stellate and marginal type cells appear to be at an earlier stage of development at this time.

In summary, there are a number of morphological changes occuring after birth in the superficial layers of the SC. The exact nature of these changes requires further study. (Supported by NIH Grant EY04478)

122.6 AN ULTRASTRUCTURAL ANALYSIS OF THE CORTICOTECTAL PROJECTION FROM THE LATERAL SUPRASYLVIAN AREA TO SUPERIOR COLLICULUS IN THE CAT. M.J. Graper* and M. Behan. (SPON: A.J. Weber). School of Veterinary Medicine and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

In the mammalian visual system, the lateral suprasylvian area (LS) of cerebral cortex represents an important source of visual input to the superior colliculus (SC). Segal and Beckstead (J. Comp. Neurol., 225:259-275, 1984) have shown that two distinct areas of LS cortex, the posteromedial (PMLS) and posterolateral (PLLS) areas, project to superficial and deep laminae of the SC respectively. While the superficial layers of the SC are a primary target of visual input from retina and visual cortex, the deep layers receive multimodal input from a variety of different cortical and subcortical regions. Efferent targets of superficial and deep collicular layers are also different.

We are interested in the differential input of LS cortex on neurons in the superficial and deep layers of the SC, and in particular, whether the synaptic morphology of PMLS and PLLS neurons is different.

Injections of tritiated amino acids were made into PMLS or PLLS. Following a 24 hour survival, animals were perfused with mixed aldehydes and alternate sections through the SC were prepared for light (LM) and electron microscopic (EM) autoradiography. LM autoradiograms were analyzed to determine those areas of highest radioactive label. Corresponding areas from adjacent sections prepared for EM were serially thin sectioned and processed for EM autoradiography.

LM autoradiograms show the expected differential projection pattern from PMLS and PLLS in the superficial and deep layers of SC. Preliminary EM autoradiographic data indicates that there may be a difference in the morphology and synaptology of terminals from PMLS and PLLS. PMLS terminals are larger, up to 3.5µm in diameter, with clusters of centrally located mitochondria. They contain loosely packed vesicles which are predominantly pleomorphic, and make asymmetrical contacts on large caliber dendrites and axon initial segments. PLLS terminals are smaller, ranging from 0.5 - 1.5µm, have clear axoplasm, and several scattered mitochondria. They contain predominantly pleomorphic vesicles and make symmetrical contacts with smaller caliber dendrites.

It may be necessary to look upon the projection from LS cortex to SC as two distinct pathways that exert quite different effects in superficial and deep layers.

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122.7 LOCAL PROJECTIONS OF NEURONS IN THE DEEP LAYERS OF THE CAT SUPERIOR COLLICULUS: A STUDY USING PHASEOLUS VULGARIS-LEUCOAGGLUTININ (PHA-L). M. Behan and P.P. Appell*. School of Veterinary Medicine and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

The purpose of the present series of experiments was to examine the intrinsic projections of neurons in the deep layers of the superior colliculus in the cat.

Small, iontophoretic injections of the neuroanatomical tracer Phaseolus vulgaris-leucoagglutinin (PHA-L) were made in the superior colliculus in layers deep to the stratum opticum. After a six day survival, animals were perfused transcardially with mixed aldehyde fixatives. The tissue was sectioned at 80μm, reacted immunocytochemically for PHA-L, and alternate sections prepared for light and electron microscopy.

The injection sites usually measure less than a millimeter in diameter, and contain a maximum of 25 labeled cells in any section. Many neurons at the injection site can be classified easily. Almost all labeled neurons are small (10-15μm in diameter), and resemble the vertical, horizontal, and multipolar neurons described in previous Golgi studies of this area. Axonal arborizations and synaptic terminals are also clearly labeled with PHA-L. As previous investigators have determined that there is virtually no uptake of PHA-L by fibers of passage, it is probable that the arborizations described in this study originate from labeled cells at the injection site.

One of the most interesting findings is the extent of axonal projections in the superior colliculus. Following injections of PHA-L in the deep layers in the middle of the colliculus, labeled axons and terminals are found throughout the rostrocaudal and mediolateral extent, and in all superficial and deep laminae. A few axons and terminals are also found in the contralateral colliculus. Outside the borders of the superior colliculus, there are extensive axonal arborizations in the ipsilateral periaqueductal grey, and the mesencephalic reticular formation.

Are these axons derived from interneurons or are they the local collaterals of projection neurons? While it is not yet possible to answer this question with certainty, it is likely that both neuronal types are labeled. Regardless of their origin, these preliminary data suggest that very localized regions of the superior colliculus can influence extensive areas of the mesencephalon. We are now examining the ultrastructure of PHA-L labeled synaptic terminals and their postsynaptic targets.

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122.8 MORPHOLOGY AND PHYSICLOGICAL CHARACTERISTICS OF SUPERIOR COLLICULUS NEURONS IN THE HAMSTER THAT PROJECT FROM THE SUPERFICIAL TO THE DEEP LAYERS. P.R. Hess*, Z.K. Allen, M.M. Nikoletseas, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

An issue of considerable importance with respect to the organization of the mammalian superior colliculus (SC) is the extent to which cells in the superficial laminae communicate with the deep layers. Conventional anatomical tracing methods have been unable to provide a definitive answer to this question so we have used intracellular recording and horseradish peroxidase (HRP) injection techniques to label SC cells and define their interlaminar axonal projections.

We have recovered 65 superficial layer SC cells whose axons were well enough filled with dense reaction product to determine whether or not they projected to the deep layers. Of these, 44.6% (N=29) gave off one or more axon collaterals that descended at least as far as the intermediate gray layer. Not all morphological cell types projected to the deep layers with equal frequency: 33.3% of the recovered marginal cells, 17.7% of the stellate cells, 76.9% of the narrow field vertical cells, 71.4% of the widefield vertical cells, 20% of the horizontal cells and 50% of the neurons that we could not classify according to this scheme had axonal projections to the deep laminae. Those neurons with deep going axon collaterals generally had their cell bodies in the lower stratum griseum superficiale (SGS) or stratum opticum (SO). Neurons whose axons were restricted to the superficial layers usually had their cell bodies in the upper portion of the SGS.

As might be expected on the basis of previous correlations between the physiological and morphological characteristics of superficial layer SC neurons in hamster (Mooney, R.D. et al., J. Neurosci., 5:2989, 1985), most of the cells that projected to the deep layers were non-directional, but movement sensitive.

These data thus indicate that only a portion of the sensory processing accomplished by the superficial SC laminae is conveyed to the deep layers and that the interlaminar pathway does not equally involve all cell types in the SGS and SO.

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122.9 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE SUPERIOR COLLICULUS CELLS THAT PROJECT TO THE LATERAL POSTERIOR NUCLEUS IN THE GOLDEN HAMSTER. M.M. Nikoletseas*, R.D. Mooney and R.W. Rhoades (SPON: R. Waziri). Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The projection from the superficial layers of the superior colliculus (SC) to the lateral posterior nucleus (LP) in rodent and the pulvinar nucleus, its homologue in cat and monkey, has been the focus of considerable attention for a number of years. Nevertheless, there is relatively little detailed information regarding the physiological and morphological properties of the SC neurons that give rise to this pathway. This is especially true in rodents, and in an effort to fill this gap in our information regarding extra-geniculostriate visual pathways, we have used intracellular recording and horseradish peroxidase (HRP) injection techniques to characterize, both physiologically and morphologically, tecto-LP cells in hamster.

We have recovered 17 SC cells that were antidromically activated at low current intensities from LP stimulating electrodes. These neurons were remarkably uniform in both their physiological and structural properties. The average antidromic latency to LP shocks was 3.2 ± 1.5 ms and, with one exception, all of the recovered neurons had their cell bodies in the lowermost stratum griseum superficiale or stratum opticum (SO). One tecto-LP cell had its soma in the upper stratum griseum intermediale, but its dendrites extended into the overlying SO. Thirteen (76.5% of the sample) of the recovered neurons were widefield vertical cells. Nearly all of these were movement sensitive, but not directionally selective. Two of these cells had heavily filled axons that could be traced to LP. The rest of the sample was comprised of three narrow field vertical cells and one large stellate neuron.

The structural and functional homogeneity of tecto-LP projection neurons in hamster stands in sharp contrast to the superficial layer cells that give rise to the projection from SC to the lateral geniculate nucleus (LGNd) in this species (see Mooney, R.D. et al., this meeting). A variety of structural and functional cell types contribute axons to the tectogeniculate pathway, but only one of the 18 SC-LGNd cells we have recovered so far was a widefield vertical cell.

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122.10 MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF THE SUPERIOR COLLICULUS CELLS THAT PROJECT TO THE DORSAL LATERAL GENICULATE NUCLEUS IN THE HAMSTER. R.D. Mooney, M.M. Nikoletseas* and R.W. Rhoades. Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The superficial layers (the stratum griseum superficiale-SGS, and stratum opticum-SO) of the mammalian superior colliculus project to a number of thalamic targets. While retrograde tracing and electrophysiological methods have been employed to assess the physiological and morphological characteristics of subsets of tectothalamic projection neurons, only a very small number of experiments have examined both of these variables for individual SC cells. We have used intracellular recording and horseradish peroxidase (HRP) injection techniques to characterize the SC neurons which innervate the dorsal lateral geniculate nucleus (LGNd) in the hamster.

We have recovered 18 SC cells that were antidromically activated at low currents by stimulating electrodes positioned in LGNd. The average latency to LGNd stimulation was 2.11 ± 0.70 ms. All of the recovered neurons had their cell bodies in the SGS and all but three were located in the upper two-thirds of this layer. Not all of the morphological cell types in the superficial SC laminae contributed equally to the sample of recovered neurons: 38.9% were marginal cells, 33.3% were narrow field vertical cells, 16.7% had stellate morphology, 5.6% were widefield vertical cells and 5.6% were horizontal cells. Two thirds of the marginal cells were movement sensitive and/or directionally selective while one-half of the narrow field vertical cells responded nearly equally to stationary and moving stimuli and exhibited no preference for stimulus direction. This was also the case for the horizontal and widefield vertical cells that we recovered. One of the stellate cells had weak and variable visual responses and the other two were equally responsive to stationary, flashed and moving stimuli.

These data suggest that a variety of cell types in the superficial laminae of the hamster's SC contribute to the tectogeniculate projection, but that the dominant types in this pathway may be marginal and narrow field vertical neurons. Our data indicate further that several types of visual information are conveyed by this pathway and that it may not be appropriate to consider this tectal efferent projection as a single information channel.

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122.11 PROPERTIES OF LATERAL GENICULATE CELLS WHICH ARE INFLUENCED BY THE SUPERIOR COLLICULUS

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The retinal output, in mammals, is carried to the cortex along two parallel channels. It passes either through the Superior Colliculus (SC), or through the Lateral Geniculate Nucleus (LGN). In broad terms, the geniculo-cortical path is involved in fine discrimination of discrete target, while the colliculo-cortical system is associated with ambient vision and eye movements. Furthermore, neurons of the visual dorsal layers of the SC send axons to the LGN. To test the relationship between these two paths, two light sources are presented in the visual field. The first one is a light source, remotely located (the conditioning stimulus), the second one is the central stimulus which is located in the Receptive Field (RF) of a geniculate cell. Thus, the influences of the conditioning stimulus upon responses to the test stimulus are studied at the geniculate level, prior to, during, and after collicular inactivation.

Experiments were carried out on rabbits which were anesthetized, paralyzed and prepared for single units recordings from the lateral geniculate nucleus.

Results may be summarized in the following way: 1) All geniculate cells (30%) which reacted to collicular inactivation had their RF close to the optic axis. 2) It appears that the conditioning stimulus facilitates geniculate responses if the RF is located eccentrically. 3) Cells that decreased their responses had their RF distributed throughout the visual field. From data obtained so far (N=75) it may be inferred that the colliculo-geniculate fibers influence mostly the central vision. This conclusion is further supported by our previous observation that cells with concentric RF, that is, those which transmit successive contrasts, are particularly sensitive to the inactivation of the superior colliculus.

Supp. CRSNG, FCAR.

122.12 **PROPERTIES OF TECTAL CELLS PROJECTING TO DIFFERENT REGIONS OF THE PULVINAR IN RABBITS.** C.Casanova*+ and S. Molotchnikoff. (SPON: R.D. Freeman) Dept de Sciences biologiques, Universite de Montreal, Montreal, P.Q., Canada, H3C 3J7.

Electrophysiological techniques were used to study the tecto-pulvinar pathway in rabbits, which had previously only been studied anatomically. The animals were anesthetized with urethane and prepared for single unit recordings. Stimulating electrodes were placed in the anterior or posterior part of the pulvinar nucleus and in the optic tract. Using glass micropipettes a total of 563 neurons were recorded throughout the superior colliculus (SC). Of these, thirty-two were identified as tecto-pulvinar cells on the basis of antidromic activation (constant latency, persistence of response to high frequency stimulation, positive collision test). The latencies of the antidromic spikes were distributed between 0.5 and 6.0 ms; mean= 2.76 ± 1.37 ms. Interestingly, there appeared to be a marked difference in the response properties of collicular cells which responded to stimulation of anterior and posterior pulvinar. When the anterior part of the pulvinar was stimulated, the majority (91%) of the tecto-pulvinar cells recorded were visual and usually located in the superficial layers of the SC. However, when the posterior region was stimulated, tecto-pulvinar units were rarely visual (22%) and they were found in the deep layers of the SC. It thus seems that there is a segregation of the SC projections such that the visual layers project to the anterior part of the pulvinar and the multimodal layers to the posterior region.

Additionally, trans-synaptic responses were recorded in the SC following stimulation of the pulvinar (latencies between 1.5 and 14 ms; mean= 4.8 ± 2.6 ms, N= 143). These trans-synaptic responses cannot be attributed to possible activation of the ganglion fibers located near the pulvinar because they were also recorded in rabbits with a degenerated contralateral optic tract (mean= 5.7 ± 3.3 ms; N=23). As noted with the antidromic responses, there was a difference between the properties of the collicular neurons activated trans-synaptically, depending on which region of the pulvinar was stimulated. More precisely, 70% of the tectal cells driven by stimulation of the anterior part of the pulvinar were visually responsive, while only 40% when the posterior part was stimulated. Thus it seems that the two pulvinar regions receiving a tectal input send back information to the SC (sensorimotor loop), probably along an indirect pathway involving cortical areas, although a more direct pathway cannot be ruled out.

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122.13 A COMPARISON OF MAO AND ACHE STAINING PATTERNS IN THE MIDBRAIN WITH SPECIAL REFERENCE TO THE SUPERIOR COLLICULUS.

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The superior colliculus (SC) displays laminar specific patterns of acetylcholinesterase (AChE) staining which has, in part, given rise to ideas concerning compartmentalization of its afferents, efferents, and internal structure. The relationship between AChE and other biochemical markers, however, remains to be established. Thus, we sought to establish the spatial relationship between AChE and monoamine oxidase (MAO) staining in the hope of finding systematically related markers that would facilitate our understanding of functional compartmentalization in the SC.

Tissue was obtained from hooded rats, ferrets, belted rabbits, and mongrel cats. Fixation was by mixed-aldehyde perfusion. Serial sections were stained alternately for AChE by the copper ferricyanide method (Tsuji, S., Histochem. 42:99, 1974) and for MAO by the coupled peroxidase method (Kitahama, K., et al., Brain Res. 324:155, 1984). AChE staining in the SC was distributed homogeneously in the superficial gray layer with a slight dorsal to ventral density gradient. In the deeper layers, AChE staining was organized in a series of enzyme-positive patches distributed so as to form an interrupted band within the layer. In serial reconstruction these patches appear as elongated, partly cross-linked longitudinal bundles. Similar features were observed in all four species.

Overall, the distribution of MAO and AChE in the SC were quite similar with only a few noticeable differences. In the superficial gray layer, both MAO and AChE staining were distributed homogeneously although MAO had a ventral to dorsal density gradient. In the dorsal tier of the intermediate gray layer, patches of MAO and AChE were overlapping, but in the ventral tier they were not systematically related. In both intermediate and deep layers, AChE staining was primarily in the caudal SC, whereas MAO was found throughout the rostro-caudal extent of the SC. Furthermore, in deeper layers and periaqueductal gray, MAO staining exhibited a characteristic U-shaped band symmetrical about the midline where AChE staining was weak.

In the cholinergic afferents to the SC, we found a dichotomy of MAO-AChE staining. In these areas, AChE staining was very heavy and MAO staining was either weak (pedunculopontine tegmental nuc., lateral dorsal tegmental nuc.) or very weak (parabigeminal nuc.).

In light of the overall similarity of MAO and AChE staining in the SC, it is possible that spatially overlapping inputs utilize different transmitters. Whether or not these two independent systems can influence the same neuron remains to be determined.

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122.14 **MULTISENSORY INTEGRATION IN SUPERIOR COLLICULUS NEURONS IS DETERMINED BY MODALITY-SPECIFIC RECEPTIVE FIELD PROPERTIES**

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The receptive field properties of visual, somatosensory and auditory neurons in the superior colliculus of the cat have been well described and are essentially the same for unimodal and multimodal cells. However, multimodal cells are known to integrate inputs from different sensory modalities so that their responses to stimuli from one modality are markedly enhanced or depressed when a stimulus from another modality is also presented. Thus, their efferent messages represent a "synthesis" of multisensory inputs. At present, it is not known whether or not this synthesis disrupts the fundamental receptive field properties characteric of a specific modality of input (e.g., visual). The present experiments were initiated to examine this possibility by evaluating the receptive field properties of visual cells in the absence and then in the presence of an auditory stimulus.

Cats, chronically prepared for extracellular recording, were anesthetized with ketamine HCl, paralyzed and artificially respired with a mixture of N20 and 02. Neurons in the deep laminae of the superior colliculus were isolated, their receptive fields mapped and their modality-specific receptive field properties (e.g., direction selectivity) were determined using electronically controlled stimuli (visual: galvanometer driven mirror projected onto translucent hemisphere; auditory: broadband noise generator routed through a hoop-mounted speaker). Single-modality tests (visual only, auditory only) appropriate to evaluate a given response property were presented alone and in combination with a stimulus from another modality (visual and auditory together) in an interleaved fashion.

In most of the neurons examined, the auditory stimulus could enhance or depress visual responses, depending on "rules" previously described (see Meredith and Stein, J. Neurophysiol. 56:640, 1986). However, under the present experimental conditions, the receptive field properties of a neuron appeared to be immutable: directionally selective cells retained directional assymmetries; velocity selectivities and spatial sensitivities were also maintained during combined visual-auditory tests. Apparently, integration resulting from combined-modality stimulation enhances or depresses the activity of a cell but does not disrupt the fundamental receptive field properties that define how specific stimulus parameters will be encoded.

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122.15 **BEHAVIORAL STUDIES OF MULTISENSORY INTEGRATION IN THE CAT** B.E. Stein, W.S. Huneycutt and M.A. Meredith, Depts Physiol & Anat. Med Coll Va/Va Commonwealth Univ, Richmond, Va. 23298.

Recent physiological experiments have demonstrated that visual, auditory and somatosensory inputs converge on, and are integrated by, individual neurons in the superior colliculus (SC). This convergence often results in activity which exceeds that produced by either of the stimuli presented alone. This 'response enhancement' is dependent on the spatial and temporal coincidence of the stimulus combination. In contrast, the same stimulus combination presented out of spatial and/or temporal synchrony can often depress neuronal activity. Since most multisensory SC cells have projections to premotor areas, their enhanced or depressed responses should be reflected in overt, SC-mediated, behaviors. The present experiments were designed to test this postulate directly.

The behavioral apparatus consisted of 7 LEDs (40 ms duration, range=0.0013-0.011 ft-candles) and 7 speakers (broad-band noise burst; 40 ms duration, range=27-34 dB SPL) arranged in 30° intervals (90° left to 90° right) on a 90cm diameter semicirular track. Two cats were trained to fixate directly at 0° and to approach each stimulus when presented alone, or when presented simultaneously at the same locus (regardless of the particular eccentricity). The animals received a food reward from trays mounted beneath each LED-speaker pair. Two other cats were trained in a similar fashion, but were taught to ignore the auditory stimulus and to respond only to the visual stimulus even when both stimuli were presented stimultaneously at different loci. Once these tests were completed both pairs of animals were trained and tested on the opposite task. Each animal had a total of 20 trials of each stimulus condition (visual, auditory, visual/auditory) at each position (n=7) and at each stimulus intensity (n=5) (total trials = 2100 to 2800/animal).

In all animals, and at all stimulus eccentricities, correct responses to combinations of low intensity spatially coincident stimuli far exceeded that evoked by either stimulus presented alone. This behavioral response enhancement was multiplicative and could exceed a five-fold increment over that produced by the most effective single-modality stimulus. In contrast, when the visual and auditory stimuli were presented at the same time but at different loci, responses to these combinations were reduced below that evoked by the visual stimulus alone. This occurred even though the animals were taught to ignore the auditory stimulus. These observations are consistent with physiological data from multisensory SC neurons and indicate that the same rules that govern multisensory integration at the level of the single neuron also govern attentive and orientation responses of the intact, behaving animal. Supported by NIH grant NS 22543.

122.16 SINGLE UNIT ACTIVITY IN THE DEEP LAYERS OF THE SUPERIOR COLLICULUS IN BEHAVING RATS. D. A. Weldon and P. J. Best. Department of Psychology, University of Virginia, Charlottesville, VA 22903.

Multiple unit recordings in rats (Malmo & Malmo, EEG & Clin. Neurophysiol., 42:501, 1977) and single unit recordings in hamsters (Rose, Exp. Neurol., 87:225, 1985) have demonstrated relationships between neural activity in the superior colliculus and head movements. In order to investigate this phenomenon further, we measured single unit activity in the deep layers of the superior colliculus as rats performed a visuomotor orientation task in which head movements were under experimental control.

Long-Evans hooded rats were trained in an apparatus containing four cue lights and adjacent spouts through which 20% sucrose reinforcement was provided. Orientations towards the spout located adjacent to an illuminated light were reinforced. After stable performance was obtained, each rat was anesthetized with sodium pentobarbital and received a cranial implant including a moveable microelectrode assembly consisting of ten 25 μm wires (modified after Kubie, Physiol. Behav., 32:115, 1984) and electrodes positioned for electrooculogram recording. When retested in the behavioral task, a miniature 12 v light was affixed to the head to permit accurate measurement of head position and movement. Electrophysiological and behavioral data were stored on videotape and later analyzed off-line. Each unit was tested for visual, auditory, somatosensory, and vestibular responsivity, as well as for a relationship with specific behavioral events.

The activity of some deep collicular units was related to movements of the head in specific directions. Other units were silent until the rats engaged in specific postures involving neck extension, when the neural activity attained average rates of 25 spikes/second with bursts of up to 120 spikes/second. In several cells in which neural activity occurred during rearing behavior, the neurons also fired 1) when the animal was held such that neck extension was required for the animal to walk from the experimenter's hand to a platform and 2) when the animal drank from the lower spouts in the testing apparatus in a way that involved neck extension. Several neurons were responsive to somatosensory stimuli applied to the snout and vibrissae when the freely moving animal actively approached the stimulation probe; some of these neurons were unresponsive when the probe was presented while the animal was held in the experimenter's hand. These results indicate a role of the rat superior colliculus in head movements during visuomotor orientation and in sensorimotor integration.

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122.17 RETINOTECTAL PROJECTIONS IN RAY-FINNED FISHES: PHYLOGENETIC AND TAXONOMIC IMPLICATIONS. C. S. von Bartheld* and D. L. Meyer* (SPON: G. Rager). Dept. of Neuroanatomy, University of Göttingen, 3400 Göttingen, Federal Republic of Germany.

Retinotectal projections were studied after intraocular injections of horseradish peroxidase or cobaltous lysine in 33 different actinopterygian species (ray-finned fishes). The distribution of retinorecipient layers in the contralateral optic tectum was analysed. In addition, the degree of differentiation of the stratum periventriculare, and the presence of ipsilateral retinotectal projections was examined.

Retinofugal fibers are labeled in the stratum opticum (SO), stratum fibrosum et griseum superficiale (SFGS), stratum griseum centrale (SGC), stratum album centrale (SAC) and stratum periventriculare (SPV). Some species lack the projection to SO, others lack the projection to SGC, and a third group of fishes lack both projections. Five different patterns of retinorecipient tectal strata are distinguished. These patterns correlate with the species' taxonomic position and naturally fall into 5 groups within the actinopterygians:

Type I	(Non-teleost-actinopterygians)	: SO - SFGS - SGC/ SAC/SPV.
Type II	(Osteoglossiformes)	: - SFGS - SAC/SPV.
Type III	("Prototeleostei")	: SO - SFGS - SGC - SAC/SPV.
Type IV	(Siluroidei)	: - SFGS - SGC - SAC/SPV.
Type V	(Neoteleostei)	: SO - SFGS - SAC/SPV.

For the group of teleosts with the type-III pattern, we suggest the term "Prototeleostei". The retinotectal projection patterns provide a useful indicator for phylogenetic relationships.

The degree of stratification in the SPV decreases from the less to the more advanced ray-finned fishes. Stratification in the SPV - hitherto not described in teleosts - is prominent in most osteoglossomorphan teleosts, similar to the nonteleost actinopterygians (e.g. Polypterus, Acipenser). Ipsilateral retinotectal projections are present in nearly all "lower" actinopterygian orders, but not in the more advanced teleosts (Neoteleostei).

Preliminary studies on the distribution of acetylcholinesterase in the optic tectum indicate that the lamination pattern differs in different groups of ray-finned fishes. Further species need to be examined to possibly demonstrate a correlation of retinotectal projection patterns and acetylcholinesterase distribution.

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122.18 RELATIONSHIP BETWEEN ISTHMO-TECTAL AND RETINO-TECTAL FIBERS IN THE FROG RANA PIPIENS. E. Gruberg, M. Mote*, R. Waldeck* and M. Wallace* Biology Dept., Temple Univ., Philadelphia, PA 19122

The nucleus isthmi and the retina both project to the superficial layers of the frog tectum. Loss of n. isthmi leads to loss of tectally mediated visually-guided behavior. Isthmo-tectal and retino-tectal fibers appear to have an exclusive anatomical relationship. We explored the relationship in 3 ways: 1) we assessed whether other extrinsic inputs also terminate in the superficial tectum; 2) we investigated if there was any overlap of retino-tectal and isthmo-tectal pathways; 3) we determined whether other fiber systems cross in the supraoptic decussation with n. isthmi fibers.

1) We either applied bibulous paper soaked in a solution of 25% horseradish peroxidase (HRP) plus 2% lysolecithin to the surface of the tectum or injected 25% HRP solution into the tectum. After survival periods of 3 to 7 days we determined the distribution of retrogradely labelled cells. When HRP was restricted to the most superficial layer virtually all stained cells were found in the contralateral retina and contralateral n. isthmi. With deeper penetration of HRP, contralateral retina and both n. isthmi were stained. In the deepest penetration of HRP a significant number of cells were stained in other areas, particularly posterior thalamic areas, in addition to contralateral retina and both n. isthmi. We suggest that in the most superficial tectal layers the retina and n. isthmi are virtually the only extrinsic inputs.

2) We unilaterally injected n. isthmi with HRP (staining for orthogradely labelled fibers) and injected both retinas with tritiated-proline. We found an admixture of retinal and n. isthmi fibers after n. isthmi fibers decussate.

3) At the midline we cut the posterior half of the optic chiasm and the overlying supraoptic decussation and applied HRP to the cut. Only n. isthmi and retinal fibers were stained. Thus, no other fibers cross at the supraoptic decussation. In addition, animals with such cuts lost visually-guided threat avoidance behavior while visually-guided prey catching was unaffected.

We conclude that isthmo-tectal and retino-tectal fibers have an intimate and exclusive relationship. Supported by NIH grant EY04366.

122.19 SINGLE-UNIT ANALYSIS OF THE OPTIC PRETECTAL NUCLEUS, LENTIFORMIS MESENCEPHALI IN RANA PIPIENS. Katherine V. Fite, Carol Kwei-Levy and Lynn Bengston. University of Massachusetts, Amherst, MA.

The large-celled pretectal nucleus, lentiformis mesencephali, (nLM) receives a major projection from the retina and appears to be critically involved in horizontal optokinetic nystagmus (hOKN) in amphibians (Montgomery et al, 1982). Previously, several neurophysiological investigations have reported directional-specific unit responses in the pretectal region (Katte & Hoffman, 1980; Cochran et al, 1984) of ranid species, many with sensitivity to horizontally moving optokinetic patterns. Thus, we anticipated that nLM would contain a high proportion of such units, also with temporal-to-nasal sensitivity, since hOKN shows a strong asymmetry under monocular viewing conditions.

The present study was based upon a quantitative, computer-assisted single-unit analysis of visually responsive units from nLM under monocular conditions, with electrode recording sites recovered histologically for each unit (N=80). Eight directions of pattern movement at 3 stimulus velocities were presented to each unit (vertical, horizontal and oblique vectors; 6, 15 and 25 degrees/sec.). Surprisingly, only 26% of the total were found to be directionally sensitive, while 70% of the total were responsive to pattern movement, in general. Most units were spontaneously active and among the directionally sensitive units, no preference was observed for any direction of pattern movement. No systematic changes occurred in unit responses as a function of pattern velocity. Injection of picrotoxin into the contralateral eye 30 minutes prior to recording correlated with a 50% reduction in the number of directionally sensitive units and a larger proportion of movement-sensitive units than was observed in normal animals (86%). Thus, picrotoxin appears to reduce directional sensitivity.

These results are consistent with our previous 2-deoxyglucose studies (Fite et al, 1986) which showed an equivalent uptake of 2-DG during either temporal-to-nasal or nasal-to-temporal OKN stimulation. The lack of directionally selective units in nLM indicate that the directional nature of OKN reponses must originate elsewhere; alternatively, that other neural structures modulate the output of nLM such that the asymmetry of hOKN with monocular viewing involves more complex circuits than previously imagined. Results obtained from our 2-deoxyglucose studies strongly suggest that the accessory optic nucleus, nBOR, shows enhanced uptake of 2-DG only in the nasal-to-temporal direction of pattern movement. Since the primary efferent projection of nBOR is to nLM, we postulate that an inhibitory pathway from nBOR to nLM exists which may play a major role in the hOKN asymmetry. At present, studies are in progress to test this hypothesis.

AGING AND DEMENTIA: TRANSMITTERS

123.1 CORTICAL CHOLINERGIC RECEPTORS IN AGING RHESUS MONKEYS. M.V. Wagster†, L.C. Walker†, P.J. Whitehouse§, K.J. Kellar§§ and D.L. Price†. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Alzheimer Neuroscience Center, Univ. Hospitals of Cleveland, Cleveland, OH 44106; §§Dept. of Pharmacology, Georgetown Univ. Sch. of Med. & Dent., Washington, DC 20007.

In the cortex of the rhesus monkey, cholinergic and serotoninergic receptor binding declines significantly with age [Wagster et al., Soc. Neurosci. Abstr. 12:1469, 1986]. In this study, the effect of age on binding patterns of cortical cholinergic receptor subtypes was examined in rhesus monkeys (Macaca mulatta). Using in vitro receptor autoradiography (ARG), the distributions of cholinergic muscarinic receptors ([^{3}H] N-methyl scopolamine [NMS], [^{3}H] pirenzepine, and [^{3}H] oxotremorine) and nicotinic receptors ([^{3}H] acetylcholine) were mapped in the temporal cortices of 17 rhesus monkeys ranging in age from 2-22 years. The ligand, [^{3}H] NMS was selected to measure high- (M2) and low- (M1) affinity agonist cholinergic muscarinic receptor subtypes. The pattern of distribution in the temporal cortex revealed a greater concentration of M1 receptors in superficial cortical layers than in deep layers, although M2 receptors were concentrated in deep layers. M1, but not M2, receptor binding site concentrations were significantly decreased with age (ANOVA, $p = 0.0017$). In cortex, [^{3}H] pirenzepine ARG revealed a nonsignificant trend for M1 receptors to decrease with age. The pattern of distribution of [^{3}H] pirenzepine binding sites was identical to that of the low-affinity [^{3}H] NMS binding sites. The pattern of distribution of [^{3}H] oxotremorine binding for the M2 cholinergic receptor subtype in cortical layers was similar to that of high-affinity [^{3}H] NMS binding sites. M2 receptors were significantly decreased with age in the temporal cortex (ANOVA, $p < 0.0001$). The ligand, [^{3}H] acetylcholine (using atropine to block muscarinic receptors), was used to assess nicotinic receptors in the temporal region. Nicotinic receptor binding decreased significantly with age (ANOVA, $p = 0.0081$). In summary, M1 and M2 receptor binding occurred across all layers of temporal neocortex in macaques, but M1 receptor binding was highest in superficial cortical layers, and M2 receptor binding was highest in deeper cortical layers. The number of muscarinic and nicotinic receptor binding sites decreased with age in this cortical region. The age-related decline in binding site concentrations for both muscarinic receptor subtypes (M1 and M2) was uniform across cortical layers and cortical nicotinic receptor binding appeared to parallel this pattern.

Tissues obtained from the Regional Primate Research Center at the University of Washington, NIH grant RR 00166.

123.2 CHOLINERGIC RECEPTORS IN HIPPOCAMPUS OF AGED RHESUS MONKEYS. M.D. Applegate*†, M.V. Wagster†, L.C. Walker†, P.J. Whitehouse§, K.J. Kellar§§ and D.L. Price† (SPON: E.H.M. Koo†). †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Alzheimer Neuroscience Ctr., Univ. Hospitals of Cleveland, Cleveland, OH 44106; §§Dept. of Pharmacology, Georgetown Univ. Sch. of Med. & Dent., Washington, DC 20007.

Using in vitro receptor autoradiography, distributions of nicotinic and muscarinic cholinergic receptors were mapped in several regions of hippocampus and in the tail of the caudate of 17 rhesus monkeys (Macaca mulatta) ranging in age from 2-22 years. The age range and number of monkeys in each of the four groups were: Group I, two years old (n = 3-5); Group II, 6-7 years old (n = 2-3); Group III, 13 years old (n = 2); and Group IV, 18-22 years old (n = 6-7). [^{3}H] Acetylcholine (with atropine to block muscarinic receptors) was used to label nicotinic receptors. [^{3}H] Pirenzepine (M1), [^{3}H] oxotremorine (M2), and [^{3}H] N-methyl scopolamine (M1 and M2) were used to label muscarinic receptor subtypes. In the hippocampus, nicotinic receptor binding was generally low; the presubiculum exhibited binding concentrations 250-600% higher than in adjacent hippocampal regions. The oldest monkeys (Group IV) showed a significant ($p < 0.01$) reduction (<60%) in nicotinic receptor binding concentrations in the presubiculum. Total muscarinic binding in the hippocampus was much higher than nicotinic binding, and a reciprocal pattern of regional M1 and M2 binding was evident. Both low and high affinity M1 binding concentrations were relatively low in the subiculum and CA3 regions of hippocampus, whereas M2 binding concentrations were high in those areas. One exception to this pattern was the mossy fiber field in the apical dendrites of CA3, which showed low levels of both M1 and M2 receptor binding. Unlike reductions that occur in muscarinic receptors in the temporal neocortex [Wagster et al., Soc. Neurosci. Abstr. 12:1469, 1986] and in the tail of the caudate (where 40% and 21% decreases in M2 and M1 receptor binding, concentrations, respectively, were found), no clear age-related changes in muscarinic receptors were observed in the hippocampus. This age-related decrease in nicotinic, but not in muscarinic, receptor binding in the hippocampus, in concert with reported decreases in muscarinic receptor binding in other brain regions, suggests a selective regional vulnerability of certain cell populations with aging.

Tissues were obtained from the Regional Primate Research Center at the University of Washington, supported by NIH grant RR 00166.

123.3 ^{3}H-PIRENZEPINE BINDING IN AGED RAT BRAIN: REGULATION OF MUSCARINIC (M1) RECEPTORS AFTER CHRONIC CHOLINERGIC DRUG ADMINISTRATION. Norman W. Pedigo, Jr. Depts. of Pharmacology and Anesthesiology, Univ. of Kentucky Medical Ctr., Lexington, KY 40536.

A senescent rat model of aging was used to assess the effects of chronic cholinergic drug treatment on muscarinic receptors in frontal cortex. Young, adult and senescent rats (3,9 and 27 months old, respectively) were treated for three weeks with either intracerebroventricular (ivt) saline (2 µl/hr), ivt methylatropine or ivt oxotremorine (3 µg/hr). Age-related and treatment-induced receptor changes were quantitated via selective binding of ^{3}H-pirenzepine (^{3}H-PZ) to the M1 receptor subtype.

M1 muscarinic receptors in rat frontal cortex show a significant decline with advanced age. The density of ^{3}H-pirenzepine binding sites (Bmax) determined in saturation studies was reduced by 25-40% in young versus senescent rats, while Kd increased in older animals. Bmax values for ^{3}H-PZ in 3,9 and 27 month old rats were 290 ± 18, 262 ± 25 and 174 ± 24 fmol/mg protein, respectively (mean ± SEM).

Young rats administered chronic ivt methylatropine exhibited a significant (40%) increase in ^{3}H-PZ binding compared to age-matched controls. In contrast, 3 month old animals treated with ivt oxotremorine showed a 30% loss of M1 receptors in frontal cortex. Similar up-regulation, but no down-regulation was noted in adult rats. However, ^{3}H-PZ binding was not altered in senescent rats treated chronically with either ivt methylatropine or oxotremorine.

Thus, there was a marked age-related impairment of muscarinic (M1) receptor plasticity in rat brain. These results support previous observations from studies using the nonselective ligand ^{3}H-QNB. Current research endeavors will attempt to correlate these receptor alterations to changes in phosphatidylinositol turnover. Such information could have important implications for the potential treatment of Alzheimer's disease patients and cholinergic drug therapy in the elderly.

Supported in part by the American Federation for Aging Research and an NIA grant (AG04991).

123.4 ENDOGENOUS ACETYLCHOLINE RELEASE FROM BRAIN HEMISPHERIC REGIONS OF YOUNG AND SENESCENT FREELY MOVING RATS DETERMINED BY MICRODIALYSIS TECHNIQUE. S. Consolo*, C.F. Wu*, G. Pepeu° and R. Bertorelli* (SPON : A. Giachetti). Istituto di Ricerche Farmacologiche"Mario Negri", Milan, Italy and °Department of Preclinical and Clinical Pharmacology, University of Florence, Italy.

The in vivo extracellular acetylcholine (ACh) release from striatum (ST), frontal cortex (FC) and dorsal hippocampus (DH) of young (2 months) and old (18 months) freely moving rats was measured by intracerebral microdialysis technique coupled with a sensitive and specific radioenzymatic method. The procedure used for implanting the fibers was essentially the same as already described by others. A thin dialysis tube (AN 69 membrane, DASCO) was inserted trasversally through both ST and FC of the same animal. In other rats, the fiber was inserted through both DH. The day after implantation the rat brain areas were perfused with a Ringer solution containing 10 µM physostigmine sulphate, at a constant rate of 2 µL/min. The perfusates were collected at 10 min (ST) and 20 min (FC, DH) intervals. The collected samples were lyophilized and the ACh was assayed by a radioenzymatic method (sensitivity 1 pmole). After 30 min of perfusion, the ACh output from the three hemispheric areas studied reached a steady state level. In the young rats, the net average output of ACh, when corrected for the recovery (49.5%) and expressed as fmoles/min was 1804 ± 134 from ST, 608 ± 61 from FC and 669 ± 64 from DH. In the old rats, the basal ACh release was 53%, 35% and 37% lower in ST, FC and DH, respectively. The effect of hemicholinium-3, a choline uptake blocker, was studied in the striatum, the area most affected by age. The marked time-dependent decrease caused by the drug (20 µg i.c.v.) in the ACh release was not statistically different in the two groups. Thus, the release mechanisms in vivo do not seem to be impaired by age and more likely the decline in ACh release from the striatum of old rats is accompanied by a reduction of ACh synthesis. (The rats, Wistar strain, were kindly supplied by I.S.F., Trezzano sul Naviglio, Milano).

123.5 Cholinergic neurotransmission in the aged rat: a behavioral, electrophysiological, and anatomical study. D.M. Armstrong, G. Buzsaki, K. Chen, R. Ruiz, R. Sheffield, and F.H. Gage. Department of Neurosciences, University of California, San Diego, LaJolla, CA 92093.

In the rat, cognitive impairments are often associated with deficiencies in cholinergic neurotransmission. In the present study we employed behavioral, electrophysiological, and anatomical techniques to investigate age-related alterations of basal forebrain cholinergic neurons. Throughout these studies young (8 months) and aged (24 months) female Fischer 344 rats were examined. Behavior: As a group aged rats show behavioral impairments compared to controls. However, some of the aged rats performed as well as young controls. Electrophysiology: Most aged rats show pathologic EEG patterns as reflected by frequent long-duration high voltage neocortical spindles present during immobility. Anatomy: Cholinergic neurons were identified by immunohistochemical techniques using antibodies against choline acetyltransferase (ChAT), the acetylcholine biosynthetic enzyme. The quantitative assessment of neuronal number and size was made on a computerized image analysis system. Thus far our studies of representative tissue sections of the medial septum (MS) and magnocellular preoptic/substantia innominata (MgPO/SI) indicate no significant decrease in cell number. Cholinergic neurons, however, are reduced in size (i.e., area) by 12% in the MS and 20% in the MgPO/SI compared to young controls. Moreover, the degree to which cholinergic neurons are shrunken appear to correlate with extent of cognitive impairment.

In the present investigation we also observed swollen ChAT-positive neurites in the neocortex of the aged rat. These neurites appear either singularly or bunched together forming a "plaque-like" structure. These latter structures appear similar to the dystrophic ChAT-positive neurites observed in autopsied tissue of patients with Alzheimer's disease. This research was supported by the J.D. French Foundation and NIH grants AG05344 and AG06088.

123.6 ALTERATIONS IN MUSCARINIC CONTROL OF STRIATAL DOPAMINE AUTORECEPTORS IN SENESCENCE: A DEFICIT IN MEMBRANE CALCIUM MOBILIZATION? J.A. Joseph, G.S. Roth*+, T.K. Dalton*, and W.A. Hunt*, The Armed Forces Radiobiology Research Institute, Bethesda, MD 20814, +Gerontology Research Center, NIA,Baltimore, MD 21224.

Numerous reports have indicated that the release of striatal dopamine (DA) is controlled by inhibitory DA autoreceptors (ATR) which are in turn regulated by inhibitory cholinergic heteroreceptors (HTR) located in close vicinity to the ATR. Muscarinic HTR (eg., via oxotremorine) activation enhances K+ induced release of DA from striatal slices from mature (M) but not senescent (S) rats. Since it has been shown that age-dependent declines in Ca++ mediated acetylcholine release can be restored by the ionophore A23187, it was of interest to determine if age-related decrements in calcium mobilization might contribute to the alterations in muscarinic control of the striatal DA ATR seen in senescence. Striatal tissue slices (300 mu) obtained from M (6 mo.) and S (24 mo.) Wistar rats were placed in chambers and superfused with a modified Krebs-Ringer medium containing (in mM) NaHCO3 21, glucose 3.4, NaH2PO4 1.3, EGTA 1, MgCl2 .93, NaCl 127, KCl 2.5 (Ph 7.4) ("Lo KCl buffer"). After a 30 min equilibration period, a 5 min baseline fraction was collected. The M & S tissue in four of the chambers was then perfused with "Hi KCl" (30 mM KCl, 1.26 mM CaCl2.2H20, NaCl 57 mM, in addition to the components described above) and 0 or 500 uM oxotremorine. In other parallel chambers A23187 (10 or 100 uM) was added to Hi KCl buffer which contained one of four Ca++ concentrations (in mM; 1.26, 6.2, 11.2, or 27.2) and no oxotremorine. Six 5 minute fractions were collected. DA release was determined using HPLC coupled to electrochemical detection. The results indicated that, as previously demonstrated, oxotremorine was not effective in enhancing DA release in striatal tissue from the senescent animals (193 + 8% peak DA enhancement young; 2 + 0.5% old). However, application of 10 uM A23187 to senescent striata evoked a 181 + 10% enhancement of K+ induced release of DA in the presence of 11.2 mM Ca++. In the presence of 100 um A23187, K+ induced release of DA was enhanced at all Ca++ concentrations in the senescent tissue. While age-related differences in enhancement of K+ induced release of DA still remained at both 10 and 100 uM A23187, they were reduced with the application of the higher A23187 concentration (eg., 10 uM A23187, 1.2 Ca++ DA peak enhancement = 153% + 6% young, 110 + 5% old; 100 uM A23187, 1.2 Ca++ = 175 + 12% young, 165% + 6.5%). (Note: Neither concentration of A23187 induced DA release with the Lo KCl.) These results suggest that a deficit in Ca++ mobilization may occur in senescence, and that this decrement may contribute to reduced control of striatal ATR's by muscarinic HTR's.

123.7 AUTORADIOGRAPHIC EVIDENCE FOR REDUCTION IN D1 DOPAMINE RECEPTOR BINDING IN AGED RHESUS MONKEY CORTEX. F. Filloux, *+ P.J. Whitehouse, ++ T.M. Dawson,+ M.V. Wagster, D.L. Price, L.C. Walker and J.K. Wamsley.+ Depts. of Psych., Pharmacol., Neurol., Univ. of Utah Sch. Med.,+ SLC, UT 84132; Neuropath. Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205-2182; and Alzheimer Neurosci. Center, Univ. Hosp. Cleveland,++ Cleveland, OH 44106.

Behavioral studies have demonstrated impaired cognitive functioning in aged non-human primates. The precise neurobiological correlates of this deterioration in cognitive ability are unclear; however, both animal and human studies have demonstrated age-related alterations in a variety of neurotransmitter systems. Surprisingly, a recent report (Rinne, Brain Res. 404:162, 1987) failed to demonstrate D1 receptor alterations in the brains of aged humans. In contrast, using [^{3}H]-SCH 23390 receptor autoradiography, this study demonstrates a highly significant reduction in D1 receptor binding in the temporal cortex of aged rhesus monkeys (M. mulatta).

Ten micron sections of hippocampus and adjacent temporal cortex were obtained from the brains of seventeen M. mulatta monkeys aged 2-22 years. Three age groups were sampled: two year-old animals; animals 6-15 years; animals 17-22 years of age. Tissue sections were incubated according to previously described methods (Dawson et al., J. Neurosci. 6:2352, 1986) in a Tris-HCl buffer containing NaCl, $CaCl_2$, $MgCl_2$, KCl and 1.0 nM [^{3}H]-SCH 23390. Labeled sections and brain paste standards were apposed to tritium-sensitive LKB Ultrofilm for two months. Quantitation of the resulting autoradiograms was performed by computerized microdensitometry.

[^{3}H]-SCH 23390 binding in hippocampal structures ranged from 0.43 fmoles/mg tissue (stratum oriens) to 4.16 fmoles/mg tissue (CA1, pyramidal layer), and did not show significant age-related changes. However, in all layers examined, cortical [^{3}H]-SCH 23390 binding was prominently reduced in the oldest compared to the youngest group. Specifically, binding was reduced from 7.93, 8.77 and 5.79 fmoles/mg tissue to 5.98, 6.56 and 4.73 fmoles/mg tissue in layers II-III, layer V and layer VI, respectively ($p<0.001$, $p<0.005$, $p<0.05$, respectively). Animals 6-15 years of age had intermediate levels of binding which approached those of the youngest animals, but which were not statistically different from receptor densities in either of the other two groups.

These results demonstrate that age-related changes in the mesocortical DA system do occur in the rhesus monkey. How this may relate to impaired cognitive functioning in non-human primates is currently unknown.

Tissues were obtained from the Regional Primate Research Center at the University of Washington, supported by NIH grant RR 00166.

123.8 AGE-RELATED AND REGIONAL DIFFERENCES IN BETA-2 ADRENERGIC RECEPTORS IN RAT CEREBELLUM: *IN VITRO* AUTORADIOGRAPHY WITH [^{125}I]CYP. E. Pearlman*, D. Lorton, R.M. Booze, H.H. Yeh and J. N. Davis. Departments of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC, and Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY. (Spon: Dorothy G. Flood).

Membrane binding studies of age-related changes in rat cerebellar beta-adrenergic receptor densities have reported increases, decreases and no changes in these receptors with age. To clarify these conflicting reports, we have used *in situ* receptor autoradiography to study age-related changes in cerebellar beta-2 adrenergic receptor densities. In addition, we compared the distribution of beta-2 receptors within several representative lobules of the rat cerebellum.

In situ receptor autoradiography was performed with [^{125}I]-cyanopindolol (CYP), a specific beta-adrenergic antagonist. The selective beta-1 adrenergic antagonist ICI-89,406 was used to localize the beta-2 adrenergic receptor subtype. Six- and 24-month old (N = 6 and 7, respectively) Long Evans hooded rats were sacrificed, the brains were quickly removed, blocked and frozen. Midsaggital cryosections of cerebella (16-um thickness) were slide-mounted for autoradiography and incubated with [^{125}I]CYP and either ICI 89,406 or 1 um dl-propranolol. The sections incubated with propranolol were used to estimate non-specific binding. Following incubation, the labeled sections were apposed to LKB ultrofilm for 30 h and the resulting autoradiographs examined using RAS 1000 Image Analyzer. Densitometric readings were made in cerebellar lobules II-III, VI and IX-X. Adjacent sections were processed histologically to aid in identifying [^{125}I]CYP-labeled lobules. A 2 (age) X 3 (lobule) X 2 (section) analysis of variances was employed to estimate the significance of the densitometric data.

We found significant overall effects related to age ($F_{1,10} = 12.5$, $p = 0.0054$), lobule ($F_{2,20} = 36.9$, $p = 0.0001$) and of an age X lobule interaction ($F_{2,2} = 5$, $p = 0.018$). The optical densities of beta-2 receptors in 24-month old cerebella were reduced by 30% when compared to those of 6-month old cerebella. Quantitative differences in beta-2 adrenergic receptor densities were evident among certain cerebellar lobules — beta-receptor density within lobule II-III was lower than that measured from lobule VI or loblules IX-X. On the other hand, no obvious differences in beta-2 receptor density were found in lobules VI and IX-X at any age examined.

These results suggest that there is an overall decline in beta-2 adrenergic receptors with age in the rat cerebellum. This change occurs with regional uniformity from lobule to lobule independent of any interlobular variations in beta-2 receptor densities.

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123.9 THE SEROTONERGIC AND DOPAMINERGIC SYSTEM IN THE AGED RAT CNS AS STUDIED BY IMMUNOCYTOCHEMISTRY WITH ANTIBODIES TO SEROTONIN AND DOPAMINE. H.W.M. Steinbusch, M.G.P.A. Van Luvtelaar*, J.G.J.M. Bol* and #J.A.D.M. Tonnaer*. Dept. Pharmacology, Free University, Amsterdam and #Dept. CNS Pharmacology, Organon Int. BV., Oss, The Netherlands.

Normal aging has been characterized neurochemically among others by a decrease of the cholinergic/serotonergic transmission. It has been observed that transmitter systems such as dopamine is involved in Parkinsonism, and acetyl choline and serotonin in Alzheimer's disease. For this study we used young adult (3 months old) and aged (28 month and older) rats for serotonin- and dopamine-immunocytochemistry. The serotonergic system in the aged rat appeared to be particularly degenerated in all layers of the frontoparietal cortex, the caudate-putamen complex, the medial preoptic region and the intermediolateral cell column of the spinal cord. In these regions we did not only observe a decrease in innervation, but in addition a large number of swollen varicose fiber clusters. A large decrease of serotonergic fibers was demonstrated in the hippocampus. With regard to the dopaminergic system we found a small but overall decrease in the number of dopaminergic fibers, but did not observe the appearance of highly degenerated varicose fibers like seen for the serotonergic system. No differences between young and old were demonstrated in the area of the entorhinal cortex, the medial habenulae or the caudate-putamen complex. However, there was a striking loss of dopaminergic cell bodies both in the substantia nigra, pars compacta as well as in the ventral area tegmental. The dopaminergic cells in the zona incerta and the arcuate nucleus show a decrease in immunostaining, while the dopaminergic cells in the posterior hypothalamic periventricular region show an increase in immunoreactivity.These results indicate that aging results in a local and not an overall degeneration of the serotonergic and dopaminergic system. It is known that both serotonergic as well as dopaminergic cell bodies send their axons to a whole variety of different forebrain regions. Retrograde tracing studies have shown that these cell bodies can give collaterals to various brain regions. The local degeneration of the serotonergic system in some forebrain regions and the absence of this degeneration in the dopaminergic system indicates the appearance of an intraneurotoxic compound, possibly receptor specific, for the serotonergic system, having no effect on the dopaminergic system. The specific disappearance of subpopulations of dopaminergic neurons in the substantia nigra and the ventral tegmental area in conjunction with a decrease of the cellular dopamine-immunoreactivity in the other diencephalic dopaminergic nuclei points towards the same direction, i.e. intraneuronal neurotoxic compounds, which induce a retrograde axonal degeneration. Both anterograde and retrograde tracing studies will provide further evidence for this hypothesis.

123.10 AGE-RELATED CHANGES IN IN VITRO CORPUS STRIATUM DOPAMINERGIC FUNCTION: INFLUENCE OF INTERNEURONS. D. E. Dluzen*, N. J. Laping and V. D. Ramirez (SPON: G. Jackson). Department of Physiology & Biophysics, University of Illinois, Urbana, Illinois 61801.

In the present experiment we examined the basal and stimulated in vitro efflux of dopamine from the corpus striatum of male rats as a function of three developmental periods (2-4, 11-13 and 18-24 months of age). Effluent samples were collected at 10 minute intervals (~300 ul) for 15 intervals and immediately assayed for dopamine using HPLC-EC. After a basal collection period of 5 intervals the responsiveness of these tissue preparations was evaluated following infusions of either potassium (30mM) or amphetamine (10^{-5}M).

In none of these experiments were any significant age-related changes in basal dopamine efflux observed with levels remaining relatively stable and ranging between 3-7 pg/mg/min. In Experiment I the potassium stimulated dopamine efflux from corpus striatum of 18-24 month old animals was significantly reduced (Peak Response = 6.0+.63 pg/mg/min, N=8) compared to the other two age groups, who failed to differ from one another (2-4 month = 32.2+11.8, N=5 and 11-13 month = 30.4+9.1, N=4). To asses whether this marked decrease in responsiveness of the corpus striatum from 18-24 month old animals represented a specific alteration in dopaminergic function or a generalized, non-specific decrement to potassium stimulation, in Experiment II we examined the response of these preparations to amphetamine, a specific activator of dopaminergic terminals. Amphetamine stimulated dopamine efflux from the corpus striatum of 18-24 month old animals was actually increased (Peak Response = 26.6+2.9, N=4) compared to 2-4 (17.6+2.9, N=4) and 11-13 (17.1+2.3, N=4) month old animals. In Experiment III corpus striatum tissue fragments from 2-4 and 18-24 month old animals were superfused with medium containing 10^{-6}M tetrodotoxin (TTX) to block interneuronal influences and subsequently challenged with infusions of potassium. In contrast to the results from superfusions with normal (TTX-free) medium (Experiment I), in the presence of TTX both age groups responded similarly to the potassium infusion (Peak Response: 2-4 month = 19.7+6.9, N=4 versus 18-24 month = 15.5+5.4, N=6).

These results suggest that age-related decrements in in vitro potassium stimulated dopamine efflux as obtained in 18-24 month old male rats may not necessarily be a direct result of an alteration in dopaminergic function per se, but seem to be due to changes in control of dopaminergic function exerted by interneurons.

123.11 AGE-RELATED CHANGES IN PRIMATE NEUROCHEMISTRY. G.L. Wenk, L.Cork, R. Struble, D. Pierce, and D. Price. Departments of Psychology and Neuropathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Deterioration in sensory, motor and cognitive functions is frequently associated with old age. Because these functions depend on the integrity of cortical processes, their impairment with age may be due to structural or functional alterations that may be expressed by changes in neurochemistry. Recent studies in humans have revealed decrements in various biochemical measures that correlate with age. In the present studies, the concentrations of monoaminergic neurotransmitter levels and the density of their receptors were determined in the brains of rhesus monkeys ranging from 2 to 37 years of age. Major changes that occur with increasing age include: (1) a decrease in cortical levels of dopamine and its major metabolites, homovanillic acid and dihydroxyphenylacetic acid, that is correlated with the increased presence of senile plaques, and (2) a decrease in cortical levels of serotonin and serotonergic (type 2) receptors. Significant, age-related changes were not found in measures of the (1) acetylcholinergic system, including choline acetyltransferase activity, and nicotinic and muscarinic receptors, (2) norepinephrine and beta-adrenergic receptors, or (3) benzodiazepine receptors. These findings demonstrate the selective neurochemical changes that occur in various neurotransmitter systems of the primate neocortex during normal aging. Supported by NIH AG 05146.

123.12 AGE-RELATED CHANGES IN DENSITY AND UP-REGULATION OF TYPES I AND II HIPPOCAMPAL CORTICOSTERIOD RECEPTORS. J.C. Eldridge*, D.G. Fleenor*, D.S. Kerr*, L.W. Campbell*, and P.W. Landfield. (SPON: J.H. Robinson). Dept. of Physiol and Pharmacol, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

The rat hippocampus contains a high density of receptors for corticosteroids (cf. review in McEwen et al, 1975; in: The Hippocampus, Plenum). Because of occupation by endogenous steriods, receptor analyses have used prior adrenalectomy (ADX). Prolonged ADX up-regulates corticoid binding in hippocampus (McEwen et al, 1974, Brain Res.), while diminished binding occurs with advancing age (Sapolsky et al, 1985, Brain Res.) or exposure to stress (Sapolsky et al, 1984, Endocrinology). Recent studies have indicated two distinct binding components in hippocampus: Type I receptors (CR) which exhibit high affinity and saturation during basal adrenal activity, and Type II sites (GR) which exhibit lower affinity and approach saturation only at elevated plasma corticoid levels (Reul and de Kloet, 1985, Endocrinology). However, the separate receptor types have not yet been studied extensively in response to prolonged ADX or aging.

Either single or paired hippocampi were dissected from Fischer 344 male rats for single point binding assays. Cytosols were prepared and incubated with 20 nM ^{3}H-Corticosterone (CORT). Type II binding capacity was assessed by co-incubation with 100-fold excess of non-radioactive RU-28362, a synthetic steroid that has specificity for Type II sites. Type I binding was calculated from the difference between total receptor capacity (using 500-fold excess dexamethasone) and binding displaced by RU-28362. Non-specific binding was also determined with non-radioactive CORT.

The quantity of Type I binding at 2 days post-ADX was significantly less in aged rats (23-26 mo-old) than young rats (4-6 mo-old). Type II receptor content did not differ with age at this point. Between 3 and 7 days post-ADX, a significant up-regulation of both receptor types occurred in young animals, although less consistently for Type I. In aged rats, Type II up-regulation was less than in young rats, while increases in Type I binding did not occur. Because of variability of Type I up-regulation, however, further work is needed on the latter point. In young animals, a further up-regulation of Type II, but not Type I, binding was exhibited after 11 days post-ADX.

These results indicate that neither decline of hippocampal CORT receptor binding in aging nor up-regulation after ADX is equivalent for the two receptor types. Type II binding exhibited more pronounced up-regulation, whereas Type I receptors appeared more affected by aging, at 2 days post-ADX and during prolonged ADX. (Supported by grants AG-04207 and DA-03637. We thank Roussel-Uclaf for assay materials).

123.13 UP-REGULATION OF OPIATE RECEPTORS IN MATURE AND AGED WISTAR RATS FOLLOWING CHRONIC NALOXONE. J. L. Neisewander, A. J. Nonneman, S. A. McDougall*, and M. T. Bardo. Department of Psychology, University of Kentucky, Lexington, KY 40506.

Previous research has shown a compensatory increase in 3H-naloxone binding sites, known as up-regulation, following chronic naloxone in adult rats. It is not known, however, whether aged rats are also capable of up-regulating opiate receptors. The present experiment therefore assessed whether there are age-dependent differences in up-regulation of 3H-naloxone binding sites in 19 mature (112-119 days) and 20 aged (27 months) male Wistar rats. Half of each age group was implanted subdermally with two slow-release pellets containing naloxone (10 mg free base in each). The other half received a sham surgery, for which an incision was made but no pellets were implanted. Ten days later the animals received a second surgery where the pellets were removed or another sham surgery was given. The day immediately following pellet removal or sham surgery, the rats were decapitated and the brains and spinal cords were removed. The brains were hemisected and the following areas were dissected from the left hemisphere: cerebellum, hindbrain, midbrain, diencephalon, hippocampus, striatum, olfactory tubercle/nucleus accumbens, and prefrontal cortex. These tissues were homogenized in 0.5 M tris buffer (pH 7.4) and incubated at 0° C for three hours with 1 nM 3H-naloxone in the presence or absence of 100 nM levallorphan tartrate. Each sample was washed over GF/B glass fiber filters and radioactivity was determined by liquid scintillation spectrometry.

There was a significant main effect of pellet treatment in all areas except cerebellum, indicating that chronic naloxone treatment produced up-regulation in these areas regardless of age. There was also a significant main effect of age in spinal cord, midbrain, striatum, and olfactory tubercle/nucleus accumbens, which showed that aged rats had fewer binding sites in these areas than mature rats. Interestingly, there was a significant age by pellet interaction in the hippocampus. A Newman-Kuels test showed that there was an enhanced up-regulation in hippocampus of aged naloxone-treated rats relative to mature naloxone-treated rats. There was no difference in binding sites between the sham groups in this region.

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123.14 ACETYLCHOLINESTERASE IN CHOLINERGIC NEURONS INCREASES IN ALZHEIMER'S DISEASE. Younkin, L. H., Palmert, M. R., Usiak, M., and Younkin, S. G. Depts. of Pathology and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

In a previous study of 26 cases of Alzheimer's disease (AD) and 14 controls well matched for age and postmortem interval, we assayed acetylcholinesterase (AChE) and evaluated cholinergic neurons in the nucleus basalis of Meynert (nbM). In AD there was a significant 61% decrease in the number of cholinergic neurons, an insignificant 19% decrease in nbM AChE, and a significant 266% increase in nbM AChE per cholinergic neuron. To eliminate AChE contributed from the region surrounding the nbM, we reevaluated 13 AD and 11 control cases by taking punches (3 mm in diameter) from the center of the nbM in the frozen tissue blocks previously examined. In AD the mean AChE per cholinergic neuron measured in these central nbM punches increased over 2-fold. We have also employed a quantitative immunocytochemical approach to evaluate AChE in cholinergic somata. In these experiments, sets of AD and control sections well matched for age and postmortem interval were processed in parallel. Thaw mounted 12 um cryostat sections were fixed 5 min in 10% buffered formalin, rinsed in water, and exposed serially to 1% normal goat serum (NGS) in 0.1 M Tris pH 7.6, 0.15 M NaCl (TBS) for 5 min X3, 10% NGS/TBS for 10 min, a 1:500 dilution of anti-human AChE antibody in 1% NGS/TBS for 16 h, 1% NGS/TBS for 5 min x3, ^{125}I-labelled goat anti-rabbit IgG in 1% NGS/TBS for 1 h, and 5 copious TBS rinses. Each section was dipped in Kodak NTB-2 emulsion, exposed for 48 hours, developed, stained for AChE, and the grains over cholinergic somata counted. To assess non-specific binding of radiolabelled secondary antibody, grains were counted over cholinergic somata in sections processed identically except for the omission of primary antibody. The primary antibody employed was an affinity-purified rabbit polyclonal that we raised against affinity-purified human red blood AChE (provided by T. L. Rosenberry). In parallel with the assessment described above, a series of standard sections (prepared by mixing human cerebral cortical brain paste with known amounts of AChE) was examined to establish that, for AChE levels like those in the sections examined, the amount of radiolabelled antibody bound is proportional to the AChE present. Grain counts over cholinergic somata showed (a) acceptable neuron to neuron variability (SEMs for grains/soma fell to less than 5% of the mean when 20-40 neurons were assessed) and (b) a significant increase in the number of specific grains (primary and radiolabelled secondary antibody - secondary only) over cholinergic somata when 7 AD cases were compared with 6 controls. (Supported by AG-06656 and a grant from the American Federation for Aging Research)

123.15 VARIABILITY OF GANGLIOSIDE CONTENT IN NUCLEUS BASALIS OF ALZHEIMER PATIENTS. P.B. Crino, M.D. Ullman*, B.A. Vogt, L. Volicer and E.D. Bird. E.N. Rogers Mem. Vet. Hosp., GRECC, Bedford, MA 01730, and Depts. of Psychiatry, Anatomy and Pharmacology, Boston Univ. Med. Sch., Boston, MA 02118.

Dementia of the Alzheimer type (DAT) is characterized by neurofibrillary tangles, neuritic plaques, neuronal cell loss, and reactive gliosis in specific brain regions such as nucleus basalis, hippocampus, and some neocortical areas. Because gangliosides are components of neuronal and glial cell membranes and because they may have been detected in neurofibrillary tangles, changes in regional distribution of brain gangliosides in DAT would be expected. We have measured gangliosides from affected and unaffected brain regions of five neuropathologically and clinically diagnosed DAT patients and from three controls.

Gangliosides GM1, GD1a, GD1b, and GT1b were quantified by HPLC of their perbenzoylated derivatives. They were extracted by solvent partition and isolated on a reversed-phase sample preparation cartridge. The isolated gangliosides were perbenzoylated at 45°C for 24 hours in 5% benzoyl chloride in 25% toluene in pyridine. The derivatives were quantified by their UV absorption at 230 nm and expressed as nmoles ganglioside/mg protein.

We have found that in hippocampus and areas 28 and 24, there were no consistent differences in ganglioside content between control and DAT tissues with little variability between subjects. In contrast, there was a large variability (up to 25 times) in ganglioside content of nucleus basalis in the DAT brains, although mean ganglioside concentrations were not significantly different from controls. This finding was replicated by TLC analysis. Ganglioside concentration did not correlate with age at disease onset, age at death, sex, post-mortem interval, and hemisphere analyzed. All DAT brains had pronounced deposition of plaques and tangles in the region of the nucleus basalis. Amyloid angiopathy and temporal lobe atrophy were noted in two brains. Ganglioside variability in nucleus basalis may reflect neuropathological heterogeneity of DAT.

123.16 A "NATURAL" ANIMAL MODEL OF PARKINSONISM. V.M. Trulson and M.E. Trulson. Dept. Anat., Coll. Med., Texas A&M Univ., College Station, TX 77843.

Previous studies have proposed the use of various experimental animal models of Parkinsonism. These include chemical (6-hydroxydopamine, 6OHDA) or mechanical destruction of the nigrostriatal dopamine (DA) system and administration of the apparently specific DA neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Destruction of the nigrostriatal DA system by these methods has been shown to produce abnormal motoric function. However, these behavioral changes do not mimic Parkinsonism in humans. We examined the aged mouse as a potential model for studying Parkinsonism. Four types of behavioral measures were taken in normal and aged mice, and those treated with either 6OHDA or MPTP. These tests included locomotor activity, roto-rod performance, ergometric activity and videotape records. In addition, both in vivo and in vitro electrophysiological recordings from DA-containing neurons in the substantia nigra were obtained from control mice, aged mice, 6OHDA and MPTP-treated mice. Locomotor activity measures revealed that 6OHDA treated mice showed no significant change from control animals (+6.4%) while both MPTP (-36.9%) and aged mice (-59.8%) showed a significant decrease from control values. Aged mice also moved significantly slower (-44.2%) than control animals, while 6OHDA and MPTP-treated mice showed no change. Aged mice fell off the roto-rod significantly more (+82.3%) than control, 6OHDA (+16.7%) or MPTP (+29.9%) treated mice. Ergometric activity was observed at regular intervals (mean = 7.2 sec) in aged mice. Video analysis revealed that this activity consisted of body tremors and head weaving. By comparison, little ergometric activity was observed in control, 6OHDA or MPTP-treated animals. When it did occur, video analysis revealed that it was associated with grooming. DA-containing neurons recorded in mice that had received MPTP or 6OHDA displayed the characteristic electrophysiological and pharmacological properties seen in control animals, with the exception that many fewer cells were encountered. However, aged mice showed altered electrophysiological (longer duration action potentials and slower discharge rates) and pharmacological (decreased responsiveness to DA agonists) properties. The reason for these differences among animal models of Parkinsonism may be due to the fact that, when a subpopulation of DA-containing neurons is destroyed by MPTP or 6OHDA in young animals, aside from the changes in the central DA system, the brain is still a "young brain." On the other hand, numerous neural changes have taken place in aged mice. These changes may be in terms of both afferent and efferent projections of the DA-containing neurons, as well as possible changes in membrane and receptor properties of individual cells due to the aging process.

123.17 **BENEFITS OF RAPID VS. DELAYED AUTOPSY IN HUMAN BRAIN CATECHOLAMINE AXONAL MORPHOLOGY.** C.R. Gutman*, R.M. Booze, and J.N. Davis. (SPON: G. Marsh) V.A. Medical Center and the Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Departments of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27705.

The aims of this study were: 1) to identify and classify tyrosine hydroxylase immuno-reactive (TH-IR) axons in normal aged human brain tissue, 2) to examine the regional distribution of six different types of TH-IR neurites and 3) to determine the role of post-mortem delay in axonal pathology.

Normal brain tissue was obtained via rapid autopsy (<1 hr) and routine autopsy (mean delay 5 hrs). Tissue blocks were collected from the superior frontal cortex (Brodmann area 9), hippocampal gyrus, and calcarine cortex (Brodmann area 17). The blocks were immersion-fixed in 4% paraformaldehyde (24 hours, 4°C) and stored in PBS. Adjacent Vibratome (50 um) sections were processed for H&E, Nissl, AChE, or TH-IR. A naive observer sampled 25 fibers from each TH-IR section and classified them into distinct fiber type categories based on their morphology (r^2 test-retest=.95, interobserver=.90).

Of the 650 fibers counted less than 1% were unclassifiable and 1-2% occurred in unusual clusters consisting of several fiber types. These rare fibers were not included in the analyses. All other fibers were easily classified into one of the six remaining classes. Of these six fiber types, autopsy delay significantly decreased the frequency of fine fibers with axonal varicosities (fiber type 2). The decreases were significant in the hippocampus and calcarine cortex with decreases of 27% and 26% respectively.

In summary, we have identified six distinctly different types of TH-IR fibers in human brain tissue. Our data demonstrates that even a relatively short post-mortem delay selectively alters distribution of fiber morphologies. These results suggest that post-mortem delays in fixation may compromise conclusions regarding axonal pathology.

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123.18 **CATECHOLAMINE AXONAL MORPHOLOGY IN ALZHEIMER'S DISEASE: EXAMINATION OF THE SYMPATHETIC INGROWTH HYPOTHESIS.** R.M. Booze, C.R. Gutman*, and J.N. Davis. VA Medical Center and the Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Depts. of Medicine (Neurology) and Pharmacology, Duke Univ. Durham, NC 27705.

We have hypothesized that the loss of cholinergic neurons in Alzheimer's disease results in the abnormal growth of peripheral sympathetic fibers (Booze and Davis, 1986). Sympathetic ingrowth occurs in experimental animal models in response to cholinergic denervation of the hippocampus. We have tested this hypothesis using rapid autopsy tissue obtained from normal and Alzheimer's disease patients and studying alterations in fiber morphology.

Tissue was obtained within one hour post-mortem from the superior frontal cortex (Brodmann area 9), the hippocampal gyrus, and the calcarine cortex (Brodmann area 17). The tissue blocks were immersion-fixed for 24 hours, Vibratome-sectioned, and processed for tyrosine hydroxylase immunocytochemistry (TH-IR). Sections were further processed for Thioflavin-S to neuropathologically confirm Alzheimer's disease. Additional adjacent sections were processed for Nissl, H & E, and AChE. All TH-IR sections were coded and 25 fibers from each section were surveyed and classified into one of six distinct fiber-type categories based on fiber morphology. A total of 925 TH-IR fibers were counted.

Statistical analyses of the categorical fiber count data found significant overall shifts in the relative proportions of fibers in Alzheimer's brain tissue. When analyzed by region, only the hipppocampus demonstrated significant changes in the fiber category profile. These changes were characterized by a decrease in long (up to 2 mm) TH-IR fibers and an increase in short, tortuous, axons with thick-caliber axons.

In summary, we have found shifts in fiber morphology in Alzheimer's disease that are quantitatively and qualitatively different from those occurring as a consequence of delays in tissue processing (Gutman, Booze, and Davis, this meeting). This specific shift in hippocampal catecholaminergic fiber morphology could represent sympathetic ingrowth in Alzheimer's disease. However, the lack of a specific marker for sympathetic fibers in human brain tissue precludes definite conclusions at this time.

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123.19 CEREBROSPINAL FLUID QUINOLINIC ACID ALTERATIONS IN NORMAL AGING AND ALZHEIMER'S DISEASE. M.M. Mouradian*, M.P. Heyes*, S.P. Markey*, T.N. Chase (SPON: J.R. Walters), Experimental Therapeutics Branch, NINCDS, Lab of Neurophysiology and Lab of Clinical Science, NIMH, Bethesda, MD 20892.

Excitatory amino acid transmission has recently been implicated in the pathogenesis of Alzheimer's disease. Quinolinic acid, an endogenous tryptophan metabolite, has potent excitotoxic properties and a high affinity to the N-methyl-D-aspartate receptor. Quinolinic acid is found in the human central nervous system, and is not derived from the systemic circulation. In this study quinolinic acid levels were measured in the cerebrospinal fluid of 19 patients with Alzheimer's disease and 14 neurologically normal volunteers. Spinal fluid was collected between 8 and 9 am after overnight fast and complete bedrest, at sequential 1 ml aliquots and frozen immediately. Quinolinic acid was assayed by negative chemical ionization gas chromatography/mass spectrometry as the dihexafluoroisopropanol ester using [^{18}O]-quinolinic acid as internal standard. There was no significant gradient in quinolinic acid levels between the first (5.67 $\pm$ 0.50 ng/ml) and thirtieth (4.63 $\pm$ 0.95 ng/ml) aliquot in five individuals. Quinolinic acid values increased with normal aging as measured in 14 controls with an age range of 22 to 81 years ($r = .655$, $p < .02$). Patients with Alzheimer's disease (mean age 64 $\pm$ 1.5 years, range 53-80) had significantly lower quinolinic acid levels (3.73 $\pm$ .48 ng/ml) compared to 9 age matched (mean age 64 $\pm$ 3.2 years, range 54-81) healthy volunteers (5.90 $\pm$ 1.07 ng/ml), (37% lower, $p < .05$). There was no significant correlation between quinolinic acid levels and symptom duration, age of onset, WAIS-R, Mattis Dementia Rating Scale, or Memory Quotient.

Whether the observed alterations in quinolinic acid in lumbar spinal fluid reflect changes in its central metabolism or transport remain uncertain. Nevertheless, the present results could cast doubt on the suggestion that an accumulation of this excitotoxin in the central nervous system accounts for the neuronal degeneration in Alzheimer's disease.

123.20 CORRELATIONS BETWEEN SOMATOSTATIN-LIKE IMMUNOREACTIVITY AND QUANTITATIVE EEG IN ALZHEIMER PATIENTS. H. Soininen*, J. Partanen*, J. Jolkkonen*, V. Laulumaa* and P.J. Riekkinen. Department of Neurology and Clinical Neurophysiology, University of Kuopio, 70210 Kuopio, Finland.

The most consistent neurochemical abnormalities in Alzheimer's disease (AD) are deficits in cholinergic and somatostatinergic systems. Reduced levels of somatostatin-like immunoreactivity (SLI) in cerebral cortex and also in CSF have been reported in several studies. A recent study demonstrated correlation between CSF SLI and cognitive impairment and glucose utilization measured by PET. We were interested in neurochemical substrates of EEG and analysed correlations between CSF SLI and variables of quantitative EEG (QEEG) in 24 patients with probable AD of mild to moderate severity.

The beta percentage ($r = 0.53$, $p < 0.05$) and mean frequency in alpha and theta range ($r = 0.49$, $p < 0.05$) correlated significantly with CSF SLI. However, in mild patients there appeared several other significant correlations: CSF SLI correlated positively with beta ($r = 0.71$, $p < 0.05$) and alpha ($r = 0.67$, $p < 0.05$) power, negatively with theta power and positively with alpha/theta and alpha/delta ratios, as well as with mean and peak frequency parameters.

The amount of beta activity is reduced in AD, and this is one of the main findings in the beginning of the disease. Interestingly we observed a significant correlation between beta percentage power and CSF SLI. To our experience CSF SLI is reduced already in early stages of AD and the observed association between beta activity and CSF SLI further supports the hypothesis that the disturbance of somatostatinergic cortical neurons is an early phenomenon in AD.

123.21 NEUROPEPTIDES AND NEUROPATHOLOGICAL CHANGES IN THE AMYGDALA IN ALZHEIMER'S DISEASE. J. Unger*, T. McNeill, L. Lapham* and R. Hamill. Depts. Neurology, Pathology, Univ. Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Alzheimer's disease (AD) is clinically marked by a progressive decline in intellectual acuity and memory as well as deficits in emotional, motivational and psychosocial behavior. Neuropathological studies have indicated the involvement of several neurotransmitter systems including acetylcholine and norepinephrine and in particular one neuropeptide, somatostatin. Since it is known that the amygdala, an important component of the limbic system, contains a large population of somatostatinergic neurons, partially co-localized with neuropeptide Y (NPY), we investigated the distribution and morphology of these neurons in AD. Further, we examined the distribution of classical neuropathological changes, neuritic plaques (NP) and neurofibrillary tangles (NFT) in subregions of the amygdaloid complex to determine if particular subnuclei of the amygdala may be more susceptible to the pathology of AD. Brain tissue was obtained within 11 hours postmortem from 10 patients with clinical signs of dementia and autopsy confirmed AD and seven normal controls of similar age. Serial sections were either stained with Bodian's silver method for NP or processed for SOM- and NPY-immunohistochemistry. Sizes of SOM- and NPY-IR perkikarya from 100 cells per case were morphologically analyzed using an IBM Bioquant IV computer system. The distribution curves, based on the number and size of SOM- and NPY-IR neurons in controls were identical, supporting the finding of a high rate of co-localization for both peptides. In AD, the curves showed an increase in the number of smaller neurons (<150 μm^2) for both SOM and NPY. However, the reduction of SOM immunoreactive cells was not as severe as reported for the cortex and is consistent with radioimmunological studies that showed only modest quantitative changes for SOM and NPY in the amygdala in AD. Subregional analysis revealed similar size reductions in all subnuclei. In contrast there was a characteristic pattern of NP distribution observed in 8 of the 10 AD cases. High density of NP was observed in the cortical, assessory basal, granular nuclei and transitory zone, moderate density in the medial and central nuclei and few NPs were seen in the basal and lateral nuclei. Double labeling with thioflavin showed some SOM- and NPY-IR fibers closely associated with NP but most of the IR fibers and neurons were found in areas between plaques. Supported by PHS Grant AG03644 and DFG, Un 59/1-2 (JU).

123.22 CHANGES IN CORTICOTROPIN-RELEASING FACTOR IMMUNOREACTIVE NEURONS IN THE PARAVENTRICULAR NUCLEUS OF ALZHEIMER'S DISEASED BRAINS. J.C. Pearson and L. Jennes. Dept. of Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435.

Alzheimer's diseased (AD) and control brains have been examined for cytoarchitectural differences, distribution of corticotropin-releasing factor (CRF)-immunoreactive neurons, and the presence of amyloid protein (Congo Red stain) in the paraventricular nucleus (PVN) of the hypothalamus. Human PVN may be cytoarchitecturally subdivided into parvocellular and magnocellular parts similar to those described in the rat. In control brains, CRF-immunoreactive (CRF-IR) neurons are scattered throughout the anterior, dorsal, and lateral parvocellular subnuclei. In the periventricular subnucleus, CRF-IR is contained in fusiform somata and in strands of varicose fibers. In the medial parvocellular subnucleus, CRF-IR neurons are densely packed and show extreme heterogeneity in soma shape and immunoreactive staining intensity. The magnocellular subnuclei make up a relatively small part of the PVN in human hypothalamus. The large neurons within the posterior magnocellular subnucleus show no CRF-immunoreactivity. In Alzheimer's diseased brains, cresyl violet stained sections show a dramatic reduction in the number of neurons and an increase in the number of glial cells in the PVN when compared to control brains. AD brains also show a significant reduction (approximately 80%) in the number of CRF-IR neurons in the PVN when compared to control brains. The greatest reduction in CRF-IR neurons is found in the medial parvocellular subnucleus. The number of CRF-IR fibers is also reduced in the PVN of diseased brains. Many existing fibers consist of swollen and irregularly spaced varicosities with thin intervaricose segments and resemble axons undergoing degenerative reaction. In Congo Red stained sections, amyloid protein deposits are located throughout the PVN of AD brains but not controls. The present results suggest that CRF-IR neurons in the hypothalamic PVN are strongly affected in the Alzheimer's disease process and may undergo degeneration similar to that which has been described for cortical CRF-producing neurons.

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123.23 RECEPTOR CHANGES IN HIPPOCAMPUS OF ALZHEIMER'S DISEASE. D.L. Debowey*, W.F. Maragos, Z. Hollingsworth*, J.T. Greenamyre, A.B. Young and J.B. Penney. (Spon: M.B. Bromberg) Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

There is extensive cellular pathology in the hippocampal region of Alzheimer's disease (ALZ) brains with many neurofibrillary tangles (NFTs) in CA1, subiculum and entorhinal cortex. Receptor studies have varied in the degree of change found depending on the assay used and the brains examined. No studies have directly compared multiple receptors to cellular pathology in the same regions of ALZ brains. Therefore, we have measured receptors in stratum moleculare (SM) and stratum pyramidale (SP) of CA1, dentate gyrus (DG), subiculum (SUB), presubiculum (PRE) and layers 2 (EC2) and 4 (EC4) of entorhinal cortex in 9 ALZ, 9 age and post-mortem-delay matched control and 7 similarly matched demented, non-Alzheimer's cases using quantitative receptor autoradiography. Serial cryostat sections were assayed for NFTs with congo red stain, muscarinic receptors with 1 nM [3H]-QNB (QNB), benzodiazepine receptors with 25 nM [3H]flunitrazepam (FLU), dissociative anesthetic receptors with 20 nM [3H]TCP (TCP), NMDA receptors with 200 nM [3H]glutamate plus 2.5 μM cold quisqualate both in 50 mM Tris-acetate buffer (NMA) and in 50 mM Tris-HCl buffer with 2.5 mM $CaCl_2$ (NMC) and total glutamate receptors with [3H]glutamate in the Tris-HCl with calcium buffer (GLU). All assays were performed, autoradiograms generated and data analyzed by observers blind to the diagnoses. Statistical analyses were determined by one way ANOVA.

Binding for all ligands was highest in DG and CA1, high in PRE, SM, and EC2 with less binding in EC4 and SUB. In ALZ brains there were significant binding reductions of 20-30% for QNB in SP and SUB; of 20-30% for FLU in SM, SP, SUB, PRE and EC2; of 30% for TCP and NMA in SM and SP; of 45% for GLU in SP; and of 50-60% for NMC in SM, SP and SUB. There were good correlations between the NMDA receptor changes and NFTs. The non-Alzheimer's brains had QNB and FLU reductions in SUB, PRE, EC2, and EC4 with NMA reductions in EC2 and EC4.

The results suggest that all the receptors measured were abnormal in ALZ hippocampus, but that NMDA/TCP receptors were more affected than others. The greater NMDA receptor changes measured in the presence of chloride suggest that Cl^- may reveal a set of binding sites that are absent from ALZ brains.

Supported by the Arbogast foundation, the Fraternal Order of the Eagles, Roy Gettel, and USPHS grants NS15655 and AG06155.

123.24 AGE-RELATED IMPAIRMENTS IN CONTINUOUS NON-MATCHING TO SAMPLE MEMORY PERFORMANCE ARE LESS ROBUST THAN IMPAIRMENTS PRODUCED BY DISRUPTION OF CHOLINERGIC TRANSMISSION. M.J. Pontecorvo, M.F. White*, D.B. Clissold* and L.H. Conti*. (Spon: L.R. Steranka). Nova Pharmaceutical Corporation, Baltimore, Maryland 21224.

Young (4 months) and aged (24 months) rats were trained in two experiments to perform a two choice tone/light continuous non-matching to sample (CNM) working memory task. In both experiments the rats were reinforced for responding on one lever if the stimulus was the same as on the previous trial (match), and reinforced for responding on the second lever if the current stimulus differed from the previous (non-match). Thus, the rats were required to remember across the intertrial interval (retention interval) which stimulus was presented most recently.

In Experiment I, young and aged rats were trained to criterion at short (1, 2.5 sec) retention intervals, then tested for asymptotic performance with retention intervals of 2.5, 10 and 20 sec. Under these conditions no age-related differences were observed. Both young and aged rats acheived the initial learning criterion ($A' > 0.80$, with 1 sec retention intervals) in less than 30 days, and acheived a stable, accurate asymptote ($A' > 0.85$ at 20 sec). In contrast to this lack of an age-related impairment, disruption of cholinergic transmission in these young rats by administration of the muscarinic antagonist scopolamine produced a significant impairment that was particularly evident at long retention intervals. Similar effects have also been observed in our lab following lesion of cholinergic neurons in the nucleus basalis magnocellularis.

Experiment II examined performance of young and aged rats on a more difficult version of the task. Rats were trained as in Experiment I. The ratio of match to non-match trials was then reduced, increasing potential confusion from previous trials (proactive interference), and the retention intervals were changed to 2.5 and 40 sec. As in Experiment I, no age differences were observed in acquisition or performance at the 2.5 sec retention interval. However, when the 40 sec retention interval was introduced, aged rats were both more variable (between subjects) and less accurate than the young rats. Asymptotic levels of performance (in progress) will be reported.

In summary, age-related impairments in CNM memory performance were not observed under conditions that are sensitive to disruption of cholinergic transmission. However, age-related impairments were observed under more difficult experimental conditions. Thus, there are at least quantitative differences between the memory impairments produced by aging and cholinergic dysfunction in this task.

AGING AND DEMENTIA: FUNCTION I

124.1 ENVIRONMENTAL ENRICHMENT AND BEHAVIOR: A LIFESPAN APPROACH. T.L. Petit and E.J. Markus. Div. of Life Sciences, Univ. of Toronto, Scarborough, Ont. M1C 1A4, Canada.

Environmental input plays an important role in the development of mammalian neuronal systems. It is of interest to examine the degree of environmental plasticity past early development as well as the lasting effects of these environmentally induced changes at different ages.

We examined the short and long term effects of environmental enrichment on learning and long-term retention in young (1-4 month) and aged (24-30 month) male rats.

The animals were in one of three regimes:

1) Control: standard housing.
2) Previously enriched: an environmental enrichment period followed by three months of standard housing.
3) Recently enriched: three months of environmental enrichment.

The animals were tested on a series on symmetrical mazes (Davenport et al., Behav Res Meth Instru 2:112-118, 1970) prior to, and following the final three months of housing

Preliminary results show:

1. Young animals are more effected by environmental enrichment than aged.
2. Long-term retention is best preserved in previously enriched animals kept in standard housing.
3. In both young and aged rats previous environmental enrichment improved learning even three months after the enrichment period.
4. There is a decay in the effect of environmental enrichment: recently enriched rats performed better than previously enriched rats.

124.2 POST-NATAL HANDLING ATTENUATES AGE-RELATED CHANGES IN THE ADRENOCORTICAL STRESS RESPONSE AND SPATIAL MEMORY DEFICITS IN THE RAT. M.J. Meaney, D.H. Aitkens*, C. Berkel*, S. Bhatnagar*, A. Sarrieau* and R.M. Sapolsky. Douglas Hospital Research Centre, Dept. Psychiatry, McGill Univ., Montreal, Canada H4H 1R3 and Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305.

Age-related changes in the adrenocortical axis of the rat result in an increased exposure to adrenal glucocorticoids, both as a result of increased basal levels and of steroid hypersecretion following stress. These increased glucocorticoid levels have been implicated in the age-related degeneration that occurs in the hippocampus, a principle corticoid target region in the brain. Adult rats handled (H) daily for a brief period of time (15 min) for the first three weeks of life show more efficient adrenocortical negative-feedback (reduced corticoid secretion during and following stress) associated with increased glucocorticoid receptors in the hippocampus, a critical brain region for the corticoid-induced inhibition of CRF and ACTH release.

In the present experiments H and NH Long-Evans rats were examined at 6, 12, and 24 months of age. All animals exhibited an age-related hypersecretion of corticosterone (CORT) following the termination of immobilization stress (delayed suppression of ACTH secretion), although this effect was significantly greater in the NH animals. NH animals also showed an age-related increase in basal CORT levels, suggesting that cumulative CORT exposure with age was greater in the NH animals. Cell counting in the hippocampus showed pronounced neuron loss in the CA_1 and CA_3 cell fields in the 24-month old NH animals, with only slight loss in the 24-month old H animals. The performance of the aged 12 and 24-month old H animals in the Morris' Water Maze (a spatial memory task that is highly dependent on hippocampal function) was significantly superior to that of the same-aged NH animals, and not reliably different from the younger animals (either H or NH). These data reveal an age-related spatial memory impairment in the NH animals, but not the H animals.

Taken together, these data suggest that individual differences in age-related hippocampal cell loss and cognitive impairments are related to differences in the adrenocortical stress response. The underlying mechanism for this relationship appears to involve the neurotoxic effects of increased glucocorticoid exposure on hippocampal neurons.

124.3 EFFECTS OF CHRONIC PROGESTERONE AND ESTRADIOL ON CYCLICITY OF MICE:IMPLICATIONS FOR AGING. S. G. Kohama* P. C. May, C. E. Finch (SPON: W. O. McClure). Department of Biology, U.S.C., Los Angeles, CA. 90089-0191.

Increases in progesterone (P) due to pseudopregnancy or P administration, increases the incidence of short cycles (Flurkey et al., Biol Repro, 1987). Orally administered estradiol (oE2) on the other hand accelerates the loss of cyclicity (Kohama et al., NSci Abst. 395.2, 1986). We tested the effects of P+oE2 to see if the hormones had an antagonistic influence on the cyclicity of mice. Young (5 month) C57BL/6J mice were given the following steroids: E2 implants (yielding serum levels of 15-25 pg/ml, iE2), P (70 ng/ml), oral E2 (80 pg/ml, oE2), P+oE2 (same doses) and appropriate controls. During treatment, the E2 and oE2 groups displayed persistent vaginal cornification, while the P and P+oE2 groups had vaginal cytology typical of low E2 levels. After 6 weeks, treatment stopped and cyclicity was evaluated for 5 months.

Cyclicity after treatment was evaluated on a monthly basis examining % cyclicity (contingency table analysis, CATMOD), cycles/mo (general linear model, GLM), and cycle length analysis, the mean number of 4, 5, 6 or >6 day cycles/mo/treatment (multivariate analysis, MANOVA).

Analysis of % mice cycling (CATMOD) revealed effects of treatment ($p<0.001$) and time ($p<0.001$). The estradiol treated groups (iE2, oE2 and P+oE2) lost cyclicity with aging faster than the other groups. The cycles/mo, showed differences due to treatment ($p<0.01$), time ($p<0.001$) and the interaction of treatment and time ($p<0.01$). At mo 2 and 3, the P group had more cycles than controls and iE2, whereas oE2 and P+oE2 cycled less than controls.

Cycle lengths were compared by MANOVA. The 4 and 5 day cycle length categories showed effects due to treatment, time and an interaction. During mo 2 and 3, the P and P+oE2 groups had more 4 day cycles than the respective control and E2 groups. All other treatments during this period resulted in predominantly 5 day cycles, except for the E2 implants which reduced cyclicity. Thus, chronic P leads to the prolonged reinstatement of short cycles, reversing age-related cycle lengthening.

This study was supported by NIA Training Grant T32-AG00093, and Grant AG-00443.

124.4 LORDOSIS RESPONSES IN AGING FEMALE RATS: EFFECTS OF PROLONGED ESTRADIOL TREATMENT. K. C. Chambers. Repro. Biol. & Behav., Oregon Regional Primate Research Center, Beaverton, OR 97006

Although most investigators have not found decreases in lordosis responses in aging female rats, Peng, Chuong and Peng (Neuroendocrinology 24:317-324, 1977) found that 41% of the old females given high doses of estradiol benzoate (EB) and progesterone (P) showed a decrease in lordosis responses to male mounts. In the following study, the lordosis responses of 20 young sexually naive and 20 aging retired breeder female Fischer 344 rats were examined. Since prolonged exposure to E has been shown to accelerate age-related changes in the reproductive system of female rodents (Finch et al., Endocrine Reviews 5:467-497, 1984), the effects of prolonged estradiol treatment on the lordosis responses of the young and aging females also was determined. Ovariectomies were performed when the younger females were 2 months old and the older females were 12-13 months old. Two weeks after surgery, all of the females were given a treatment of EB and P (10 µg/150 g of body weight of EB on 2 consecutive days and 0.5 mg/150 g of body weight of P 24 hours later). One week later sexual behavior testing was initiated. Females were given 1 test per week for 3 weeks while under EB and P treatment (tests 1-3). Tests lasted 20 minutes or until the males ejaculated. Both the younger and older females then were randomly divided in equal numbers into 2 groups: oil and EB. The EB-treated females received 10 µg/150 g of body weight of EB on 2 consecutive days per week for 18 weeks and the oil-treated females received an equal volume of oil per body weight on 2 consecutive days per week during the same period. The females were not tested behaviorally during this time. Three EB-treated older females died during the course of the treatment. On the 19th week, all of the females were given a treatment of EB and P. One week later sexual behavior testing was initiated. The females again were given 1 test per week for 3 weeks while under EB and P treatment (tests 4-6). The oil-treated older females had a significantly lower lordosis to mount ratio than the oil-treated younger females on test 1 but not tests 2 and 3. The lordosis to mount ratio of the oil-treated older females also was lower than the oil-treated younger females on test 4. Whereas the EB-treated older females had a significantly lower lordosis to mount ratio than the EB-treated younger females on test 1 they did not differ on test 4. Both groups of older females did not differ on test 1 but the EB-treated females had a higher lordosis to mount ratio on test 4. These data suggest that after an absence of E, older females require more exposure to E than younger females. Repeated exposure to E does not accelerate the decreases found in lordosis responses of older females.

This study was supported by grants HD-20970 and RR-00163.

124.5 BEHAVIORAL, PHYSIOLOGICAL, AND HISTOLOGICAL ASSESSMENT OF FETAL HYPOTHALAMIC TISSUE TRANSPLANTED TO DORSAL THIRD VENTRICLES OF AGED MICE. D. Ingram*, M. Talan, E. Bresnahan, J. Hengemihle*, S. Kobayashi*, H. Kametani*, and F. Gage. Gerontology Res. Ctr., NIA, NIH, Baltimore, MD 21224 and Dept. of Neuroscience, School of Medicine, UCSD, San Diego, CA 92093.

Decline in hypothalamic function is hypothesized as a mechanism of mammalian aging (Everitt, A., Exp. Gerontol., 8:265, 1973). Previous reports have demonstrated that fetal neural grafts can improve behavioral performance in aged rat hosts (Gage, F., et al., Science, 221:966, 1983). Relatively less research in neural tissue transplantation has been directed toward the aged mouse as a model. Therefore, we are investigating in inbred mice the feasibility of grafting fetal hypothalamic tissue from C57BL/6J donors to male hosts of the same genotype. Specifically, from 15-16 day old fetal brains, we dissected a basal diencephalonic area (< 1 sq mm) from the approximate anatomical location of the adult median eminence. Pieces of this tissue were transplanted via cannula into dorsal third ventricles of anesthetized aged (26-mo old) hosts using appropriate stereotaxic coordinates. Control groups include: young (6-mo) unoperated mice for age comparisons; aged sham-operated (cannulae lowered) and unoperated controls; and aged operated hosts that received fetal neocortical tissue or nonneural tissue (fat and muscle) taken from the fetal groin area. At 30 mo of age, all mice were subjected to an age-sensitive performance battery (Ingram, D., Exp. Aging Res., 9:225, 1983; Talan, M. & Engel, B., Exp. Gerontol., 19:79, 1984), including open-field activity, tight-rope test, rotorod test, water consumption, body temperature, oxygen consumption, cold tolerance, runwheel activity, and running speed test, and were then sacrificed. The hypothesis being tested was whether viable hypothalamic grafts would restore or prevent further age-related performance decline compared to controls. Although we have observed many examples of histologically viable grafts, i.e., well-developed neurons, minimal or no gliosis, interfaced to adult tissue, little evidence of functional effects have emerged from this on-going study. Preliminary analysis indicates that the most likely candidates for observing effects of this treatment were those tests reflecting more clearly defined hypothalamic involvement, e.g., body temperature and water consumption. Thus, this technique has proven feasible in mice for conducting further functional analysis of its effects on aging rate in behavioral and physiological performance. Grafting into younger (18 mo) mice may be a more productive approach to more efficiently prevent hypothalamic decline before senescent processes have had major impact. As such, we will be testing hypothalamic involvement in governing aging rate in a mammalian model.

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124.6 OLD RATS ARE CAPABLE OF ADEQUATE PERFORMANCE ON SPATIAL AND AVOIDANCE TASKS. J. Rick *, N.W. Milgram and G.O. Ivy. (SPON: C. Bielajew). Life Sci. Div., Scarborough Campus, U. of Toronto, Scarborough, ONT, M1C 1A4.

In addition to numerous morphological and biochemical effects on the brain, aging results in a number of behavioral changes. For example, aged rats have been reported to show marked deficits in spatial learning ability. We decided to further investigate such deficits in order to provide a basis for a future study involving young rats subjected to a treatment which produces morphological and biochemical changes similar to those observed in aged animals.

Mature (6-8 mo.) and old (18-22 mo.) Sprague-Dawley rats were tested for acquisition and retention of a spatial and a passive avoidance (PA) task. Using a Morris water maze we were able to assess differences in both reference and procedural memory. The latter was studied by moving the goal platform between the three tests. Five trials per day with a 30-minute intertrial interval were used to minimize the development of hypothermia. Each test consisted of a maximum of 25 trials.

The aged rats required significantly more trials to reach criterion on each of three tests and showed significantly less retention 3 days after the first test. No differences were observed on the second retention test. That the slower learning was not due to age-related deficits in motor ability can be concluded from the fact that the rats were equally capable of reaching criterion. In the PA tests, few rats of either group (50% young, 12.5% old) learned not to cross, though the groups did not otherwise differ significantly. Latency to cross increased significantly over trials for both groups. Subjects later received a second, more intense shock which produced complete avoidance in both groups within two trials.

Although the differences were less than anticipated, the old rats exhibited greater variability. While only one old rat did not learn, others were indistinguishable from mature animals. We conclude that old and mature rats are equally capable of learning and retaining memories of these tasks over periods of days or weeks, though older rats may require more time or more salient stimuli.

124.7 GLUCOSE RELATIONSHIPS WITH MEMORY AND SLEEP IN OLD RATS. P.E. Gold, W.S. Stone, S.M. Martin*, R.J. Rudd* and N.C. Salustri*. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Aging is accompanied by both memory and sleep deficits. Recently, we found significant correlations between the decline in paradoxical sleep parameters and the decline in memory performance in individual old rats. In previous studies we also found that age-related memory impairments could be reversed by epinephrine, which may enhance memory through its hyperglycemic actions, in rodents and by glucose itself in elderly humans. In the study using elderly humans, we also found that the response to a glucose tolerance test predicted the performance of individual subjects on a battery of memory tests; those individual subjects with poor glucose control had significantly poorer performance on the memory tests than did same-age subjects with good glucose control. The present experiment was designed to ask: (1) whether glucose would ameliorate the paradoxical sleep deficits (previously associated with memory impairment) in aged rodents and (2) whether the response to a glucose tolerance test would predict memory in aged rodents as it does in humans.

Twelve 2-year-old male Sprague-Dawley rats were trained on a one-trial inhibitory avoidance task and tested for retention 24 hrs later. EEG and EMG electrodes were then surgically implanted for cortical recordings. After recovery, 3 hr baseline sleep samples were obtained from each animal. On separate days, the rats were then injected with saline or glucose (100 and 500 mg/kg, counterbalanced) and sleep samples were again obtained. After these trials, a small incision was made in the rats' tails and blood glucose levels were obtained using a Glucometer. A single injection of glucose (500 mg/kg) was then administered, and blood glucose was monitored over the next 2 hours.

The results indicated that: (1) Glucose (500 mg/kg) significantly increased paradoxical sleep bout duration to levels approaching those of young rats. (2) Retention performance for inhibitory avoidance training was negatively correlated with change in blood glucose after the glucose injection (e.g., at 10 min, $r = -0.81$, $p < 0.01$); at 2 hrs, $r = -0.76$, $p < 0.05$).

These findings demonstrate that glucose administration to rats attenuates age-related sleep impairments which are closely associated with memory impairments. In addition, the results indicate that in rats, as in humans, glucose regulation is negatively correlated with memory performance in individual animals. Such results are consistent with the general view that glucose utilization may be an important contributor to age-related changes in sleep and memory. [Supported by the Office of Naval Research (N00014-85-K0472) and the American Diabetes Association.]

124.8 **HABITUATION RETENTION: AGE DIFFERENCES IN YOUNG NZB/B1NJ MICE PARALLEL SENESCENCE-RELATED CHANGES IN C57BL/6NNIA MICE.** M.J. Forster, K.C. Retz, T.L. Johnson*, M.D. Popper*, and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

Relatively young mice of several autoimmune-prone strains show senescence-like declines in learning/memory (Forster et al., Soc. Neurosci. Abstr., 11:722, 1985) and sensorimotor capacity (Forster et al., Soc. Neurosci. Abstr., 12:175, 1986). In the current experiments, we investigated the possibility that autoimmune mice show senescence-like changes in habituation retention. Separate age groups of non-autoimmune C57BL/6Nnia (1.5, 3, 6, 12, or 26 mo) and autoimmune NZB/B1NJ mice (1.5, 3, or 6 mo) were given 20-min sessions in computerized animal activity monitors (Omnitech Electronics) on each of 8 consecutive days. Habituation retention was considered to be the decrease in total daily activity as a function of the 8 daily sessions. Within-session habituation was addressed by sampling locomotor activity within 5-min periods during each session. Horizontal (total distance traveled) and vertical activity (rearing) within the 39.5 X 39.5 X 30.5 cm activity chamber were recorded. In addition, the floor of the apparatus was divided into 9 equal zones (approximately 13.2 X 13.2 cm) by the recording system, and the total time spent by the mice within the center zone and the 5 noncorner zones were measured independently. Horizontal (total distance) and vertical components of activity showed overall decline over the life spans of each strain. Those declines occurred between 6 and 26 months for C57BL/6Nnia mice, but between 1.5 and 6 months for the NZB/B1NJ mice. Age-related changes in habituation retention were most evident when the spatial components of activity were considered. The times spent in the center and noncorner zones showed a marked decrease as a function of test session in 1.5-, 3-, and 6-month-old C57BL/6Nnia mice, whereas the 26-month old C57BL/6Nnia mice showed a much slower rate of change in these measures over the 8 consecutive test sessions. Session-related decreases in horizontal and vertical components of locomotor activity showed a similar, but less marked change with age. NZB/B1NJ mice showed a senescence-like pattern of age differences in spatial components of activity between 1.5 and 6 months, but failed to exhibit session-related changes in horizontal activity components at any age tested. The NZB/B1NJ mice showed significant within-session and 1-h habituation retention at all ages. These findings indicate that for selected components of locomotor activity, age-related changes in habituation retention are accelerated in NZB/B1NJ mice. [Supported by NIH-NIA grant R23-AG06182 (M.J.F.), NIH-BRSG award SO7 RR05879 (T.C.O.M), and NHLBI grant T35 HL 07465].

124.9 AGE DIFFERENCES IN PURKINJE CELLS AND RATE OF CLASSICAL CONDITIONING IN YOUNG AND OLDER RABBITS. D. S. Woodruff-Pak and J. B. Sheffield* Departments of Psychology and Biology, Temple Univ., Phil., PA 19122

Significant progress has been made in the understanding of the neural circuitry involved in classical conditioning of the eyelid response in rabbits. This model system has important implications for learning and memory in aging because large age differences have been demonstrated in eyelid conditioning in rabbits and humans. In a hypothetical model of the neuronal system that could serve as the essential memory trace circuit for this response, it has been postulated that one of the sites of the memory trace is at Purkinje cells in cerebellar cortex. Purkinje cell loss with age has been demonstrated in rats, monkeys, and humans. The purpose of this study was to examine Purkinje cells in young (Y) and older (O) rabbits which had been behaviorally trained in the trace eyelid classical conditioning paradigm.

Twelve New Zealand white rabbits were used in the study: 6 Y of a mean age of 3 months; 6 O ranging in age from 27-50 months with a mean age of 40 months. They were trained in a paradigm in which an 85 dB, 1 KHz, 250 msec tone CS was presented and followed 750 msec after its onset by a 2.1 N/cm^2, 100 msec corneal airpuff US. The trace interval between the offset of the CS and the onset of the US was 500 msec. Y rabbits took an average of 471 trials to attain learning criterion, and O rabbits took 1169 trials. Animals were sacrificed with an overdose of Nembutal and perfused through the heart in saline followed by 10% formalin. Brains were maintained in formalin for 2 or more days and then embedded in an albumin-gelatin mixture. Every fourth 80 μm section through the cerebellum was mounted for reconstruction. Tissue was stained with cresyl violet. Six sections for each rabbit were used. Images were obtained from each section on the left and right HVI area and the vermis. Counts of Purkinje cells in a single row 0.5 mm wide were made on 18 photographs for each animal by two investigators who were blind to the age of the animal. Inter-rater reliability was .82, and an average of the two counts was used. Comparisons of the total number of Purkinje cells counted for each age group revealed a highly significant difference ($t=4.3$; $p < .001$) with older rabbits having fewer cells. Molecular cell counts in the same animals indicated no age differences suggesting that differential tissue shrinkage in Y and O brains was not affecting the age difference in Purkine cell number. The correlation between Purkinje cell number and age was $-.77$ ($p<.005$). The correlation between trials to criterion and Purkinje cell number was $-.79$ ($p < .005$). A partial correlation was computed partialling out the variance due to age, and the resulting relationship between Purkinje cell number and trials to criterion was $r = -.61$ ($p < .025$). These results suggest that differences in Purkinje cells in Y and O rabbits may be related to age differences in rate of classical conditioning.

124.10 SENILE PLAQUES AND BEHAVIOR IN AGED SQUIRREL MONKEYS. E. Schwam[1], L.C. Walker[2], B. Buckwald[1]*, F. Garcia[1]* and J. Sepinwall[1]. [1]Hoffmann-La Roche Inc., Nutley, NJ 07110 and [2]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

A relationship between senile plaque density and cognitive decline has been reported in patients with Alzheimer's Disease (Tomlinson et al., 1970) and needs to be explored more fully in nondemented aged individuals. A nonhuman primate model of this relationship would provide an important methodology for developing effective treatments.

The behavior of four 23-year-old squirrel monkeys (Saimiri sciureus) with varied drug and experimental histories was evaluated on three tasks: choice reaction time, spatial learning, and delayed matching-to-sample. Acquisition and maintenance behavior differed among the animals as reflected in an overall ranking of performance shortly before death.

Brain sections at levels of the rostral temporal lobe and prefrontal cortex revealed the presence of neuritic plaques (silver stain) consisting of abnormal, swollen neurites around an amyloid (thioflavin-T stain) core. In sections through the level of the anterior temporal lobe, plaques were most frequent in the inferior temporal cortex. Occasional plaques were seen near the cingulate sulcus and in parahippocampal cortex, insular cortex, hippocampal formation and amygdala. In sections through prefrontal cortex, most plaques were located in the orbitofrontal cortex, frontal opercular cortex, and occasionally in cingulate cortex. Compared to a 31-year-old rhesus monkey, plaques were smaller (20-55 μm vs. $\leq$ 100 μm) and less dense (4-5/mm^2 vs. 8/mm^2 in regions of greatest density) in the aged squirrel monkeys. Plaque distribution and composition were comparable in the two species. No plaques were found in a group of three, eight-year-old squirrel monkeys.

In this limited sample of aged squirrel monkeys, the largest number of plaques (mean = 18.5/section) was found in the monkey with the poorest overall performance on the behavioral tasks. The density of plaques in the other three aged monkeys ranged from 3.25 - 9 plaques/section and was not related to performance.

While these results suggest that plaque density must increase beyond a certain threshold to become a predictor of performance, it is also possible that performance on specific tasks is affected preferentially by pathological changes in certain brain areas. Studies utilizing larger numbers of animals are clearly required to resolve these issues. Nevertheless, the present finding of a possible relationship between neuritic plaques and behavioral deficits in the aged squirrel monkey supports the utility of this model as a research strategy to find treatments for cognitive impairments in aged humans.

124.11 **DEFICITS ON OLFACTORY IDENTIFICATION AND DISCRIMINATION TASKS IN THE EARLY STAGES OF ALZHEIMER'S DISEASE.** J.P. Kesslak, C.W. Cotman, H. Chui*[1], H. Fang*[1], S. Van den Noort and G. Lynch. Department of Psychobiology, University of California, Irvine, CA, 92717, [1]Department of Neurology, University of Southern California, Los Angeles, CA.

The pathology of Alzheimer's disease (AD) is characterized by early and severe loss of neurons in the entorhinal cortex and associated structures of the primary olfactory cortex. To determine the extent of olfactory dysfunction resulting from this neural loss, odor identification and discrimination tests were administered. The Smell Identification Test (SIT) required identification of common odors, was well standardized and had a high correlation with threshold detection tasks. The match-to-sample test required odor discrimination, using uncommon odors to minimize dependence on verbal ability. Subjects in the early stages of AD (n=18) were diagnosed according to NINCDS-ADRDA guidelines. Also tested were subjects with Parkinson's disease (PD, n=14), multiple sclerosis (MS, n=14) and age matched controls (n=18).

Scores for each olfactory test were normalized to account for the different levels of chance performance. On the SIT subjects with AD scored below normal (52% correct) but only two subjects were in the anosmic range. There was a severe deficit on the match-to-sample test for subjects with AD (19% correct). The subjects with PD were severely impaired on the SIT (25% correct), with 10 out of 14 scoring as anosmic. Only the 4 nonanosmic PD subjects were administered the match-to-sample test (27% correct) and they scored below controls. Control and MS groups performed at normal levels on the SIT (respectively, 84% and 83% correct) and significantly better on the match-to-sample test (respectively, 65% and 55% correct) than the AD and PD groups. The correlation coefficient between scores on the SIT and match-to-sample test was quite low (r^2= .27). Scores for AD subjects on the SIT and match-to-sample test had low correlation coefficients with scores on the mini mental status test (respectively, r^2=.001 and .160). The SIT and match-to-sample test were found to have no significant correlation with either age or sex.

The results of the SIT indicate that AD subjects, at least during the early stages, are able to discriminate and identify common odors although they have a quantitative deficit in this regard. The more dramatic impairment on the match-to-sample test may reflect a problem in short-term memory or difficulty in processing novel odors or both. The results encourage the idea that refined tests of olfactory function, in conjunction with other tests, may provide a simple means of detecting and monitoring select neurodegenerative diseases.

124.12 FAMILIAL ALZHEIMER'S DISEASE: NEUROPSYCHOLOGY AND NEUROIMAGING. Gary D. Miner, Linda A. Miner*, William S. Yamanashi*, and Seppo Saksanen*. Alzheimer Disease & Related Geriatric Disorders Laboratory, Oral Roberts University School of Medicine, Departments of Pharmacology & Radiology, Tulsa, Oklahoma; and Familial Alzheimer's Disease Research Foundation, Tulsa, Oklahoma.

Subjects from two Familial Alzheimer Disease (FAD) families have been studied with a battery of neuropsychological tests with a goal of finding early 'pre-disease' risk factors. Tests of first-order capabilities such as visual perception, reaction time, or motor ability, which might be closer to measuring central nervous system disability than abstract-conceptual abilities, were included in the test battery. Additionally MRI brain imaging has been done on some of these subjects. The first of the subjects tested has been diagnosed with FAD 2 years following the neuropsychological testing. Analysis of 6 of the neuropsychological tests (Lezak's Tinkertoy Test; Benton's Controlled Word Association, Judgment of Line Direction, Facial Recognition, & Visual Retention; and Shipley's Verbal Abstractions) indicate that the experimental subjects apparently fall into 2 groups, whereas the control subjects score within a single group. Additional measures of possible early subtle changes in behavior have been made: a 'tapping test', and the 'Torque test'. The variable torque occurred more frequently in the low-scoring at-risk group than in the not-at-risk group (46% compared to 10%). The relationship among neuropsychological, neuroradiological and other indexes are not well understood yet for Alzheimer's disease. The clinically diagnosed FAD subject and another subject showing cortical atrophy by MRI both appear in the at-risk group, providing evidence that there may be pre-morbid neuropsychological risk-factors for FAD. This study has really only begun. Some directions are emerging which may prove useful in building an algorithm to be tested (1) over time, as family members are diagnosed, and (2) as additional members are tested and (3) as new FAD families are located. Therefore, this report must be considered tentative in nature, but hopeful as a beginning.

124.13 MULTIPLE MORPHOLOGICAL AND BIOCHEMICAL MEASURES COMPLETELY DISTINGUISH PATIENTS WITH ALZHEIMER'S DISEASE. Rochester Alzheimer's Disease Project: R. W. Hamill, E. D. Caine, P. D. Coleman, T. A. Eskin, D. G. Flood, R. J. Joynt, L.W. Lapham*, T. H. McNeill, and C. L. Odoroff*. Depts. of Neurology, Psychiatry, Neurobiology and Anatomy, Neuropathology, and Biostatistics, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

In spite of the fact that there are statistically significant differences between cases with Alzheimer's disease (AD) and aged-matched controls for many morphological and biochemical measures considered separately, there are still AD cases in most studies that are within the normal range for the measure taken. It seems apparent that any measure showing any degree of overlap between AD and control is not a singular marker of AD. The general problem of overlapping values for AD cases and controls is particularly apparent when examining older cases. This issue assumes added importance for patients who meet the neuropathological criteria of AD but apparently lack clinical features.

This failure to find complete separation between AD cases and controls for any single morphological or biochemical measure led us to consider that multiple disease markers may provide a more convincing diagnosis of AD and may better correspond to the observed behavioral devastation. In testing this possibility, we have examined the middle frontal gyrus, an area which often shows overlap between AD and control cases for any single measure. There were 7 AD cases ranging in age from 59 to 97 years, and 7 controls ranging in age from 63 to 84 years. Measures taken for this analysis included those that are traditional: 1) counts of senile plaques and neurofibrillary tangles, 2) counts of neurons, and 3) choline acetyltransferase (CAT) activity, and those that are somewhat less traditional: 1) soma size of somatostatin-containing neurons and 2) dendritic extent.

Each of these measures alone resulted in some, but not complete, separation of AD and control subjects. However, a multivariate analysis (Biplot) utilizing these measures produced complete separation of all of the AD subjects from all of the controls. These data suggest that perhaps the best substrate for diagnosing dementia of AD is the summed effect of changes in a number of morphological and biochemical measures.

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124.14 NEUROPSYCHOLOGICAL IMPAIRMENT IN PATIENTS WITH EARLY- AND LATE-ONSET ALZHEIMER'S DISEASE. D. Salmon, W. Heindel*, D. McCullough*, N. Butters, and I. Grant. Depts. of Neurosciences and Psychiatry, Univ. of Cal., San Diego, La Jolla, CA 92093 and VA Medical Center, San Diego, CA 92161.

Recent investigations of the neuropsychological features of dementia of the Alzheimer type (DAT) have revealed different patterns of impairment in those patients who developed the disease before (early onset) or after (late onset) age 65. Specifically, early-onset DAT is reportedly characterized by greater language impairment than is late-onset DAT, while visuospatial deficits are reported to be more prevalent in late-onset than in early-onset DAT.

To further examine the relationship between age-at-onset and neuropsychological performance in DAT patients, we examined 27 early-onset (age-at-onset=59.6) and 41 late-onset (age-at-onset= 71.5) patients with a battery of tests which assessed language, visuospatial, memory and problem solving abilities. The patient groups did not differ in years of education, duration of symptoms, or overall level of dementia.

The performances of these well-matched early- and late-onset DAT patients were not significantly different on any component of the extensive neuropsychological test battery. In addition to equivalent deficits on tests of verbal and nonverbal memory and problem solving, the two DAT patient groups were impaired to the same degree in a number of language abilities including confrontation naming, fluency and vocabulary. Similarly, equally deficient performances were elicited from the early- and late-onset patients on tests of visuospatial functions including complex figure drawing and the WISC-R Block Design subtest. Similar results were obtained with separate analyses which included only those patients whose duration of illness was limited to 3 or 4 years, or only the very early-onset (mean age-at-onset=56.8) and late-onset (mean age-at-onset=78.1) DAT patients.

These results suggest that the patterns of neuropsychological deficits evidenced by early- and late-onset DAT patients do not differ when the duration of symptoms and overall level of dementia of the two groups are equated. Thus, in terms of neuropsychological functioning, presenile and senile DAT appear to represent a unitary disorder.

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125.1 DISCHARGE OF BASAL FOREBRAIN PROJECTION NEURONS DURING WAKING AND SLEEP. R. Szymusiak and D. McGinty. Dept. Psych., U.C.L.A., Los Angeles, CA and Neurophysiol. Res., V.A. Med. Ctr., Sepulveda, CA.

The basal forebrain (BF) is the origin of several ascending and descending projection systems. Little is known about the discharge correlates of identified BF projection neurons in behaving animals. We have described a population of unidentified BF neurons which exhibit elevated discharge rates during slow-wave sleep (SWS), and low rates during alert wakefulness (Brain Res. 370: 82-92, 1986). These sleep-active neurons (SANs) were recorded throughout the lateral preoptic area/substantia innominata (LPO/SI) and ventral globus pallidus (VGP). We report here attempts to antidromically activate SANs and other BF cell types by stimulation of ascending and descending efferent pathways.

Three adult cats were prepared for chronic sleep-waking and unit recordings. Microwires (32u diameter) were aimed at the LPO/SI and VGP. Bipolar stimulating electrodes were placed in the midbrain reticular formation (MRF) and corticopetal fiber tracks; the external capsule (EC), and anterior cingulate bundle (ACB).

Of 128 neurons studied, 31 were in the LPO/SI and 97 were in the VGP. Antidromic responses were recorded in 2 LPO/SI cells and in 57 VGP cells. Sleep waking discharge of driven cells was correlated with antidromic latency (ADL). Eleven cells had ADLs <5 ms, and had high discharge rates during waking with low rates in SWS. Cells with short ADLs were dorsally located. The remaining 48 cells had ADLs >5 ms. Long ADL neurons were characterized by low rates during waking (Table I); several were silent during periods of exploratory locomotion. These cells were not responsive to startle stimuli, or to the sight and ingestion of palatable food. Approximately 25% of long ADL cells had elevated rates during grooming, compared to other waking behaviors. All long ADL cells had peak discharge rates in SWS (Table I), but several had peak rates <1 spike/s. There was no anatomical segregation of cells identified as having ascending or descending projections.

These results demonstrate that, in one BF subregion, SANs are a source of both ascending and descending projections, and that they have more slowly conducting axons than adjacent projection neurons.

TABLE I SLEEP-WAKE DISCHARGE AND ANTIDROMIC LATENCIES

Stimulation Site	N	Antidromic Latency	Discharge Rates Waking	SWS	REM
ACB	11	11.2±1.2	0.2±0.1	2.8±0.3	1.4±0.3
EC	18	15.8±1.8	0.2±0.1	2.1±0.4	1.0±0.3
MRF	19	14.9±2.2	0.1±0.1	1.8±0.4	0.7±0.2

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125.2 EVIDENCE FOR OLFACTORY INPUTS TO THE SEPTUM. C. Reyher*, University of Berlin, West Germany; J.H. McLean and M.T. Shipley, Dept. of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267 (Spon: R. Cardell)

A unique feature of the olfactory system is its remarkably direct and heavy innervation of telencephalic limbic structures. The main and accessory olfactory bulbs have dense and partially reciprocal connections with the hippocampal formation (entorhinal cortex, hippocampal rudiment) and the cortical amygdaloid nuclei. Direct hypothalamic inputs to the bulb are well established and the piriform cortex has reciprocal connections with the hypothalamus. Early neuroanatomists reported olfactory projections to the septal region in several vertebrate species but subsequent tract-tracing experiments failed to support this view. Here, we report that there is a dense, focal projection to the septal area from a restricted subfield of the anterior olfactory nucleus (AON).

Discrete, iontophoretic injections of WGA-HRP were made in architectonic subdivisions of AON. The injections resulted in anterograde and retrograde labelling of olfactory bulb and olfactory cortical connections previously described by others. With focal injections in the dorsomedial transition area (AONdm) and dorsal peduncular cortex (DPC) there was dense anterograde labelling in the medial half of the lateral septal nucleus. The labelling directly abutted but did not encroach upon the medial septal nucleus. This projection was confirmed by placing WGA-HRP injections in the septum. A discrete population of neurons was retrogradely labelled in AONdm and DPC. The apical dendrites of these neurons are directed towards the molecular layer of AON, a zone which receives a dense terminal projection from the main olfactory bulb.

Classical reports of axons from the olfactory bulb to the septum were based on descriptive methods, and the observations were largely confined to non-mammalian vertebrates. The present findings suggest that an olfacto-septal circuit may exist, but that the projection arises from third order neurons in AON rather than second order neurons (mitral and tufted cells) in the olfactory bulb.

Based on these results, we hypothesize that olfactory bulb mitral/tufted cells activate a discrete population of dorsomedial AON neurons; these AON neurons project to a specific zone in the septum and, thus, act on multiple limbic circuits.

The AON projection does not terminate in the medial septal nucleus, but an analysis of the terminal field in relation to sections stained for the cholinergic enzyme ChAT suggests that olfactory inputs may be able to modulate medial septal neurons. Medial septal neurons play a key pacemaker role in hippocampal theta rhythm. During investigatory sniffing behavior, hippocampal theta, olfactory bulb theta and sniffing are temporally correlated. The AON to septum projection could function to link olfactory bulb theta to hippocampal theta when the two structures become phase locked during olfactory learning (Macrides, *et al.*, 1982).

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125.3 SUBNUCLEAR ORGANIZATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS: A CYTOARCHITECTURAL, CONNECTIONAL AND IMMUNOCYTOCHEMICAL STUDY. M.M. Moga, T.S. Gray and C.B. Saper. Dept. Anatomy, Loyola Univ., Maywood, IL 60153; Dept. Pharm. Physiol. Sci. and Neurol., Univ. of Chicago, Chicago, IL 60637.

The bed nucleus of the stria terminalis (BST) is closely associated with the amygdala, its major source of afferent input, and, like the amygdala, has been implicated in neuroendocrine regulation and reproductive behaviors. Traditionally, the BST has been divided into medial and lateral subdivisions. Recently, investigators have noted additional subdivisions within the BST (i.e., DeOlmos et al., '85; McDonald, '83) but there has been no complete description of the BST subnuclei. In this study, we examine BST subnuclei for their Nissl cytoarchitecture, WGA-HRP labeled afferent and efferent connections, and neuropeptide immunocytochemistry.

Subnuclei of the BST can be grouped into lateral, medial and preoptic divisions. Lateral BST subnuclei are extensively connected with autonomic nuclei. For example, the posterior lateral (PL) subnucleus projects to the nucleus of the solitary tract (NTS), ventrolateral medulla (VLM) and parabrachial nucleus (PB). Dorsal lateral (DL) BST projects heavily to PB and contains numerous CRF- and NT-immunoreactive neurons. Ventral lateral (VL) BST receives heavy NTS input and contains a dense cluster of CRF-immunoreactive cells and fibers. Juxtacapsular (JXC) BST receives input from insular cortex.

Medial BST subnuclei can be characterized by their connections with medial portions of the amygdala and hypothalamus. For example, the anterior medial (AM) subnucleus receives heavy input from the medial amygdala. ENK-, SS- and CRF-immunoreactive neurons are scattered throughout AM. Posterior medial (PM) BST receives input from the posterior medial amygdala and projects to ventromedial hypothalamus. Substance P fibers are particularly dense within PM. Posterior intermediate (PI) BST is interconnected with the ventromedial hypothalamus.

Preoptic BST (PO) projects strongly to the brainstem. PO may be divided into medial and lateral portions. Many of the neurons in the lateral part of PO are immunoreactive to CRF and galanin.

The subnuclear organization of the BST may reflect a diversity of function, heretofore, unstudied.

125.4 PATTERNS OF ACTIVATION EVOKED BY STIMULATION OF THE RAT LIMBIC FOREBRAIN. K.M. Carnes and J.L. Price. Dept of Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The 14C-2-deoxyglucose (2-DG) method has been used to define the patterns of activity which are produced by repetitive, train stimulation of the amygdala, piriform cortex and olfactory bulb in the awake, unrestrained rat. Low levels of stimulation, which did not produce generalized convulsive behavior, resulted in patterns of 2-DG uptake which reflect the majority of known primary projections from each of these structures. Thus, moderate stimulation of the basolateral amygdala activated the infralimbic, agranular insular and perirhinal cortical areas, the bed nucleus of the stria terminalis (BNST), the substantia innominata and other nuclei of the amygdala. More lightly activated areas included the nucleus accumbens, lateral septum, substantia nigra and entorhinal cortex. There was little or no evoked activity in the thalamus or hypothalamus. Equivalent stimulation of the piriform cortex activated other parts of the olfactory cortex, including the entorhinal cortex, the olfactory bulb and, to a lesser extent, the amygdalohippocampal area and the rostral hippocampus. Low level stimulation of the olfactory bulb produced substantial uptake of 2-DG only in the olfactory cortex and the nucleus of the horizontal diagonal band.

In contrast, intense stimulation of each of the three regions invariably activated the other two areas, together with a consistent constellation of other brain structures. Furthermore, regardless of the site of stimulation, similar convulsive behaviors were observed, including motionless stares, "wet-dog shakes", head jerks with chewing, salivation, and episodes of rearing with bilateral forelimb clonus and loss of posture. In addition to the areas activated by less intense stimulation, the structures most prominently labeled were the prelimbic area, the mediodorsal, midline, ventromedial (principal and basal divisions) and posterior nuclei of the thalamus, the anterior, lateral and ventromedial areas of the hypothalamus, the olfactory tubercle and nucleus accumbens, the medial and posteroventral parts of the caudate/putamen, the lateral habenular nucleus, the substantia nigra and ventral tegmental area, and the hippocampal formation. In many cases these structures were labeled bilaterally.

It is not yet clear which of these structures may be labeled directly from the stimulation and may therefore be considered causative for the convulsive behaviors, and which structures may be activated as a result of those behaviors. However, it is apparent that seizures produced by stimulation of different parts of the limbic forebrain are associated with a remarkably consistent pattern of both brain activity and behavior.

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125.5 INVESTIGATION OF CONNECTIONS FROM THE DIAGONAL BAND OF BROCA TO THE SUPRAOPTIC NUCLEUS IN THE RAT. E. L. Weiss and R. W. Rieck, Dept. of Physical Therapy, Louisiana State University Medical Center and Dept. of Anatomy, Tulane Medical School, New Orleans, LA 70112.

The nucleus of the diagonal band of Broca (DBB) is one of several nuclei within the basal forebrain which contributes projections to neocortical and subcortical structures. Recent physiological evidence also indicates that the supraoptic nucleus (SON) of the hypothalamus receives a projection from the DBB. Thus, the DBB is a limbic structure that may play a fundamental role in the regulation of cardiovascular function. The present study utilizes several anterograde tracing techniques to determine if the nucleus of the DBB gives rise to a direct projection to SON. Neuroanatomical tracers which included either tritiated amino acids, wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) or phaseolus vulgaris-leucoagglutinin (PHAL) were placed within the dorsomedial portion of the horizontal limb of the DBB. Following the deposition of the tracers, anterogradely transported label was located within both a dorsal and a ventral descending pathway. Terminal label associated with the dorsal descending pathway was apparent within thalamic nuclei, including the paratenial nucleus, the mediodorsal nucleus and the habenular nuclei. Label associated with the ventral pathway extended caudally into the medial forebrain bundle, the magnocellular preoptic area and the lateral hypothalamic area. Anterogradely transported label was located directly dorsal and lateral to SON. Thus, in autoradiographic cases, reduced silver grains were located immediately dorsolateral to SON, however, reduced silver grains were never apparent directly over SON. Corresponding results were obtained following the placement of WGA-HRP within the DBB. Thus, labeled fibers were located dorsolateral to the supraoptic nucleus but terminal label was not apparent within SON. Similarly, following restricted injections of PHAL within the horizontal limb, numerous PHAL immunoreactive fibers (PHAL-IR) were adjacent to SON. Although PHAL-IR fibers did approach the nuclear boundary of SON, PHAL-IR fibers were not found within the nucleus. These data indicate that although the DBB provides a descending projection which terminates within an area immediately dorsolateral to SON, perikarya within SON do not receive a direct projection from the DBB. It, however, is possible that perikarya within SON give rise to dendritic arborizations which extend outside the nuclear boundary into the DBB-terminal zone. Further investigation is indicated to determine if descending fibers from the DBB come into contact with dendritic processes of neurons which have cell bodies located within SON. Supported by LSUMC-SAHP Intramural Grant(ELW) and BRSG 533112 (RWR).

125.6 STUDIES ON THE ORGANIZATION OF THE EFFERENT CONNECTIONS OF THE DIAGONAL BAND IN THE RAT. T. R. Stratford and D. Wirtshafter, Dept. of Psychology, University of Illinois at Chicago, Box 4348, Chicago, Illinois 60680.

The nucleus of the diagonal band (DB) is located in the basal forebrain and perhaps is best known for containing large numbers of putatively cholinergic cells. The nucleus does, however, contain a central core consisting of cells which do not appear to contain either choline acetyltransferase (ChAT) or acetylcholinesterase (AChE). While the efferent connections of the DB have been extensively investigated, few studies have examined whether the cells of origin of these various projections might be differentially distributed within the nucleus. In the present study we injected several fluorescent retrograde tracers into structures receiving DB efferents and used the pharmacohistochemical detection of AChE to partially map the internal structure of the nucleus of the DB.

Injections of tracers into the median raphe nucleus (MR) resulted in heavy labeling of cells within the apparently non-cholinergic core of the DB as did injections confined to the interpeduncular nucleus (IPN). Double-labeling studies indicated that many of the cells projecting to the MR sent collaterals to the IPN. While disruption of the stria medullaris at the habenula did not apparently affect the transport of the tracers, lesions of the medial forebrain bundle (MFB) completely abolished the labeling of neurons in the ipsilateral DB. In contrast to injections confined to the midbrain, injections into the hippocampus resulted in the intense labeling of a band of cells within the DB, most of which were located ventral to the MR/IPN projecting cells. Most of these cells were relatively large and many appeared to contain AChE. A similar band of large cells was observed in the caudal DB dorsal to the MR/IPN projecting cells following injections into the retrosplenial cortex (RSpl). Multiple tracer injections demonstrated that very few, if any, of the large cells in either the dorsal or ventral laminae appeared to send collaterals to the MR.

In short, the nucleus of the diagonal band appears to have a laminated structure which is observable when stained for ChAT or AChE or when labeled by retrograde tracers. The most ventral lamina consists of large AChE containing cells, a large proportion of which project to the ipsilateral hippocampus. A central core of relatively small cells which do not stain intensely for AChE appears to project, in part, to the MR and/or IPN via the MFB. The dorsal lamina contains large AChE staining cells from which arise a possibly cholinergic projection to the ipsilateral RSpl.

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125.7 NEURONS IN THE MEDIAL SEPTAL NUCLEUS INNERVATE CHOLINERGIC NEURONS IN THE DIAGONAL BAND, HISTAMINERGIC NEURONS IN THE MAMMILLARY BODY, AND SEROTONERGIC NEURONS IN THE NUCLEUS RAPHE DORSALIS. A DOUBLE-LABEL STUDY COMBINING PHA-L-TRACING AND ENZYME IMMUNOCYTOCHEMISTRY. F.G. Wouterlood*, H.W.M. Steinbusch and T. Watanabe (SPON: ENA). Depts. Anatomy (FGW) and Pharmacology (HWMS), Free Univ., Amsterdam, The Netherlands, and Dept. Pharmacology (TW), Tohuku University Med. School, Sendai, Japan.

By previous autoradiographic tracing, neural connections have been found between the medial septal nucleus (MSN) and telencephalic, diencephalic and mesencephalic nuclei (Swanson, L.W. and Cowan, W.M., J.Comp.Neurol.,186:621, 1979). We reinvestigated these connections with the Phaseolus vulgaris-leucoagglutinin (PHA-L)-tracing technique. In 6 female rats, PHA-L was injected in the MSN. A two-color, double-peroxidase immunocytochemical technique (Wouterlood,F.G.,Bol,J.G.J.M. and Steinbusch,H.W.M., J. Histochem. Cytochem.,35, 1987) was used to detect simultaneously the transported PHA-L and target neurons immunoreactive against one of the following substances: choline acetyltransferase (ChAT), histidine decarboxylase (HDC) or serotonin Alternating, frozen brain sections were incubated with one of the following combinations of primary antisera: goat anti-PHA-L mixed with rat anti-ChAT, goat anti-PHA-L mixed with rabbit anti-HDC and goat anti-PHA-L mixed with rabbit anti-serotonin. Secondary antisera (IgG) were also mixed (donkey anti-goat with rabbit anti-rat or swine anti-rabbit). Following incubation in goat-PAP, reaction was performed with Nickel-diaminobenzidine, staining the PHA-L-labeled fibers blue. Subsequently, the sections were incubated with rabbit-PAP and reacted with diaminobenzidine, staining the neurons containing the second antigen brown. PHA-L-labeled fibers originating in the MSN descend through the vertical (VDB) and horizontal (HDB) limbs of the diagonal band of Broca to the hypothalamus. In the VDB and HDB, they form varicosities, closely apposing ChAT-immunopositive neurons. Also ChAT-immunopositive neurons in the medial part of the nucleus accumbens and deep in the olfactory tubercle are innervated by PHA-L-labeled fibers. Descending fibers further course caudally either loosely organized, or contained in the medial forebrain bundle. Nearly all the HDC-immunoreactive neurons in the posterior hypothalamic region are apposed by varicosities on PHA-L--labeled fibers. Labeled fibers cross in the supramammillary decussation, course dorsally to the mesencephalic central grey, and reach the nucleus raphe dorsalis. Here, varicosities on the fibers appose serotonin-immunoreactive cell bodies and dendrites. Appositions between varicosities on PHA-L-labeled fibers and cell bodies immunoreactive against the second antigen suggest the existence of synaptic contacts.

125.8 BASAL FOREBRAIN PROJECTIONS TO THE HYPOTHALAMUS AND BRAINSTEM IN THE RHESUS MONKEY. K.K. HREIB and D.L. ROSENE. Boston Univ. Sch. of Med., Boston, MA 02118.

To investigate the projections of the basal forebrain (BF) to the hypothalamus (HYP) and brainstem, tritiated amino acid injections (TAA) were made in the BF of three rhesus monkeys and injections of fluorescent retrograde tracers (FRT) were made in the medial HYP, the mamillary complex (MC) and the ventral tegmental area (VTA)--substantia nigra (SN). One TAA injection involved the ventral part of the septal area (SA), the vertical limb of the diagonal band (VDB) and the bed nucleus of the stria terminalis (BNST) and produced dense label throughout the HYP except the ventromedial nucleus (VMH) and the medial mamillary nucleus (MMN). In the brainstem, heavy label occured over VTA, SN-pars compacta (SNc), nucleus centralis superior (CS), dorsal raphe (DRa), pontine and mesencephalic reticular formation (RF), the ventral part of central gray (CG), parabrachial nuclei (Pb), and the locus coeruleus. Another injection limited to the BNST produced labeling mainly in the medial preoptic area, the arcuate nucleus (Arc), tuberomamillary nucleus and the lateral hypothalamic area. In the brainstem, label was observed over SNc, CS, mesencephalic RF, ventral CG and Pb. Another injection located in the substantia innominata (SI) included the nucleus basalis. Anterograde label from this case was mainly observed in SNc, and Pb. Thus, all the TAA injections showed projections to the SNc and Pb. None labeled the MMN or VMH, only the SA-VDB labeled VTA and the SI does not project to the HYP. In the BF separate FRT injections placed in the MC and VTA-SN labeled neurons mainly in the ventral intermediate septal nucleus (VIS), some in the lateral SA and few in VDB and SI. An FRT injection in VMH and Arc labeled neurons only in VIS. Examination of the size and distribution of these retrogradely labeled neurons indicated that most are parvocellular neurons of the BF. Thus in contrast to the well-known projections of the cholinergic magnocellular neurons to cortex and our own description of projections to the medial dorsal thalamic nucleus and the amygdala, it appears that at least some of the hypothalamic and brainstem projections originate largely from the non-cholinergic parvocellular population.

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125.9 POSSIBLE INTERDIGITATION OF BASAL FOREBRAIN AFFERENTS IN THE MEDIODORSAL THALAMIC NUCLEUS OF THE RAT. J.P. Ray* and J.L. Price, Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The medial part of the mediodorsal nucleus of the thalamus (MDm, including both the medial and central segments in the rat) receives afferents from several different parts of the basal forebrain, especially the deep layers of the piriform and entorhinal cortices, the amygdaloid nuclei, the ventral pallidum (including the polymorph zone of the olfactory tubercle) and other parts of the substantia innominata. Previous investigations have indicated that fibers from each of these structures tend to be concentrated in different parts of MDm, although there is apparent overlap at the edges of each projection (e.g. Groenewegen et al., '86; Russchen et al., '87). In order to determine the organization of these afferents within MDm more precisely, a series of experiments is being done in which two anterograde tracers, ^{3}H-leucine and the lectin Phaseolus vulgaris leucoagglutinin (PHA-L) have been injected into different parts of the basal forebrain in the same animal. After appropriate survival, the brains were fixed and serially sectioned; alternative series of sections were prepared for autoradiography, PHA-L immunohistochemical staining, or both. The results suggest that while there is a variable degree of separation of fibers from different basal forebrain structures, there is substantial segregation into interdigitating patterns even when the fibers appear to occupy the same region within MD.

Paired injections into distinctly different structures such as the posterior piriform cortex and the polymorph zone of the olfactory tubercle tend to label two adjacent zones in MDm, which are well segregated. On the other hand, projections from different parts of the same structure tend to have interdigitating patterns of termination. For example, injections in the anterior and posterior parts of the polymorph zone label fibers in the central segment of MD. The fibers from the more anterior part are concentrated ventrally and those from the posterior part dorsally, but in the middle region where both projections are represented the autoradiographic and PHA-L labeled axons tend to be grouped into small clusters surrounded by the other label, indicating interdigitation rather than overlap. Similarly, fibers labeled from paired injections in the different parts of the posterior piriform cortex occupy approximately the same region of MDm, but tend to be clustered into small interdigitating patches.

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125.10 BASAL FOREBRAIN CHOLINERGIC AND NON-CHOLINERGIC PROJECTIONS TO THE THALAMUS AND BRAINSTEM IN CATS AND MONKEYS. A. Parent, Y. Smith, D. Paré and M. Steriade. Lab. of Neurobiol. and Lab. of Neurophysiol., Fac. of Med., Laval Univ., Québec, Canada.

It is generally thought that the thalamus is regulated by various brainstem neuronal aggregates, some of which being cholinergic. In this study we combined the retrograde transport of WGA-HRP with choline acetyltransferase (ChAT) immunohistochemistry in search for other control systems, particularly those of the basal forebrain that could influence thalamic activity through direct inputs as well as through indirect connections with the brainstem. Thus, WGA-HRP was injected (1) into virtually all sensory, motor, and intralaminar nuclei, and into the rostral pole of reticular nucleus (RE) in 27 cats, (2) into the mediodorsal nucleus (MD) in one macaque monkey (Macaca sylvana) and (3) into the peribrachial area (PB) of the brainstem tegmentum in 4 cats. Furthermore, the fluorescent tracers fast blue and nuclear yellow were injected (1) into various thalamic nuclei and PB, and (2) into sensorimotor cortex and PB in 8 squirrel monkeys (Saimiri sciureus). The WGA-HRP experiments revealed that neurons in diagonal band nuclei and substantia innominata in cats project massively to the rostral pole of RE and to MD, less importantly to anteromedial nucleus (AM), and only slightly to ventromedial nucleus (VM). Approximately 7-15% of all retrogradely-labeled neurons disclosed after RE, MD and AM injections were also ChAT-positive while no double-labeled (HRP/ChAT) cells were found after VM injections. The basal forebrain projection to MD was also documented in the macaque monkey. Injections of WGA-HRP into PB in cats led to profuse retrograde cell labelling in nuclei of stria terminalis and anterior commissure and in substantia innominata but no double-labeled cells were found. Fluorescent tracer experiments revealed that a significant number of basal forebrain neurons send axon collaterals to both RE and PB, whereas no branching neurons were found after injections involving PB and other thalamic nuclei or PB and cerebral cortex. These results reveal that cholinergic and non-cholinergic neurons in the basal forebrain project to the thalamus as well as to the brainstem tegmentum. Since recent data showed that the brainstem in turn projects to the basal forebrain, it is possible that, in addition to their direct influence upon many thalamic nuclei, the brainstem neurons in PB modulate the excitability of RE, MD and AM indirectly through basal forebrain neurons.
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125.11 RADIAL-ARM MAZE DEFICITS PRODUCED BY COLCHICINE ADMINISTERED INTO THE AREA OF THE NUCLEUS BASALIS ARE AMELIORATED BY CHOLINERGIC AGENTS. Ronnie L. McLamb*, S. Shaw*, Brian Rogers*, Peter Pediaditakis*, G.J. Harry, and Hugh A. Tilson, Lab. Behav. Neurol. Toxicol., NIEHS, Research Triangle Park, NC 27709.

One approach to the study of cognitive dysfunction in animal models has been to inject excitotoxicants such as kainic or ibotenic acid into the nucleus basalis, an area which gives rise to cholinergic neurons projecting diffusely to the neocortex. The following research determined the effects of injecting colchicine, a neurotoxicant that binds to tubulin and disrupts axonal transport, on acquisition and performance of a working memory task and responsiveness to pharmacological agents. Fischer-344 rats were given bilateral injections of colchicine (1.25 or 2.5 ug/site) into the area of the nucleus basalis. Three weeks later, the rats were food-deprived and trained in a radial arm maze task. Colchicine interfered with the rate of acquisition of the maze. Subsequent experiments found that systemic administration of cholinergic agents, including physostigmine, RS-86 and nicotine, decreased the number of errors made by colchicine-treated rats. Levels of acetylcholine transferase, NE, DA, DOPAC, 5-HT, and 5-HIAA were decreased in the frontal cortex. Such changes were not observed in the hippocampus or corpus striatum. Light microscopic assessment of tissue stained with cresyl violet and Luxolfast blue indicated limited tissue damage near the site of injection. Colchicine produced a dose-dependent increase in the size of the lateral ventricles. These data indicate that intracerebral administration of colchicine into the area of the nucleus basalis adversely affects acquisition and performance of a radial arm maze task. This animal model may be useful in the study of compensatory processes associated with neural degeneration and the development of rational approaches for the treatment of cognitive disabilities. (BR is supported by the Toxicology Curriculum, University of North Carolina, Chapel Hill, NC).

125.12 ENKEPHALIN UNILATERALLY MICROINJECTED INTO THE VENTRAL PALLIDUM/NUCLEUS BASALIS INDUCES CIRCLING. T.C.Napier and K.Marx*. Dept. of Pharmacology, Loyola Univ. of Chicago, Stritch Sch. of Med., Maywood, Il 60153.

The ventral pallidum (VP; including the substantia innominata) and nucleus basalis (nB) regions of the rat basal forebrain are homologous to the primate nucleus basalis of Meynert. These regions contain a high density of neuronal fibers which demonstrate enkephalin-like immunoreactivity. Since the VP/nB receives inputs from brain areas involved in motor function (e.g. the substanta nigra), and sends efferents to the mesencephalic locomotor region, we proposed that VP/nB opioid peptides may alter measures of locomotion. The present study was designed to test this hypothesis using a rotating animal model.

Male Sprague-Dawley rats were anesthetized with pentobarbital and bilaterally implanted with 26 gauge guide cannulaes. Two weeks after surgery, the rats (5-8/treatment group) were habituated to the rotation apparatus for at least 30 min. The rats then received a microinjection into the VP/nB of either saline (vehicle) or [D-ala^{2},D-leu^{5}]-enkephalin (ENK), at a rate of 0.1 ul/min for a total volume of 0.5 ul. Amphetamine (1 mg/kg i.p.) was administered immediately and the rats were replaced into the apparatus for determination of circling behaviors. The number of 360^{o} turns was quantified for 75 min after amphetamine administration.

Rats treated with intra-VP/nB saline plus amphetamine demonstrated classical amphetamine-induced locomotor increases and stereotypic behaviors. Unilateral microinjections of 0.1 nM ENK into the VP/nB did not alter these behaviors nor cause unilateral circling. ENK treatments ranging from 0.3 to 10 nM caused a significant, dose dependent increase in both the number of contralaterally rotating rats and the rate at which they circled. A maximum response for both parameters was obtained at a dose of 3.3 nM ENK where 80% of the rats tested circled contralaterally, with a mean rate of 9$\pm$1.4 rotations per min. These preliminary results support the following hypotheses: (1) the VP/nB is directly involved in motor function, and (2) opioid peptides in the VP/nB alter amphetamine-induced locomotion.

125.13 CAUDAL HYPOTHALAMIC PROJECTIONS TO THE ROSTRAL FOREBRAIN WITH SPECIAL REFERENCE TO AFFERENTS OF CHOLINERGIC NEURONS.
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Recent autoradiographic evidence concerning the organization of corticopetal projections from the lateral hypothalamus (Saper, 1985) has suggested that these ascending fibers establish contact with several regions of the rostral forebrain, including areas with cholinergic projection cells. We examined this possibility using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L), in which the detailed morphology of fibers and terminals may be distinguished at the light microscopic level.

Discrete iontophoretic PHA-L injections were sterotaxically delivered to the tuberal and caudal aspects of the lateral hypothalamus, the zona incerta, and fields of Forel, according to the method of Gerfen and Sawchenko (1984). Following survival periods of 7-10 days, brains were fixed via transcardial perfusion, sectioned at 40 um in the coronal plane, and processed for detection of the lectin by either the avidin-biotin peroxidase (ABC) or indirect fluorescence technique. PHA-L containing fibers and terminal varicosities were found within the medial and lateral septal nuclei, nucleus of the vertical limb of the diagonal band (VDB), dorsal aspect of the horizontal limb of the diagonal band nucleus (HDB), bed nucleus of the stria terminalis, sublenticular substantia inominata (SI), ventral pallidum, central amygdaloid nucleus, and the anterior hypothalamus.

To determine the extent to which PHA-L terminals contact cholinergic cells, sections were processed using different double labelling protocols for the simultaneous detection of cholinergic cells and PHA-L labeled terminals. These included the double fluorescence method (FITC/RITC), and the ABC technique utilizing the same (DAB-intensified/DAB; Gorcs et al., 1986), or separate (DAB/BDHC; Levey et al., 1986) markers. PHA-L stained fibers and terminals were found in close proximity to choline acetyltransferase labeled cell bodies and dendrites in the lateral aspect of the VDB, the dorsal portion of the HDB, the SI, and the lateral hypothalamic area. Subsequent electron microscopic investigation may confirm synaptic contact, and support the hypothesis that the direct corticopetal projections of the lateral hypothalamus are paralleled by a multisynaptic corticopetal system involving cholinergic neurons. Also, as the lateral hypothalamus is composed of a chemically heterogenous population of cells, studies are underway to reveal the neurotransmitter specificity of the projecting neurons.

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125.14 HIPPOCAMPAL INNERVATION OF CHOLINERGIC NEURONS IN THE VENTRAL STRIATUM (NUCLEUS ACCUMBENS) OF THE RAT. A DOUBLE-LABEL STUDY COMBINING PHA-L-TRACING AND IMMUNOHISTOCHEMISTRY OF CHOLINE ACETYLTRANSFERASE.
G.E.Meredith, F.G.Wouterlood and H.J.Groenewegen. Dept. Anatomy, Vrije University, Amsterdam, Netherlands.

A relatively small population of neurons exhibiting choline acetyltransferase (ChAT) immunoreactivity, and thus presumably cholinergic, is distributed over the entire striatum, including the nucleus accumbens (Acc). According to pharmacological studies, striatal cholinergic transmission is influenced by excitatory amino acids, which may imply that the cholinergic neurons receive input from corticostriatal fibers. However, there is as yet little anatomical evidence to substantiate this hypothesis. Presently, we report our light microscopical findings on the anatomical relationship between hippocampo-striatal fibers and ChAT-immunopositive neurons in Acc. We employed a double-labeling strategy, including marking of the hippocampal fibers by injecting the lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) in different parts of the hippocampus, and staining sections through Acc for both PHA-L and ChAT.

PHA-L was injected microiontophoretically (5 #A, positive pulsed current, 20 min.) into the subiculum or the CA1 region of deeply anesthetized female rats. Following survival times of 5-14 days, the animals were reanesthetized and perfused (3% paraformaldehyde in phosphate buffer). Vibratome sections (40 #m) were processed according to a double-label protocol (see Wouterlood et al., J.Histochem.Cytochem., 35, 1987) using a goat antiserum against PHA-L and a rat monoclonal antibody against ChAT (provided by Dr.F.Eckenstein). Nickel-enhanced diaminobenzidine (Ni-DAB) was used to stain the PHA-L-labeled fibers (blue-black color), while DAB was used to stain ChAT-immunoreactive neurons brown. The distribution of ChAT-immunopositive neurons in Acc was studied with the aid of an Apple II computer equipped with a digitizing tablet. The present analysis indicates that the distribution of ChAT-immunoreactive neurons in Acc is inhomogeneous, but not clustered. The density of ChAT-immunopositive cells is relatively low (approximately 25 cells/mm^2), yet constant along the rostrocaudal axis. There are differences in the density from medial to lateral. Following injections of PHA-L in the hippocampus, either in the dorsal or the ventral subiculum or the CA1 region, varicosities on PHA-L-labeled fibers occur in close apposition with the dendrites or perikarya of ChAT-immunoreactive neurons in Acc. These close appositions may represent synaptic connections, and at present this possibility is being studied at the ultrastructural level. We conclude that cholinergic neurons in Acc may receive direct allocortical input.

125.15 CHOLINERGIC INNERVATION OF THE MONKEY AMYGDALA: AN IMMUNOHISTOCHEMICAL ANALYSIS USING A POLYCLONAL ANTISERUM TO CHOLINE ACETYLTRANSFERASE.
J.L. Bassett and D.G. Amaral. The Salk Institute, La Jolla, CA, 92037.

The monkey amygdaloid complex receives its primary cholinergic input from cells located in the basal forebrain (Mesulam et al., 1983, 1984; Russchen et al., 1985). The basal forebrain is a heterogeneous region, however, and both cholinergic and noncholinergic neurons are known to project to the amygdala. Nonspecific anterograde tracers are therefore inadequate to define the regions of the amygdala that receive cholinergic termination. With the development of antisera to the specific cholinergic marker choline acetyltransferase (ChAT), a precise map of cholinergic innervation now can be generated. The pattern of ChAT immunoreactivity in the macaque monkey (Macaca fascicularis) amygdala was studied in 5 adult animals. Series of coronal sections were immunohistochemically prepared using the peroxidase antiperoxidase method with a polyclonal antiserum (kindly donated by F. Eckenstein) directed against ChAT.

The density of ChAT staining varied substantially in different regions of the amygdala and the pattern of immunoreactivity was similar to that observed in acetlycholinesterase histochemical preparations. The greatest density of ChAT positive fibers and terminals was seen in the basal nucleus, particularly in the posterior portion of its magnocellular subdivision (Bmg). A decrease in labeling was evident in the parvicellular region of the basal nucleus and in the paralaminar nucleus, particularly near their medial and lateral borders. The nucleus of the lateral olfactory tract (NLOT) also contained dense immunoreactivity. In contrast to Bmg, however, where ChAT immunoreactivity appeared fairly uniform, labeling in the NLOT consisted of varicose fibers that formed pericellular plexuses outlining the somata and proximal dendrites of cells. ChAT immunoreactivity in the accessory basal nucleus ranged from moderately dense in the superficial and magnocellular regions to light in the parvicellular region. The lateral nucleus generally was lightly stained though its ventrolateral region was moderately labeled.

The medial and lateral subdivisions of the central nucleus were clearly distinguished in ChAT preparations. Whereas the lateral division was darkly stained and contained numerous varicosities, the medial division contained mainly stained fibers passing through with apparently low terminal density. Of the remaining corticomedial nuclei, the amygdalohippocampal area demonstrated the greatest density of immunoreactivity; the posterior corticomedial nucleus was moderately labeled and progressively lighter staining was seen in the anterior cortical and medial nuclei. Only the portion of the periamygdaloid cortex deep to the sulcus semiannularis contained appreciable ChAT immunoreactivity.

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126.1 6-OHDA LESIONS OF NUCLEUS ACCUMBENS BLOCK THE STIMULANT EFFECTS OF NICOTINE IN RATS. C. Ksir and E.J. Cline*. Department of Psychology & Neuroscience Program, University of Wyoming, Laramie, WY 82071.

After previous exposure, nicotine produces a dose-related increase in locomotor activity in rats. The locomotor stimulation produced by amphetamine and by the opiates has been associated with the mesolimbic dopamine system, which projects from the A10 dopamine cell group in the midbrain ventral tegmental area (VTA) to the nucleus accumbens. Nicotinic cholinergic binding sites are found in the VTA, and there is a recent report that nicotine releases dopamine from the accumbens. Pert and Chiueh (1986 Neuroscience Abstracts) reported that infusions of cytisine, a nicotinic agonist, into the VTA produced locomotor activation. The current study was designed to determine whether the mesolimbic dopamine system is necessary for the expression of the locomotor stimulation produced by nicotine.

Beginning 5 days before surgery and resuming 2 days after surgery, each of the adult male albino rats received daily injections of 0.2 mg/kg nicotine. This type of exposure in necessary if the test injections of nicotine are to produce a robust locomotor response. The rats were anesthetized and injection needles were directed bilaterally into the nucleus accumbens under stereotaxic control. In the control group 2 ul of vehicle was injected into each side. In the lesion group the 2 ul infusions each contained 8 ug of 6-OHDA base.

On the 21st day after surgery the rats were placed into one of 16 identical photocell test cages. These cages measure locomotor activity as alternate breaks of two photocell beams, one near each end of the cage. After a one-hour adaptation period each rat was given an injection of saline and replaced in the test cage for one hour. At the end of that hour each rat was given an injection of 0.2 mg/kg nicotine and again placed in the test cage for one hour. On the 26th post-surgical day this procedure was repeated, except that the second injection was 1.0 mg/kg d-amphetamine.

Nicotine produced an increase in cage crossings during the first 20 min after the injection, and amphetamine produced an increase that lasted the entire hour. The lesion group had lower activity levels after saline than the control group, so the response to each drug was tested using analysis of covariance, with each rat's saline reponse the covariate for its drug response. Data for each rat were summed over 20 min for the nicotine analysis and over 60 min for the amphetamine analysis. ANCOVA found that the lesioned group showed a significantly lower response to both nicotine and amphetamine than the sham control group.

These results indicate that the mesolimbic dopamine system is a critical component of the normal locomotor stimulant response to nicotine seen in rats after several daily nicotine injections.

126.2 ALTERATION IN LICKING PATTERN FOLLOWING CHRONIC HALOPERIDOL: A VALID AND OBJECTIVELY VERIFIABLE MODEL OF TARDIVE DYSKINESIA. S. J. Lytle* and K. M. Kantak. (SPON: J. Stein). Laboratory of Behavioral Neuroscience, Dept. of Psychology, Boston University, Boston, MA. 02215.

Chronic neuroleptic administration can lead to Tardive Dyskinesia (TD). One prevalent theory suggests that TD is caused. by an increase in post-synaptic dopamine sensitivity in the striatum. Evidence indicates that the pars reticulata of the substantia nigra, which projects to the striatum, is involved in the motor control of the oropharyngeal musculature. Since TD is characterized by abnormal movements of the oropharyngeal musculature it is likely that alterations in licking behavior would occur in mice chronically treated with haloperidol.

Baseline licking behavior was recorded in 10 untreated mice until stable performance was achieved. Two groups of mice were then injected with physiological saline or haloperidol (IP. 1.0 mg/kg) for four months. At three-week intervals they were water deprived, habituated to the recording apparatus and tested for 15 minutes under two conditions: a) under the influence of haloperidol and b) after 72 hours of withdrawal from haloperidol. Five measures were recorded: # licks, # bouts, # licks per bout, session length, and latency to first lick.

Overall the # licks did not differ between the saline and haloperidol treated mice. The # bouts, however, were significantly elevated in haloperidol treated mice after the second month under withdrawal conditions and after the third month under spontaneous conditions. Consequently, this led to an abnormal drinking pattern as indicated by the # licks per bout measure. The saline treated mice significantly increased their # licks per bout over time while the haloperidol group showed no change over time or conditions. During the second month there was a significant difference ($p < .05$) between the groups under withdrawal conditions. At the third month the difference between saline and haloperidol # licks per bout became significant under both the spontaneous ($p < .05$) and withdrawal ($p < .01$) conditions. At the fourth month this difference on the # licks per bout between the saline and haloperidol groups became even more pronounced ($p < .01$) for both spontaneous and withdrawal conditions.

These data indicate that this procedure may represent an analogous model of tardive dyskinesia since the effects occurred only over an extended period of time, first upon withdrawal, and later under spontaneous and withdrawal conditions. In addition this design demonstrates a new valid and objectively verifiable animal model for Tardive Dyskinesia.

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126.3 EFFECTS OF CAERULEIN AND HALOPERIDOL ON AMPHETAMINE INDUCED BEHAVIORS. K.J. Mueller, E.M. Hollingsworth*, J.L. Peel* and K.L. Rewey* (SPON: J. Babitch) Dept. of Psych., Texas Christian University, Fort Worth, TX 76129.

Caerulein (CL) is an analog of cholecystokinin, a neuropeptide which has been implicated in dopamine neurotransmission. Matsubara & Matsushita (1984, 1985 and others) have reported that the combination of CL and haloperidol (a dopamine receptor blocker) can produce a long-term antagonistic effect on amphetamine (AMP) induced activity in rats. In other words, there is a reduction in AMP induced locomotions even when AMP is given several days after CL + haloperidol (HAL). The present study further examines both the acute and long-term influences of CL on AMP-induced behaviors using the gamma statistic ($\hat{\gamma}$) developed in Hollingsworth, Mueller & Cross as a measure of stereotypy. Rats were pretreated with a combination of drugs, injected with a test drug and observed in an open field. Five days later the subjects were observed again without pretreatment. On test day 1, rats were simultaneously administered one of the following pretreatments: saline (SAL)+SAL, CL (20ug/kg)+SAL, HAL (0.1 mg/kg)+SAL, or HAL+CL. 60 min. following pretreatment rats were injected with either SAL or AMP (2 mg/kg or 4 mg/kg). 25 min. after the second injection, rats were placed in an open field for 20 min. Rears, lines crossed and stereotypic locomotor activity ( ) were recorded. Five days after the first test session, the procedure was repeated but without pretreatments.

We found that 4 mg/kg AMP increased the statistic while HAL prevented this increase. These data support the usefulness of the statistic as a measure of stereotypic locomotor activity. In contrast to the results presented by Matsubara & Matsushita, no long-term effect of CL+HAL on locomotor activity was found. However, CL did produce a short-term decrease in for 4 mg/kg AMP but had no effect on lines crossed. The possible mechanism responsible for the short-term effects of CL administration are discussed in light of evidence of the dopaminergic system's role in locomotor activity.

126.4 A NEW TECHNIQUE FOR MEASURING STEREOTYPIC LOCOMOTOR ACTIVITY IN AN OPEN FIELD, E. M. Hollingsworth , K. J. Mueller, and D. R. Cross*, Psychology Department, Texas Christian University, Ft. Worth, Texas 76129

Amphetamine (AMPH) produces stereotypic behavior in rats; historically this stereotypy has been characterized as particular repetitive movements of head and limbs and licking/biting. The present series of experiments examines stereotypic patterns of locomotions which appear prior to the more traditional stereotypic behaviors. A probability statistic ( ) was derived to quantify stereotypic patterns of locomotor behavior in an open field.

AMPH-induced changes in the statistic were examined in the first experiment. 11 rats were injected SC with saline (SAL), 3.5, 5.0 and 6.5mg/kg AMPH in a repeated measures design. 25 minutes following each injection animals were placed in the open field and the route through the open field was recorded for 20 min. The overall pattern of locomotor activity was broken down into "trips". Trips were defined as the number of lines crossed until 1 of 3 criteria were met. These include when the rat: changes direction, enters center of open field, completes tour of perimeter. represents the number of sequential repetitions of trips divided by the total number of possible repetitions. Lines crossed and rears were also recorded. There were 4 days between each test. Lines crossed and rears tended to decrease as the AMPH dose increased, but tended to increase and then decrease (as the animal became too stereotypic to locomote). Upon further investigation we found that 2.8mg/kg AMPH enhanced lines crossed, rears and , but the change in was not statistically significant. Thus the dose may be near the threshold for reliably producing stereotypic patterns of locomotions.

In the second experiment 11 animals were injected with caffeine (CAF) at 0 (SAL), 5, 10, and 15mg/kg. Although CAF is a well known stimulant, it does not produce stereotypy. As expected, CAF produced a dose-dependent increase in lines crossed and rears without affecting .

A third experiment controlled for multiple exposures to the open field. 13 animals were injected with saline before each of the 4 open field tests. There was little change in lines crossed, rears or .

The present series of experiments suggest that the derived statistic does quantify AMPH-induced stereotypic patterns of locomotions. The statistic will be particularly useful in quantifying stereotypy during the transitional period between enhanced locomotor activity and more traditional stereotypic behavior. Further analysis revealed that AMPH produced specific changes in the types of patterns of locomotions. This information provides a more complete description of the behavioral response to AMPH in rats.

126.5 INCREASED MOTIVATION TO SELF-ADMINISTER APOMORPHINE FOLLOWING 6-HYDROXYDOPAMINE LESIONS OF THE NUCLEUS ACCUMBENS. David C.S. Roberts and G. Vickers*, Department of Psychology, Carleton University, Ottawa, CANADA, K1S 5B6.

We have previously shown that 6-hydroxydopamine (6-OHDA) induced lesions of mesolimbic dopamine (DA) cell bodies or DA terminals in the nucleus accumbens cause extinction of intravenous cocaine self-administration. These data are consistent with the idea that cocaine is an indirect acting agonist at DA receptors, and that the presynaptic element is necessary for cocaine to have a reinforcing effect. By contrast, the self-administration rate of apomorphine, a direct DA agonist, is not affected by 6-OHDA lesions of the accumbens. Indeed, the same lesioned animals which fail to respond for cocaine injections have been shown to self-administer apomorphine at pre-lesion rates.

If the 6-OHDA treatment produces denervation supersensitivity of DA receptors, and if these receptors mediate the reinforcing effects of apomorphine, then the lesion should increase the reinforcing action of the drug. Our previous demonstration that DA denervation fails to affect the rate of apomorphine self-administration suggests that either (1) the reinforcing action of apomorphine is NOT mediated via stimulation of DA receptors in the accumbens or (2) rate of self-administration is not sensitive to changes in reward strength.

We have recently re-investigated the effect of 6-OHDA lesions on apomorphine self-administration using a progressive ratio schedule of reinforcement. At the beginning of each session, the first response produces an injection of apomorphine (i.e. FR 1). With each subsequent injection the number of responses required to earn an injection increases rapidly. The "break-point" was established each day, and was defined as the last reinforcement ratio completed before the behavior extinguished. When tested following surgery, control animals showed no consistent change in break-point, failing to respond on average past a ratio of 15. By contrast, the 6-OHDA treated group displayed a steady increase in the break-point following the lesion, reaching a mean break-point after one week of 60. Several weeks after the lesion, we have found rats that will complete ratios of over 300. These data demonstrate that 6-OHDA-treated animals show an increased motivation to self-administer apomorphine which progresses daily after the lesion and may parallel the development of supersensitive DA receptors.

These data clearly demonstrate that some treatments can induce changes in reward value without affecting the rate of self-administration on simple schedules of reinforcement.

126.6 PRODRUGS ENHANCE THE DURATION OF ACTION OF THE DOPAMINE AUTORECEPTOR AGONIST, CGS 15855A. J.C. Berry*, W.C. Boyar, R.A. Lovell, A.J. Hutchison* and B.S. Glaeser, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey, 07901.

CGS 15855A ([±]trans-1,3,4,4a,5,10b-hexahydro-4-propyl-2H[1]benzopyranol[3,4,-b]-pyridin-9-ol monohydrochloride) has been described as a dopamine autoreceptor agonist having a short to moderate duration of action (Glaeser et al., Soc. Neurosci. Abstr. 11, part 1985). Rapid methylation of the hydroxy group on CGS 15855A may account for the short duration of action. Therefore, derivatives were synthesized by the derivatization of the hydroxy group to form the benzoate (CGS 16475A), pivaloate (CGS 16486A) and the dimethyl carbamate (CGS 16487A) prodrugs of CGS 15855A. These prodrugs were tested for their dopaminergic properties using the gamma-butyrolactone (GBL) model for dopamine autoreceptors (Walters and Roth, Naunyn-Schmiedeberg's Arch. Pharmacol. 296:5, 1976). Previously, in this model it had been shown that CGS 15855A had ED_{50} values of 0.16 mg/kg i.p. and 0.39 mg/kg p.o. (Glaeser et al., Soc. Neurosci. Abstr. 11, part 1, p 500, 1985). In these studies the prodrugs were initially administered at pretreatment times of 15 and 30 min before the administration of GBL (750 mg/kg i.p.). Doses of the prodrugs were calculated to correspond to 5 mg/kg i.p. of CGS 15855A and were based on the assumption that there was 100% hydrolysis of the ester linkage. After a 15 min pretreatment, the benzoate and pivaloate prodrugs reversed the GBL induced accumulation of DOPA by 79 and 90%, respectively. The dimethyl carbamate prodrug was inactive after the 15 min pretreatment, while apomorphine produced an 85% reversal. After a 30 min pretreatment, the benzoate and pivaloate prodrugs produced a 96 and 91% reversal, respectively, while the dimethyl carbamate prodrug did not significantly alter the GBL induced accumulation of DOPA. In related studies with the GBL model, all three prodrugs were administered orally (10 mg/kg) at a pretreatment time of 2 hr. All of the compounds significantly reversed the GBL accumulation of DOPA by 46 to 66%. In metabolite studies, pivaloate and dimethyl carbamate prodrugs were administered orally at doses equivalent to 10 mg/kg of CGS 15855A. Both compounds significantly reduced the striatal concentrations of DOPAC and/or HVA. Both compounds had a more profound effect than CGS 15855A on the reduction of striatal HVA concentrations at 1,2 and 3 hrs post administration. In a similar study CGS 15855A (10 mg/kg p.o.) reduced striatal HVA concentrations at 0.5 and 1 hr post administration (ibid). These studies suggest that certain prodrugs may enhance the dopaminergic autoreceptor agonism properties of the parent compound. More specifically, the benzoate, pivaloate, or dimethyl carbamate prodrugs of CGS 15855A showed a more prolonged duration of action as dopamine autoreceptor agonists when compared to the parent compound, CGS 15855A.

126.7 **DOPAMINE ESTERS AS POTENTIAL PRO-DRUGS FOR DOPAMINE.** **S.M. Tejani-Butt, A. D'Mello*, I. Lucki and D.J. Brunswick. Department of Psychiatry, University of Pennsylvania School of Medicine and Neuropsychopharmacology Unit, Veterans Administration Medical Center and Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.**

Dopamine (DA) is a hydrophilic neurotransmitter which due to its catechol and ionizable amine moiety is not sufficiently lipophilic to penetrate the brain. A labile, lipophilic derivative of DA which has the ability to penetrate the brain and hydrolyze to DA may have utility in the treatment of Parkinson's disease and may also serve as a tool to study central DA receptor function.

In this study, three esters of DA were synthesized in which the catechol moiety was esterified. These derivatives were the diacetyl (DADA), dibenzoyl (DBDA) and p-chlorobenzoyl (p-CBDA) esters of DA. The ester derivatives were 10-1000 fold more lipophilic than DA itself (log D = -2.8) as measured by their distribution coefficients between phosphate buffer and n-octanol at pH 7.4. The log D values for DADA, DBDA and p-CBDA were -2.0, 1.3 and 0.76, respectively. The kinetics of decomposition of the pro-drugs of DA in aqueous solution at 37° and pH 7.4 was studied to assess their stability and thus their suitability as pro-drugs for further pharmacologic evaluations. The half-lives of hydrolysis for DADA, DBDA and p-CBDA were 194, 423 and 5 min, respectively. DBDA was chosen for further investigation as it was lipophilic and had a usable rate of hydrolysis.

Radiolabeled DBDA was synthesized from (8-^{14}C)DA, and the total uptake of ^{14}C-DBDA into the brain was measured in Sprague-Dawley rats (200-210g) following i.v. injections (9mg/kg) via their tail vein. At various time points (5-240 min), the rats were decapitated and the brain removed, homogenized and its content of radioactivity measured. ^{14}C-DBDA penetrated the brain rapidly giving an uptake of 0.28±0.02% of injected dose/g tissue (n=3) at 5 min. After this time, levels of radioactivity declined with levels at 15 and 45 min being 0.23±0.004% (n=4) and 0.16± 0.02% (n=3), respectively. At 240 min, no radioactivity was detected in brain. By contrast, after i.v. administration of [8-^{14}C]-DA, radioactivity was less than 0.03% of the injected dose/g tissue at these time points.

Behavioral changes were observed when rats were injected with DBDA (12 mg/kg) via their tail vein. At this dose, rats exhibited significantly increased locomotor activity, and increased grooming and rearing behavior as compared with rats injected with saline.

Thus, we have demonstrated that DBDA is a potential pro-drug of DA that is able to penetrate the brain and can be hydrolyzed in aqueous solution at pH 7.4 to form DA. The behavioral effects observed are consistent with increased central dopaminergic activity. Based on these results, DA esters appear to warrant further investigation as potential DA pro-drugs for the treatment of Parkinson's disease. **(Supported by Research Funds from the Veterans Administration, N.I.H. Biomedical Research Support Grant, University of Pennsylvania and MH 36761).**

126.8 COMPARATIVE AUTORECEPTOR AND POSTSYNAPTIC DOPAMINE AGONIST ACTIVITY OF CGS 15855A AND CGS 15873A. J.M. Liebman, R. Gerber, G. Pastor*, B.S. Glaeser, C.A. Altar and P.L. Wood. (SPON: J.J. Welch). Neuroscience/Cardiovascular Research, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, New Jersey, 07901.

Two novel benzopyranopyridine derivatives, CGS 15855A and CGS 15873A, have been characterized as potent agonists at striatal dopamine (DA) autoreceptors, using the gamma-butyrolactone (GBL) striatal autoreceptor model (Glaeser et al., Soc. Neurosci. Abstr. 11:500, 1985) and other measures (Altar et al., Eur. J. Pharmacol. 134:303, 1987). The present experiments assessed the separation between the autoreceptor and postsynaptic DA agonist activity of these drugs in comparison with apomorphine and (±)-3-PPP.

Drugs were administered i.p. to rats and measurements of postsynaptic DA agonism were performed within the next 30 min. Stereotyped sniffing was assessed by direct observation, and reversal of reserpine-induced akinesia was measured using vibration-sensitive motor activity monitors. Striatal acetylcholine (ACh) levels were measured using the method of Wood and Peloquin (Neuropharmacology 21:349, 1981). Another group of rats received unilateral intranigral injections of 6-hydroxydopamine to induce loss of neostriatal dopamine neurons. Of these rats, only those that manifested a high degree of rotational sensitivity to apomorphine (at least 100 rotations/20 min after 0.03 mg/kg s.c. apomorphine) were used.

In normal rats, CGS 15855A and CGS 15873A were typically eight to thirty times less potent in the stereotypy, reserpine and striatal ACh level assays of postsynaptic activity than in the GBL model. The potency of apomorphine in the postsynaptic models was comparable to its potency in the GBL model, whereas (±)-3-PPP showed no apparent postsynaptic agonism. In lesioned rats, however, the potencies of all four drugs to induce rotation were comparable to those in the GBL model. These results suggest that in the presence of normal dopaminergic innervation, CGS 15855A and CGS 15873A show relative, but not absolute, autoreceptor selectivity. In a DA-denervated preparation, CGS 15855A and CGS 15873A, in common with 3-PPP, have potent postsynaptic activity.

	ED50 or active doses, mg/kg i.p.			
TEST	CGS 15855A	CGS 15873A	APO	(±)-3-PPP
GBL[a]	0.16	0.52	0.56	2.0
Stereotypy	3.1	~30	0.4	>30
ACh levels	4.0	≤3.0	0.9	-
Reserpine	1.0	4.6	0.15	>30
Rotation	~0.1	0.5	~0.08	0.7[b]

[a] From Glaeser et al., 1985; [b] (-)-3-PPP

126.9 SELF-ADMINISTRATION OF MIDAZOLAM MAY BE ASSOCIATED WITH A DECREASE IN DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS.
J.M. Finlay, C. Szostak*, C.D. Blaha, R.F. Lane and H.C. Fibiger. Div. of Neurol. Sci., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, V6T 1W5.

We have previously shown that rats will self-administer the short acting benzodiazepine midazolam. It has been proposed that intravenous self-administration of psychoactive drugs is subserved by increases in the activity of mesolimbic dopaminergic neurons. The present experiments further characterized intravenous midazolam self-administration in the rat and examined the effects of midazolam on mesolimbic dopamine (DA) release.

Following implantation of chronic intrajugular cannulae, rats were given 24 h access to two levers. Each response on the drug lever resulted in a single infusion of midazolam (0.05 mg/0.18 ml/infusion) coincident with the illumination of a cue light located directly above the lever. Responses on the control lever did not produce an infusion but did result in illumination of a cue light. All rats established a preferred level of responding for midazolam that remained stable over subsequent 24 h sessions. Rates of responding were consistently higher on the drug lever than the control lever. As previously reported, maximal responding occurred during the dark phase of the 12 h light/dark cycle. Following acquisition of stable levels of responding for midazolam, rats were tested under reversal conditions. During this phase, each response on the control lever produced an infusion of midazolam coincident with the illumination of a cue light while responses on the drug lever resulted in illumination of a cue light only. Across test sessions, rats tracked the drug-response contingencies such that rates of responding on the newly active lever were similar to levels attained during the initial acquisition of midazolam self-administration.

In vivo electrochemistry was used to evaluate the effects of midazolam on DA release within the nucleus accumbens (NAS) of the unanaesthetized rat. Repeated intravenous infusions of midazolam (0.05 mg/0.18 ml/infusion), delivered in a manner that mimicked the pattern of midazolam self-administration, resulted in a significant decrease in DA release within the NAS. Subcutaneous injections of midazolam also elicited a dose dependent decrease in accumbens DA release in the unanaesthetized rat.

The results of the present experiment: 1) support earlier work demonstrating that rats will self-administer midazolam and 2) provide preliminary evidence that self-administration of midazolam is not mediated by a drug-induced increase in DA release within the NAS.

J.M.F. is a MRC student and C.S. is a NSERC post-graduate scholar. Supported by the Medical Research Council of Canada.

126.10 STUDIES ON SELECTIVE D1/D2 DOPAMINE AGONIST-INDUCED CONDITIONED TASTE AVERSIONS IN RATS.
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A number of studies have demonstrated that injections of either direct or indirect dopamine agonists are able to produce conditioned taste aversions (CTAs) in animals. To date, however, the selective involvement of the D1 and D2 dopamine (DA) receptor subtypes in the production of CTAs has not been studied. The purpose of the present study was to investigate the ability of selective D1 and D2 agonists to induce the development of CTAs and to examine the effects of selective DA antagonists on these aversions. We also investigated the possible neuronal substrates involved in this behavior by studying D1 and D2 DA agonist-induced conditioned taste aversions in rats with lesions of the area postrema (AP).

The basic procedure for inducing CTAs was the same in all studies. Adult male rats were placed on a 23h water deprivation schedule for 9 days. On the 3rd, 7th and 9th days, rats were presented with a .1% sacharrin solution instead of water. After the first two presentations rats were injected with test compounds or vehicle. Antagonists were injected immediately after the sacharrin was removed; agonists were adminstered 15 minutes later. All water and sacharrin intakes were recorded.

The D2 agonist LY171555 (LY)(.075-.60mg/kg) and the D1 agonist SKF38393 (SKF) (1-20 mg/kg) both produced dose-dependent CTAs. The D2 antagonist haloperidol (.125 &.375mg/kg), which had no effect on its own, blocked the LY but not the SKF induced CTAs. The D1 antagonist SCH23390 (.12,-.60mg/kg) failed to induce a CTA on its own and also failed to block either the D1 or D2 agonist-induced CTAs. These studies suggest that selective stimulation of the D2 DA receptor underlies the ability of LY to induce a CTA since the aversion could be blocked by haloperidol. However, the CTA produced by SKF does not appear to be mediated via the stimulation of either the D1 or D2 DA receptor subtype.

In subsequent studies we investigated the possible neuronal substrates underlying these DA agonist-induced CTAs. Behavioral and neuropharmacological evidence indicate that the AP may be involved in some of the aversive properties of some DA compounds. Therefore, rats were prepared with thermo lesions of the AP under visual guidance using a transcisternal approach. At least three weeks later (by which time body weights had stabilized), rats were tested for the establishment of an LY or SKF induced CTA. Lesions of the AP failed to affect either agonist induced aversion. However, as expected, these lesions did attenuate a scopolamine-induced CTA to a chocolate milk solution. It therefore appears that the area postrema is not importantly involved in the induction of a CTA by either LY or SKF.

126.11 APOMORPHINE-INDUCED REVERSAL IN DIRECTION OF LATERALIZED WALL-FACING AFTER 10 DAYS OF UNILATERAL REMOVAL OF VIBRISSAE IN RATS.
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Previous experiments have shown that unilateral removal of the vibrissae (UVR) can induce neural plasticity in the crossed nigrostriatal projection. After 10 days of shaving the vibrissae on one side of the rats' face, more cells were retrogradely labeled in the contralateral substantia nigra (SN) when horseradish peroxidase was injected into the striatum on the side opposite to UVR than when it was applied on the same side as UVR or in normal animals (Huston, et al., Exp. Neurol., 93:380, 1986).

In the present experiment we examined the influence of UVR on rats' wall-facing behavior (i.e., the tendency to locomote along the perimeter of an open field). For this purpose the time the rats moved along the wall of an open field with respect to the side of UVR was measured. Two groups of rats which differed in the duration of experience with this sensory imbalance were compared. The results showed that after UVR wall-facing was lateralized towards the sensory intact side. That is, the rats preferentially moved along the wall with the vibrissae side towards the wall. This asymmetry was seen irrespective of whether the rats had been shaved for 4 hours or for 10 days. After an injection of apomorphine (APO, 0.75 mg/kg, s.c.) the animals that were shaved for 4 hours also spent more time facing the wall with the sensory intact side, whereas the rats shaved for 10 days preferentially moved along the wall with the shaved side of the face facing the wall.

The APO-induced reversal in wall-facing asymmetry found after 10 days of UVR suggests a plasticity in dopamine (DA) transmission after this kind of sensory deprivation. It is reminiscent of the APO-induced reversal in sensorimotor asymmetries that occurs after unilateral lesions of the SN, which has been related to denervation-induced DA receptor supersensitivity. The APO-induced reversal in wall-facing asymmetry after UVR could reflect "deprivation super-sensitivity" of DA receptors.

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126.12 DOPAMINE AND OXYTOCIN LINK IN THE EXPRESSION OF PENILE ERECTION AND YAWNING.
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Repeated episodes of penile erection and yawning can be induced in experimental animals by the intracerebroventricular (i.c.v.) injection of ACTH-MSH peptides, or by the systemic injection of low doses of dopamine (DA) agonists or by i.c.v. injection of nanogram amounts of oxytocin. The ability of the above unrelated substances, together with their presence in the central nervous system (CNS) to induce the above symptomatology, raises the possibility that a neuronal link between DA, oxytocin and ACTH might play an important role in the expression of penile erection and yawning. In order to test the above hypothesis, several experiments were performed. 1) The effect of DA antagonists such as haloperidol and sulpiride, on penile erection and yawning induced by the DA agonist apomorphine, by oxytocin and ACTH 1-24 was studied. Both haloperidol and sulpiride prevented apomorphine-induced responses, but not that of oxytocin and ACTH 1-24. 2) The i.c.v. injection of the oxytocin antagonist $d(CH_2)_5Tyr(Me)\text{-}Orn^8$-vasotocin was found to be able to antagonize penile erection and yawning induced by oxytocin and apomorphine, but not by ACTH 1-24. 3) The depletion of hypothalamic ACTH and -MSH by neonatal monosodium glutamate was found to be ineffective in antagonizing penile erection and yawning induced either by ACTH 1-24 or oxytocin or apomorphine. 4) Microinjection and lesion studies revealed that the paraventricular nucleus of the hypothalamus is the most sensitive brain area for the induction of penile erection and yawning either by apomorphine or by oxytocin.

Altogether, the present results indicate that DA agonists induce penile erection and yawning by releasing oxytocin in the hypothalamic paraventricular nucleus and that ACTH- derived peptides act either downhill to oxytocin and DA receptors or by a different mechanism to induce such responses. Moreover, the potency of apomorphine and oxytocin suggests a physiological role of hypothalamic DA and oxytocin in the expression of penile erection and that abnormalities in their function might be responsible of penile erection disturbances.

126.13 PENILE ERECTION AND YAWNING INDUCED BY DOPAMINE AGONISTS: SITE OF ACTION IN BRAIN. M.R. Melis and A. Argiolas. Dept. of Neurosciences, Univ. of Cagliari, Via Porcell 4, 09100 Cagliari, Italy.

The systemic administration of low doses of dopamine (DA) agonists, such as apomorphine induces recurrent episodes of penile erection and yawning in rats. In contrast to low doses, high doses of DA agonists induce hypermotility and stereotypy, but not yawning and penile erection. While the striatum and nucleus accumbens have been identified as the brain sites where DA agonists act to induce hypermotility and stereotypy, the site where DA agonists act to induce penile erection and yawning is still unknown. In order to determine such site(s) we microinjected apomorphine into discrete brain nuclei through stereotaxically implanted chronic guide cannulae. The results indicate that the most sensitive brain area for the induction of penile erection and yawning is the paraventricular nucleus of the hypothalamus (PVN). A significant effect was elicited by a dose of apomorphine as low as 5 ng. The symptomatology usually began within 5 min after microinjection, lasted for 30-50 min, and was identical to that induced by the systemic administration of the drug. Hypermotility and stereotypy were never observed after apomorphine microinjection into the PVN, even at the highest dose tested (1 ug). Microinjections of the same doses of apomorphine into the hypothalamic ventromedial and dorsomedial nuclei, preoptic area, caudate nucleus, nucleus accumbens and substantia nigra, were ineffective. LY 171555, a specific D_2-DA receptor agonist, and (+)-3PPP but not (-)-3PPP nor the specific D_1-DA receptor agonist SKF 38393, were as effective as apomorphine when injected into the PVN. Apomorhine-induced penile erection and yawning were antagonized by pretreatment with neuroleptic drugs such as haloperidol, (-)sulpiride, a specific D_2-DA antagonist, and SCH 23390, a specific D_1-DA antagonist.

The present results suggest that the PVN is the brain area where D_2-DA agonist act to induce penile erection and yawning. Moreover, since the PVN contains the cell bodies of a group of incertohypothalamic DA neurons, the above results suggest for the first time a possible involvement of the incertohypothalamic DA system in the expression of penile erection and yawning.

126.14 THE EFFECTS OF D-1 AND D-2 DOPAMINE RECEPTOR ANTAGONISTS ON THE DEVELOPMENT OF SENSITIZATION TO THE LOCOMOTOR EFFECTS OF MORPHINE AND AMPHETAMINE. P. Vezina and J. Stewart. Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, Montreal, Canada H3G 1M8.

In the present experiments, a comparison was made between the effects of various selective and nonselective dopamine (DA) receptor antagonists on the development of sensitization of the locomotor activity produced by repeated exposure to intra-VTA morphine or systemic amphetamine.

In experiment one, rats were prepared with bilateral injection cannulae aimed at the VTA and randomly assigned to one of six groups of 6-8 rats each. During the training phase, animals in each group were pretreated with either pimozide (0.5 mg/kg, i.p.), sulpiride (10 or 50 mg/kg, i.p.), Ro22-2586 (0.2 mg/kg, s.c.), SCH-23390 (0.2 mg/kg, s.c.), or saline (1 ml/kg, i.p.) before being administered each of five injections of morphine sulfate (5.0 µg in 0.5 µl saline volume/side) and placed in activity boxes for two hours. Injections were given every other day. On the test day, two days following the last morphine injection, animals were given vehicle injections and tested in the activity boxes following an intra-VTA morphine injection. Animals pretreated during training with the D-1 DA receptor antagonist SCH-23390 as well as those pretreated with the D-2 DA receptor antagonists Ro22-2586 and sulpiride showed, on this test, locomotor activity levels similar to the increased or sensitized activity levels of animals pretreated with saline. Animals pretreated with pimozide, however, were less active than all other groups on this test suggesting that while the effect of pimozide was to block the development of sensitization to the locomotor effects of intra-VTA morphine, the remaining antagonists, selective to D-1 and D-2 DA receptors, were without effect.

In experiment two, different groups of rats were trained as above but with d-amphetamine sulfate (1.0 mg/kg, i.p.). On the test day, rats were given vehicle injections and tested in the activity boxes following an injection of 0.5 mg/kg d-amphetamine. Activity levels on this test suggest that while Ro22-2586 and pimozide had no effect on the development of sensitization to the locomotor effects of d-amphetamine, sulpiride enhanced the sensitization while SCH-23390 attenuated its development.

These results a) suggest that the mechanisms underlying development of sensitization to the locomotor effects of morphine and amphetamine may differ even though these may ultimately result in similar changes in the presynaptic activity of mesencephalic DA neurons, and b) preclude an exclusive role for one DA receptor subtype in the mediation of sensitization to the locomotor effects of opiates and stimulants.

126.15 ACUTE BEHAVIORAL EFFECTS OF MPTP IN RATS ARE MEDIATED BY MPTP ITSELF AND NOT BY MPP^+. R. Schaffner*, M. Da Prada*, P. Polc*, W. Haefely (SPON: K. Bizière). Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland.

The preferential degeneration of the nigro-striatal system by MPTP in several mammalian species including primates and mice is dependent on the metabolism of MPTP to MPP^+ by monoamine oxidase (MAO). In rats, however, MPTP induces short lasting behavioral effects of the mixed dopamine (DA)/serotonin-type without influencing brain monoamines to a significant extent.

In an attempt to clarify whether the acute behavioral effects of MPTP in rats are due to MPTP itself or its metabolite MPP^+ and whether they are mediated by pre- or postsynaptic mechanisms at DAergic synapses the following experiments were performed. 1 MPTP was studied for its ability to induce turning behavior in rats with 6-OHDA lesions in the MFB and its antagonistic effect in rats against prochlorperazine (PPZ)-induced catalepsy without or with pretreatment with the selective MAO-B inhibitor Ro 19-6327 (N-(2-aminoethyl)-5-chloro-2-pyridine-carboxamide; Da Prada, M. et al., Pharmacol. & Toxicol. 60 (Suppl. I):10, 1987) and/or the preferential MAO-A inhibitor moclobemide (MOCLO). Furthermore, following the injection of MPTP (10 mg/kg ip, sc), mixed with trace amounts of ^{3}H-MPTP, the striatal content of ^{3}H-MPTP and ^{3}H-MPP^+ was determined by extraction of radioactivity from the striatum, separation of ^{3}H-MPTP and ^{3}H-MPP^+ by TLC and measurement of the radioactivity by scintillation spectrometry (Da Prada, M. et al., Neurosci. Lett. 57:257, 1985). 2 The ability of MPTP to antagonize the cataleptic state induced in rats by the tyrosine-hydroxylase inhibitor alpha methyl-p-tyrosine (AMT) was studied.

MPTP (30 mg/kg sc) induced contralateral turning and dose-dependently antagonized (10-30 mg/kg ip) the PPZ-induced catalepsy. In addition, MPTP (30 mg/kg ip) reduced AMT-induced catalepsy. The turning behavior and the anticataleptic effect of MPTP against PPZ catalepsy were potentiated by Ro 19-6327. A similar potentiation of the anticataleptic effect was observed with MOCLO or a combined pretreatment with Ro 19-6327/MOCLO. Determination of striatal accumulation of ^{3}H-MPTP and ^{3}H-MPP^+ revealed that, within one hour after the injection of ^{3}H-MPTP, ie the time of peak effect, the two MAO inhibitors markedly reduced the formation of ^{3}H-MPP^+ in the striatum of rats.

From these results it is concluded that the acute behavioral effects observed with MPTP in rats are in large part due to MPTP itself and are not dependent on its metabolic transformation into MPP^+. Furthermore, since MPTP induced contralateral turning and antagonized AMT-induced catalepsy it is suggested that these behavioral effects observed with the high doses of MPTP are mediated by a direct effect of MPTP on postsynaptic DA-receptors.

126.16 SENSITIZATION FOLLOWING REPEATED ADMINISTRATION OF THE DIRECT ACTING D_1-DOPAMINE AGONIST SKF-38393 IN NEONATALLY- BUT NOT ADULT-6-OHDA LESIONED RATS. H.E. Criswell, R.A. Mueller, and G.R. Breese. Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Recent work on several fronts has demonstrated that drugs which act on the dopaminergic system can produce sensitization or "reverse tolerance". We examined the effects of repeated administration of the specific D_1- dopamine agonist SKF-38393 in rats lesioned with 6-hydroxydopamine (6-OHDA) either as neonates or as adults. Neonatal-6-OHDA lesions are known to sensitize the animals to D_1-dopamine agonists while adult lesions primarily sensitize animals to D_2-dopamine agonists. Repeated doses of the D_1-dopamine agonist to adult rats which received 6-OHDA lesions as neonates produced a progressive increase in locomotion and several other behaviors following repeated administration of the dopamine agonist at 4 day intervals. Sensitization was still present 90 days after the last administration. Rats which received lesions as adults did not show the progressive increase in locomotor behavior following repeated administration of SKF-38393. Neonatally lesioned rats given repeated doses of SKF-38393 also showed sensitization to the locomotor effects of the adenosine antagonist theophylline and the cholinergic antagonist scopolamine. These two drugs produce increased locomotor activity in neonatally lesioned rats via a mechanism which can be blocked by pretreatment with alpha-methyltyrosine suggesting that it is mediated by catecholamine-containing neurons. Specific sensitization of D_1-dopamine receptors by repeated stimulation may underlie the reverse tolerance and appearance of new symptoms which follow chronic administration of dopamine agonists or drugs which indirectly activate the dopaminergic system. Supported by NS-21355 and HD-03110.

126.17 EFFECTS OF THE D1 AND D2 ANTAGONISTS, SCH 23390 AND SULPIRIDE, ON ORAL MOVEMENTS IN RATS. Edward D. Levin, Ronald E. See, Per Johansson*, David South*, Lars Gunne* and Gaylord D. Ellison Department of Psychology, University of California, Los Angeles, CA 90024.

Two experiments were performed to investigate the actions of the dopamine D1 receptor blocker, SCH 23390, and the D2 receptor blocker, sulpiride, on oral movement in rats. The oral movements were quantified by a human observer and a computerized video analysis system. The first experiment was a dose-response study of the effects of these two drugs (SCH 23390, 0.01-0.25 mg/kg and sulpiride, 4-100 mg/kg) on spontaneous chewing and head movement. Sulpiride was the more effective of the two drugs in decreasing chewing, while SCH 23390 was more effective in decreasing head movement. The low doses of both drugs increased the duration of head movement. The computerized analysis of the slope of movements showed that both drugs decreased the slope of very tiny movements but that only sulpiride decreased the slopes of larger movements.

In the second experiment, the actions of these two drugs were studied in relation to their abilities to block the effects of selective D1 (SKF 38393) and D2 (LY 171555) agonists. The two antagonists had similar effects in relation to the D1 agonist, SKF 38393. Both SCH 23390 (0.25 mg/kg) and sulpiride (100 mg/kg) were effective in eliminating the increases in computer-scored movement induced by SKF 38393 (3 mg/kg). Both drugs were also effective in eliminating the increase in movement slope caused by SKF 38393. In relation to the D2 agonist LY 171555, the two antagonists had differential effects. Sulpiride attenuated the decrease in chewing movement caused by administration of the D2 agonist, LY 171555 (0.1 mg/kg), while SCH 23390 exacerbated it. This differential effect was also seen with computer-scored movements. The increase in oral movement induced by the D1 agonist, SKF 38393, can be reversed by either a D1 or a D2 antagonist, while the decrease in oral movement induced by the D2 agonist, LY 171555, can be reversed by a D2 antagonist but is exacerbated by a D1 antagonist.

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126.18 LOCOMOTOR ACTIVITY STUDIES ON D1 AND D2 DOPAMINE RECEPTOR STIMULATION. W.E.Montana and K.E.Asin, Abbott Labs, Dept. 47U, Bldg. AP10, Abbott Park, Il. 60064.

The characterization of D1 and D2 dopamine (DA) receptors has led to a number of investigations on the behavioral functions of these two receptor subtypes. Although neurologically intact animals show relatively few behavioral changes in response to D1 receptor agonists, the DA depleted or denervated rat demonstrates hyperactivity following D1 receptor stimulation. We undertook the following studies to investigate and compare more closely the effects of D1 and D2 DA receptor drugs on locomotor activity in the intact and DA depleted rat. We also examined possible suppressant actions of D1/D2 agonists by studying the effects of D1 and D2 DA drugs on drug-induced hyperactivity.

Adult, male rats were treated with 1mg/kg reserpine (RES) or vehicle (V) for 5 days. Ninety minutes after the last injection rats (N=8-20) were injected with the D2 antagonist haloperidol (H)(.06-.25 mg/kg), the D1 antagonist SCH23390 (SCH) (.05 & .20mg/kg) or with V and placed into locomotor activity cages (tilt boxes) for 40min. Rats were then injected with either V, the D2 agonist LY171555 (LY)(.15-.30mg/kg,sc) and/or the D1 agonist SKF38393 (SKF)(1.25-20mg/kg). Following the agonist injections locomotor activity (LA) was measured for 150 min.

As expected, SKF produced increases in activity only in reserpinized rats; in contrast, increases in LA following LY were similar for the V and RES groups. The activity in response to LY was more potently blocked by H than by SCH in reserpinized rats, and SKF-induced LA in DA depleted rats was more potently blocked by SCH. Injections of SKF and LY at dosages which produced no or only minimal effects on LA on their own led to a robust increase in LA in RES but not V treated rats when administered simultaneously. In contrast, the actions of LY + another D2 agonist, pergolide (5ug/kg), did not appear to be synergistic. Thus, a "supersensitive" LA response in RES rats was only observed after injections of SKF, either alone or in combination with LY. Injections of H or SCH prior to LY+SKF reduced LA in a dose-dependent fashion, although H was more potent. These studies provide additional evidence for interactions between the two DA receptor subtypes in the chronically DA depleted animal.

Finally, we examined possible suppressant actions (as with apomorphine) of selective D1 and D2 agonists. Following a 40min habituation period rats were injected with either V, 2 mg/kg scopolamine HBr (S) or 12 mg/kg caffeine (C), plus LY or SKF. Injections of either the D1 or D2 DA receptor agonist reduced both S and C hyperactivity; lower doses tended to reduce the LA more potently than higher doses. These studies demonstrate locomotor suppressant actions of both selective D1 & D2 agonists.

126.19 EFFECT OF 3-PHENYL-1-ALKYLPYRROLIDINES ON LOCOMOTOR ACTIVITY IN THE RAT. S.C. Sylvestri*, A.M. Crider, and R.M. Dick. (SPON: W.M. Bourn) Division of Pharmacology and Toxicology, School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.

The 3-phenyl-1-alkylpyrrolidines (3-PAP) are chemical analogs of the neurotransmitter dopamine. Four recently synthesized 3-PAP derivatives were examined for their potential effects on locomotor activity in the rat. The compounds examined included: 3-(3,4-dihydroxyphenyl)-1-n-propylpyrrolidine hydrobromide (Cmpd. 1), 3-(3,4-dihydroxyphenyl)1-i-propylpyrrolidine hydrobromide (Cmpd. 2), Trans-3-(3,4-dihydroxyphenyl)-4-methyl-1-n-propylpyrrolidine hydrobromide (Cmpd. 3), and Trans-3-(3-hydroxyphenyl)-4-methyl-1-n-propylpyrrolidine (Cmpd.4). Previous work by others (Crider, et al., 1984, J. Pharm. Sci. 73:1585-1587) has shown that Cmpd. 1 acts as an effective dopaminergic agonist, demonstrating increased locomotor activity. The current investigation supports their findings. However, of the other compounds examined, only Cmpd. 4 showed a significant increase in locomotor activity when compared to controls. Of the remaining two compounds, Cmpd 2 demonstrated a strong trend towards decreasing locomotor activity and Cmpd. 3 had little if any effect.

127.1 ELECTRICAL STIMULATION OF THE LATERAL HYPOTHALAMIC FEAR/FLIGHT SITES IN RATS PRODUCES CONDITIONAL FREEZING. D.S. Sacks* and J. Panksepp. Dept. of Psych., Bowling Green State University, Bowling Green, OH 43403

Electrical stimulation of the anterior lateral hypothalamus (LH) produces behaviors indicative of underlying emotional states (Behav. Brain Sci. 1982, 5:407). Behaviors associated with stimulation-induced "fear" involve species-typical defensive postures coupled with vigorous escape behaviors. Whether the animal is experiencing a true affective state during such stimulation is not known. This study examined whether conditional freezing would develop to the contextual cues in which "fear" inducing brain stimulation was applied.

12 Long-Evans rats were implantated with bipolar electrodes (-1.0 from bregma, 1.2 lateral, 8.0 ventral). After 2 weeks recovery, each animal underwent 4 days of habituation to the experimental chamber. On the fifth day, the animals in the stimulation group underwent a baseline phase consisting of 5 one-minute observation periods measuring the number of freezing bouts and the time per minute that the animal was immobile. The stimulation phase, consisted of 5 successive 10 sec. periods of LH stimulation (18 to 30μA,sine wave), each separated by a 1 minute observation period. The post-stimulation phase consisted of 5 one-minute observation periods without brain stimulation. This regimen continued for a total of four days. The control group underwent the same procedure but received no electrical stimulation.

Electrical stimulation reliably increased the amount of freezing exhibited by the animals in the stimulation group during the post-stimulation phase of each of the four days of observation. The stimulation group had a mean number of 10.02 freezing bouts across the four days while the control group had .04 freezing bouts ($F(3,40)=10.61$, $p<.0001$). The stimulation group had a mean freezing time of 16.9 sec. across the four days while the control group had a mean time of .1 sec. ($F(3,40)=3.92$, $p<.02$). An increase in the number of freezing bouts during the baseline (pre-stimulation) phase of each of the last 3 days was indicative of conditioning to the experimental chamber. In the stimulation group, the mean number of post-stimulation freezing bouts increased from .4 bouts on the first day to 5.2 to 7.2 and 7.4 on the subsequent 3 days ($F(3,40)=23.041$, $p<.00001$). The stimulation also increased the mean amount of time per period the animal spent freezing. This increase was from 1.3 sec. on the first day to 6.3 to 13.9 and 12.6 on subsequent days ($F(3,40)=9.45$, $p<.00001$).

These results support the claim that electrical stimulation of the LH produces an affective state that induces conditional species typical defensive behaviors, as well as producing an externally referred aversive state that can become connected to environmental cues. We believe this aversive state is reasonably called "fear".

127.2 PRO- AND ANTI-PANIC TREATMENTS AND MEASURES OF ANXIETY IN THE RAT. A. L. Johnston* and S. E. File (SPON: W. A. Friedman) Dept. Pharmacology, The School of Pharmacy, Univ. London, London WC1N 1AX.

Agents useful clinically in the treatment of panic attacks and agents reported to selectively induce panic or anxiety in panic disorder patients were studied in two tests of anxiety: the social interaction test (File, S. E., J. Neurosci. Meths., 2: 219-238, 1980) and the elevated plus-maze (Pellow, S. et al, J. Neurosci. Meths., 14: 149-167, 1985). Anxiolytic drugs increase time spent in social interaction and in the plus-maze they increase the % of entries made onto the open arms and the % of time spent on the open arms; anxiogenic drugs do the reverse.

The anti-panic agents were: the triazolobenzodiazepines (adinazolam 2 & 5 mg/kg, alprazolam 1 & 2 mg/kg, U-43,465 16 & 32 mg/kg), imipramine (5 & 15 mg/kg) and phenelzine (9 & 18 mg/kg). Rats received 5 days pre-treatment with the triazolobenzodiazepines; only adinazolam (5 mg/kg) significantly increased time spent in social interaction and only alprazolam (1 & 2 mg/kg) significantly increased % time spent on the open arms in the plus-maze. Imipramine and phenelzine, tested after 21 days of pre-treatment, reduced the time spent in social interaction but were without effect in the plus-maze.

The pro-panic agents were: sodium lactate (60 & 120 mg/kg), isoproterenol (0.1-0.6 mg/kg) and yohimbine (1.25-5 mg/kg). Rats were also scored for panic-like behaviour following administration of these drugs and following CO_2 (5 & 20% in air). Sodium lactate, isoproterenol and yohimbine were without significant effect on the time spent in social interaction. Sodium lactate had no significant effects in the plus-maze but the highest dose of isoproterenol (0.6 mg/kg) reduced the % of time spent on the open arms and yohimbine (2.5 mg/kg) reduced both the % of entries made onto the open arms and the % of time spent on the open arms. None of the agents produced any behavioural indications of panic.

Our tests appear relatively insensitive to clinical pro- and anti-panic drugs. Effects were observed with the triazolobenzodiazepines and yohimbine (weak anxiolytic and anxiogenic profiles, respectively), however, these drugs are also known to have effects on anxiety levels in normal subjects.

127.3 INCREASED ANXIETY IN BENZODIAZEPINE WITHDRAWAL IS LINKED TO TOLERANCE TO ANXIOLYTIC EFFECTS. Helen A.Baldwin*,Kari Aranko* and Sandra E.File. MRC Neuropharmacology Research Group, The School of Pharmacy, Brunswick Square, London WC1N 1AX. Dept Pharmacology & Toxicology, University of Helsinki.

In a recent paper, (Lader M. and File S.E., Psychol Med, 1987) it was proposed that tolerance and withdrawal were both manifestations of a common mechanism of benzodiazepine dependence. In order to test this hypothesis rats were chronically treated with chlordiazepoxide (CDP) for 5 or 20 days and then tested for tolerance to the anxiolytic effects of a probe dose of CDP (5 mg/kg) in two animal tests of anxiety. Two days after the tests for tolerance the same animals were tested for withdrawal responses, 24-hours after the last dose of CDP.

In the social interaction test, after 5 days of pretreatment with CDP (5 mg/kg/day), there was a significant increase in the time spent in social interaction compared with control in rats tested with the probe dose of CDP. In the elevated plus-maze test, after 5 days of pretreatment with CDP (5 or 20 mg/kg/day), there were significant increases in the number of entries onto, and the time spent on, the open arms, in rats tested after the probe dose of CDP. Thus after 5 days of pretreatment there was no tolerance to CDP's anxiolytic effects. There were no indications of spontaneous withdrawal when the rats were tested, undrugged, for withdrawal responses.

After 20 days of pretreatment there was significant tolerance to the anxiolytic effects of CDP in both tests. There was a decrease in the time spent in social interaction ($p<0.10$) when the rats were tested, undrugged, for withdrawal responses. Similarly in the elevated plus-maze, there were significant decreases in the number of entries onto, and the time spent on, the open arms, by these rats.

In summary, after 5 days of chronic treatment there was no tolerance to the anxiolytic effects of CDP in either test and no indication of spontaneous withdrawal in these rats. However after 20 days of pretreatment there was tolerance to the anxiolytic effects of CDP and evidence for spontaneous withdrawal in both tests.

127.4 EFFECTS OF ANTIPSYCHOTIC DRUGS ON ISOLATION INDUCED INTRASPECIES AGGRESSION BY MALE MICE: A SCREEN FOR ANXIOLYTIC DRUGS. B. A. McMillen, A. H. Song* and E. A. DaVanzo*, Dept. of Pharmacology, School of Medicine, East Carolina U., Greenville, NC 27858.

Inhibition of intraspecies aggression is a sensitive behavioral model for screening potential anxiolytic drugs [1,2]. Several different aryl-piperazine antianxiety drugs potently inhibit aggressive behavior (e.g. buspirone ED50 = 3.5 mg/kg), but are weak or ineffective in other behavioral anxiolytic screens. However, classical antipsychotic drugs are known to be active in this screen [3], which may limit its usefulness. The following experiments were performed to test whether drugs that inhibit dopamine (DA) receptors have antiaggressive activity other than that secondary to akinesia induced by striatal DA receptor blockade.

Male mice (15-18 g) were housed in isolation for 3 weeks and then trained to attack an intruder (group housed) mouse. Only those mice consistently making a sustained attack within 60 sec were used for drug testing. Mice were re-tested 30 min after i.p. injection of drugs or vehicle and 180 sec used as criterion for no attack. Mice were observed for orienting movements and signs of sedation. Additional mice were tested on a rotorod for akinetic effects of drugs. Drugs tested were: haloperidol, sulpiride, SCH 23390, Ro 22-1319, BW 234U, BMY 14802 and (-)-3-PPP. Haloperidol at 1.0 mg/kg had marked akinetic effects, but only 3 of 8 mice were inhibited from attack. (-)-Sulpiride or RO 22-1319 had similar effects at 30 or 3.0 mg/kg, respectively, but the other drugs were ineffective except in large doses. Only BMY 14802 or BW 234U could inhibit aggression at large doses without inducing major motor dyskinesias, but neither of these 2 drugs bind to DA receptors. These data suggest that classical antipsychotic drugs inhibit aggression secondary to induction of akinesia: i.e. a rigid mouse cannot fight. SCH 23390, a D_1 preferring antagonist (in doses less than 0.5 mg/kg), does not inhibit aggression or motor activity in the low dose range. This result suggests that inhibition of D_1 receptors is without anti-aggressive activity. Thus, once the akinetic effects of drugs are accounted for, neuroleptics do not interact in the intraspecies aggression model for screening of antianxiety drugs. These results, combined with previous data which indicate that aryl-piperazines are potent inhibitors of intraspecies aggression, suggest that this animal model is useful for studying the effects of novel anxiolytic drugs.

1. B A McMillen et al., Naunyn-Schmeideberg's Arch Pharmacol 335:(in press), 1987.
2. B Olivier et al., Prog Clin Biol Res 167:137-156, 1984.
3. J P DaVanzo et al., Psychopharmacol 9:210-219, 1966.

127.5 REVERSAL OF NEUROLEPTIC INDUCED CATALEPSY BY NOVEL ARYL-PIPERAZINE ANXIOLYTIC DRUGS. S. M. Scott*, E. A. DaVanzo* and B. A. McMillen (Spon. R. B. Graham), Dept. of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

Buspirone and gepirone, aryl-piperazine anxiolytic drugs, reverse catalepsy induced either by dopamine (DA) receptor blockade or DA depletion (J Neural Trans 57:255 1983) by an unknown mechanism. Both drugs are agonists at 5-hydroxytryptamine (5HT)1a pre- and postsynaptic receptors, which may relate to the antiaggressive (or anxiolytic) effect of these drugs (N-S Arch Pharmacol 335: in press). To determine whether a 5HT action is involved in catalepsy reversal, aryl-piperazine drugs (and other 5HT agonists) and 5HT antagonists were tested for interactions with haloperidol-induced catalepsy assessed on a 4 point scale.

Ipsapirone and 8OH-DPAT reversed haloperidol (1.0 mg/kg s.c.) induced catalepsy. Simple aryl-piperazines, m-Cl-phenylpiperazine (mCPP) and trifluoromethylphenylpiperazine (TFMPP) were ineffective. Antagonists of 5HT receptors, methysergide or methiothepin, are known to potentiate the antiaggressive effect of gepirone or DPAT, but neither inhibited nor potentiated either drug. The beta-adrenoceptor antagonists, propranolol and pindolol, which have 5HT1a antagonist activity did not alter 8OH-DPAT reversal of haloperidol induced catalepsy. Fluprazine, an aryl-piperazine with low affinity for 5HT receptors, did not reverse catalepsy. The results demonstrate that DPAT and alkyl-substituted aryl-piperazine drugs reverse catalepsy in proportion to affinity for 5HT1a receptor binding in hippocampus (Table 1).

	Relative Potency*	IC50 [3H]-5HT nM
8OH-DPAT	0.11	1
gepirone	0.57	58
buspirone	1.0	10
ipsapirone	3.9	20
BMY 14802	13.5	170
fluprazine	no effect	1,429
TFMPP	no effect	100
mCPP	no effect	177

*compared to buspirone = 1.0

Although these data point to the 5HT1a site as the site for catalepsy reversal (either pre- or postsynaptic receptors), the inability of antagonists to interact suggests that another site may be involved. Additional rats had 25 mg/kg s.c. morphine injected to induce a different form of rigid posturing, which is presumably mediated by opiate receptors outside the corpus striatum. Neither buspirone nor gepirone could alter morphine-induced rigidity: thus, their effect on the rat extrapyramidal system did not interact with opiate induced rigidity.

127.6 **THE EFFECTS OF BUSPIRONE AND DIAZEPAM ON RAT CORTICOSTERONE LEVELS.** G. K. Matheson, D. Gage, G. White, V. Dixon & D.Gipson. Neurobiology Laboratory. Indiana University School of Medicine. Evansville, IN 47714

The effects of the anxiolytic agents buspirone and diazepam on the hypothalamic-pituitary-adrenal axis (HPAA) were studied. The HPAA effects were indicated by changes in plasma corticosterone (CS) concentrations obtained from trunk blood. CS levels were measured fluorometrically. The rats were maintained in a temperature and humidity controlled environment. The room lights were on from 0600 - 1800 hours. Both drugs were administered in normal saline. Plasma CS levels were measured 1/2, 1, 2, and 4 hours after drug administration (i.p.). The anxiolytic effects on the HPAA were studied in the mid-morning (0930-1130 hrs) when activity in the HPAA is normally low and in the afternoon (1400-1600 hrs) when the HPAA activity is high. Control levels of CS were measured at 11.5 ± 1.6 S.E. µg/dl (22) during the morning and 20.9 ± 1.2 µg/dl (28) in the afternoon. Animals subjected to a stress, i.e. angular rotation produced by spinning the rat at 30-60 rpm for one minute, 15 minutes before decapitation had morning stress control levels of CS 297% (45.6 ± 2.2 [26]) over unstressed controls. Afternoon stress control levels were increased by 138% to 49.7 ± 2.3 mg/kg (31). At high doses (10 mg/kg) both drugs produced effects that were measurable for at least one hour. The data in table 1 represents the combined 1/2 and 1 hour values. Buspirone had a greater facilitating effect on the HPAA than did diazepam (table 1). In addition, buspirone had a higher maximum effect on CS levels (477% increase after 50 mg/kg) than diazepam (288%). However, they are equipotent since the ED_{50} for buspirone was calculated to be 3.6 mg/kg (8.6 mmol/kg) and for diazepam it is 2.5 mg/kg (8.7 mmol/kg). From the stress data one sees that stress effects and buspirone actions on the HPAA were additive at the higher doses, while stress and diazepam effects were not additive.

Table 1. Percent change in plasma corticosterone levels.

Drug		Buspirone		Diazepam	
Dose (mg/kg)		1	10	1	10
Unstressed	AM	75*	328*	12	265*
Unstressed	PM	-5	166*	14	80*
Stressed	AM	10	20*	13	3
Stressed	PM	5	22*	2	2

* Statistically significant ($P \leq 0.05$).

127.7 TOLERANCE AND WITHDRAWAL TO THE ANTI-CONFLICT PROPERTIES OF DIAZEPAM FOLLOWING CHRONIC ADMINISTRATION IN RATS. R. S. Rock* and R. J. Barrett* (SPON: M. A. Blackshear). Dept. of Psychology Vanderbilt Univ. and VA Medical Center, Nashville, TN 37203

Although recent clinical evidence indicates that both tolerance and withdrawal occur to the anxiolytic properties of benzodiazepines following chronic administration, animal studies using the Geller and Seifter conflict paradigm have reported either no change or enhanced anti-conflict effects during long term administration. Using a modification of the Geller and Seifter paradigm, the present study further investigated this apparent discrepancy.

Two groups of rats (n=8) were trained to respond on a VI=2' schedule during 30 min test sessions. After stable VI responding, the conflict component was added. This consisted of three 2 min periods, signaled by a 70 db, 1000 cps tone, during which every response produced both food reinforcement and shock. The shock intensity started at .05 mA (0.5 sec duration for all shocks) and increased by .05 mA after every other response made during a tone presentation. The two matched groups were then administered either saline or diazepam (2.5 mg/kg) 30 min prior to testing for 9 consecutive days. On the 10th day no injections were given to animals in either group. On the 11th day both groups were injected with 2.5 mg/kg diazepam prior to testing.

The results showed that intially, diazepam significantly increased responding during the conflict period, but by the 8th day tolerance had developed to these anti-conflict properties. When both groups were tested without drug on the 10th day, the diazepam animals showed a significant increase in conflict, i.e. reduced responding during the tone period. On the 11th day when both groups were injected with diazepam, the chronic saline animals made more conflict period responses than the chronic diazepam animals, but when compared to the previous day's baseline the drug produced comparable changes in both groups.

The results show that tolerance develops to the anti-conflict properties of diazepam following chronic administration. Furthermore, the increased conflict observed following drug termination suggests a withdrawal state analogous to the rebound increase in anxiety observed in humans following discontinuation of benzodiazepine use.

127.8 THE BENZODIAZEPINE RECEPTOR AND HABITUATION. D.H. Aitken*, S.R. Bodnoff, B. Suranyi-Cadotte, R. Quirion, and M.J. Meaney (SPON: S. Young). Douglas Hospital Research Center, Dept. of Psychiatry, McGill Univ., Montreal, Canada, H4H 1R3.

Diazepam is effective in reducing the latency of food-deprived animals to begin eating in a novel environment. Another, non-pharmacological method of reducing fear of novelty is to repeatedly expose animals to the novel environment; animals habituate to the cues associated with novelty and no longer manifest fear. In this sense, the effects of diazepam mimic the process of habituation. Thus, we examined the possible role for the benzodiazepine receptor (BZR) in habituation learning.

Adult, male Long Evans rats were exposed to large plexiglas cages covered with beta chips and 12 evenly-spaced food pellets, for 0 or 4 days. Animals were food-deprived for 48 h and 1 h prior to testing, half of the animals in the 4-day pre-exposure group received a single i.p. injection of the anxiogenic beta-carboline FG-7142 (10 mg/kg). Pre-exposure to the testing apparatus significantly reduced the latency for animals to begin eating relative to controls. FG-7142, completely reversed the effects of habituation. In a second experiment, animals were exposed to the testing apparatus for 0,1,4, or 7 days. Half of the Day 7 animals received a single i.p. injection of the BZR antagonist, Ro15-1788 (20 mg/kg) prior to testing. A final group of non-preexposed animals received a single i.p. dose of diazepam (2 mg/kg). Both 4 and 7, but not 1, day of exposure produced latencies to begin eating that were significantly different from controls, but not from diazepam-treated animals. A single dose of Ro15-1788 was able to completely reverse the anxiolytic property of 7 days of pre-exposure to the test environment. In a separate series of experiments we examined [3H]flunitrazepam binding in post-natally handled (H) and non-handled (NH) rats who differ in their response to novelty. H animals exhibited significantly shorter latencies to begin eating in a novel environment and greater [3H]flunitrazepam binding than did NH animals. Taken together, these data indicate that the BZR plays an integral role in the anxiolytic property of habituation learning and mediate individual differences in response to novelty.

127.9 **BEHAVIORAL WITHDRAWAL FROM CHRONIC ORAL EXPOSURE TO MIDAZOLAM.** **J. TIZZANO, M. HUGGINS*, P. HOPKINS*, J. YARBOUROUGH* AND M. KALLAM. TOXICOLOGY DIVISION, LILLY REASEARCH LABS, GREENFIELD, IN 46140 AND DEPARTMENTS OF PSYCHOLOGY AND PHARMACOLOGY, UNIVERSITY OF MISSISSIPPI, UNIVERSITY, MISSISSIPPI 38677.**

The CNS depressant effects of acutely administered benzodiazepines are well documented (Greenblatt and Shader, 1974), but tolerance to these effects develops rapidly. The benzodiazepines share common pharmacological properties, but differ with regard to various kinetic parameters. Midazolam (MZ) has a pharmacological potency similar to diazepam (DZ) and a broad therapeutic range. However, MZ has a rapid onset of activity and due to rapid metabolic inactivation, a short duration of action. The pharmacokinetics of DZ consists of a rapid absorption followed by distribution and a slow elimination. The present study examined the acute, chronic and withdrawal effects of DZ and MZ on schedule controlled behavior. Sixteen male rats were trained to lever press for sweetened condensed milk on a differential reinforcement of low rates (DRL)-20 schedule. After response rates on the DRL schedule stabilized, animals were randomly assigned to either a DZ or MZ condition (n=8). Initial dose effect data were determined for DZ and MZ (0.0, 1.0, 3.0, 10.0 and 17.5 mg/kg IP.). Animals were then treated with MZ (100 mg/kg/day) in their drinking water for 21 days. Following the period of chronic drug exposure, dose effect functions were redetermined. To investigate the withdrawal effects of MZ, the drug solution was removed and the animals were tested for 10 days on the DRL schedule. Response rate and duration of response were collected via laboratory computer system. The acute effects of DZ and MZ produced a dose dependent decrease in response rate and a dose dependent increase in response duration. When dose effect functions for DZ and MZ were redetermined after 21 days of chronic exposure to midazolam, tolerance was not observed for either drug on the rate measure. However, a non-parallel shift to the right of the dose response curve was obtined with the duration measure indicating the development of tolerance. When midazolam was withdrawn from the drinking water, there was an increase in response rate and a decrease in response duration which began on the first day of withdraval and continued until the fifth day. Eventually response rates and response duration returned to control levels seen before MZ was introduced into the drinking water. These findings suggest that multiple behavioral measures are important in assessing the degree of tolerance obtained with DZ and MZ.

127.10 EFFECTS OF ANXIOLYTIC AGENTS ON CONFLICT BEHAVIOR IN ANIMALS WITH SEPTAL OR AMYGDALOID LESIONS. E. Yadin, E. Thomas, and C.E. Strickland*. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Rats were trained on a drinking conflict test in which unpunished and punished periods were alternately presented. The number of licks was recorded during each time segment which was 2 min long and repeated twice, for a total session time of 8 min. The punished period was signaled by a tone, during which a mild shock (0.2-0.3 ma, 1 sec duration) was delivered to the floor grids after every fifth lick. Animals were pretrained to a stable baseline of suppression of water-licking during the punished period and then were subjected to bilateral electrolytic lesions of the septum, the amygdala, or sham lesions. Beginning at 5 days post-lesion, animals were retested in the conflict paradigm, during 12 consecutive sessions. They were then tested on the conflict test after having been intraperitoneally injected with vehicle, chlordiazepoxide (10 mg/kg, 20 mg/kg), or buspirone (2 mg/kg).

Animals with amygdaloid lesions showed a strong release of the suppression during the signaled, punished period, an effect that diminished over successive sessions. Neither septal- nor sham-lesioned animals showed such a release of the punished response. Both doses of chlordiazepoxide produced a large increase in drinking during the punished period in the amygdala-lesioned animals while neither dose released punished behavior in the septal-lesioned animals. Only the higher dose produced a release of the punished response in the sham animals. Animals in both lesion groups showed suppressed drinking during the unpunished time period and no release in the punished segment after injection of 2mg/kg of the nonbenzodiazepine agent buspirone.

The results would appear to argue against a role for the amygdala as a primary site of action for the anxiolytic activity of benzodiazepines. The combination of release from punishment as a result of amygdaloid lesions and the enhancement of the drug effect in amygdala-lesioned animals is consistent with an interpretation that amygdala lesions remove an inhibitory influence upon a structure which is involved in the active inhibition of fear. The septum might be such a structure. The fact that we observed a diminution of the anxiolytic effects in septally lesioned animals is consistent with such a proposed role for the septum.

127.11 BEHAVIORAL AND NEUROCHEMICAL EVIDENCE FOR SEROTONERGIC MODULATION BY THE ANXIOGENIC β-CARBOLINE FG 7142. N.J. LEIDENHEIMER, B.K. YAMAMOTO AND M.D. SCHECHTER. Dept. of Pharmacology, Northeastern Ohio Universities Coll. of Med., Rootstown, Ohio 44272

FG 7142 (β-carboline-3-carboxylic acid methyl amide) is a β-carboline which binds with high affinity to the benzodiazepine receptor. It has been classified as an inverse agonist at this receptor due to its ability to produce anxiety in several species including man. The present behavioral and neurochemical study sought to investigate the possible serotonergic modulation by FG 7142.

Eight male rats were trained to discriminate between the stimulus effects of FG 7142 (5.0 mg/kg, i.p.) and its vehicle. Testing of various doses of FG 7142, i.e., 5.0, 2.5 and 1.25 mg/kg, produced 100, 78.6 and 42.9% FG 7142-appropriate responding, respectively, and yielded an ED50 of 1.41 mg/kg. Test doses of the serotonergically-active drug tetrahydro-β-carboline (THBC) at 15.0, 10.0 and 5.0 mg/kg produced 92.9, 75.0 and 43.8% FG 7142-appropriate responding.

A separate group of ten male rats were administered either FG 7142 (30 mg/kg) or its vehicle and decapitated thirty minutes later. Their brains were rapidly removed, frozen and hippocampi dissected out. Hippocampal tissue was analyzed for serotonin (5-HT) and 5-hydroxyindoleacetic acid (5HIAA) content by HPLC with electrochemical detection. Tissue indoles are expressed as ng/mg protein (± SEM).

	Vehicle	FG 7142
5-HT	0.74(.06)	1.06(.14)*
5HIAA	1.35(.05)	1.67(.18)

*Significantly different from vehicle, $p<0.05$.

In the present study the discriminative stimulus produced by FG 7142 generalized to THBC. It is unlikely that this generalization to THBC is mediated via the benzodiazepine receptor since THBC shows little affinity for this receptor. Since THBC has been shown to increase 5-HT transmission and FG 7142, as shown here, increases hippocampal levels of 5-HT, it is possible that the similarities of the FG 7142 and THBC cues are based on their abilities to enhance 5-HT transmission.

127.12 **RAUBASINE, A NON-BENZODIAZEPINE COMPOUND WITH ANXIOLYTIC PROFILE .** B Delbarre, G.Delbarre and F.Calinon*.Faculté de Médecine, 37000 Tours, FRANCE.

The efficiency of BZD is established in four distinct behavioural effects : anticonvulsivant, muscle relaxant, anxiolytic and sedative-hypnotic properties. Recently the various actions of BZD have highlighted the role of noradrenergic mechanisms.To elucidate the role of noradrenergic system we have compared on the classical test for BZD, clonidine an alpha-2 presynaptic agent and raubasine an alpha-1 blocking agent which interacts directly at BZD sites.

To test the role of adrenoceptor in the sedative activity of BZD we have used the sleeping time in chicken: sedative effect of clonidine (0.750 mg.kg-1), diazepam (3mg.kg-1), chlordemethyldiazepam (CDD) (4mg.kg-1) and alprazolam (0.750 mg.kg-1) are antagonized by rauwolscine and not by raubasine. Similarly, in the wire test used to test myorelaxant activity of BZD, rauwolscine (0.1 to 0.5 mg.kg-1) antagonizes effect of diazepam, CDD, alprazolam and clonidine. Raubasine (5-10 mg .kg-1) is ineffective alone or to prevent sedative activity of clonidine or BZD. Most of the clinically used BZD display some degree of anticonvulsivant activity . Furthermore, the relative potencies of various benzodiazepines in protecting mice against PTZ-induced seizures correlate well with their relative clinical potencies as anxiolytics. On this test raubasine (5-10 mg.kg-1) but not clonidine (0,2 - 3 mg.kg-1) antagonizes PTZ-induced seizures. In mice, convulsive properties of harmane and yohimbine potent endogenous inhibitors of BZD receptor binding, are antagonized by diazepam (1 mg.kg-1), CDD (1 mg.kg-1) alprazolam (0,150 - 0,30 mg.kg-1) and by raubasine (5 to 10 mg.kg-1) wich interacts directly at BZD sites. Clonidine (3 mg.kg-1) is ineffective.These results may support the hypothesis that harmane and yohimbine specifically act at the BZD receptor. On this test, BZD act at very small doses.

Sedative and myorelaxant activities of BZD and clonidine antagonized by rauwolscine, a presynaptic alpha-2 receptor blocker, may be explained by an action of BZD on alpha-2 presynaptic adrenoreceptor. Convulsive activity of PTZ , which correlates well relative clinical potency of drug as anxiolytic, is antagonized by raubasine and BZD but not by clonidine. Moreover, convulsive activity of yohimbine and harmane with anxiety like effect in humans and animals are antagonized by small doses of BZD and by raubasine but not by clonidine.

In conclusion, sedative and myorelaxant activity of BZD may be explained by an interaction with alpha-2 receptors and anxiolytic activity with alpha-1 adrenoceptors.

127.13 **IS THE 5-HT$_{1A}$ AGONIST, 8-OH-DPAT, AN ANXIOLYTIC?** D. N. Johnson, R. T. Ruckart*, B. F. Kilpatrick, J. H. Porter, H. F. Villanueva*. A. H. Robins Co., Research and Development Division, and Virginia Commonwealth University, Richmond, VA 23261-6609.

Although GABA is involved in the mechanism of action of the benzodiazepines, the newer nonbenzodiazepine anxiolytics (buspirone, ipsapirone) have been proposed to act via a serotonergic (5-HT$_{1A}$) mechanism. Buspirone and ipsapirone are potent partial 5-HT$_{1A}$ agonists: RU-24969, a potent 5-HT$_{1B}$ agonist, does not have anxiolytic activity.

Recently, 8-OH-DPAT (8-hydroxy-2[di-n-propylamino]tetralin) has been reported to be a potent and selective 5-HT$_{1A}$ agonist (Gozlan et al., Nature 305:140, 1983). This study was undertaken to determine if 8-OH-DPAT possessed anxiolytic activity as determined by standard in vivo and in vitro anxiolytic testing procedures.

8-OH-DPAT affected serotonergic systems in displacement studies in vitro, with IC$_{50}$ values of 1.2 nM for 5-HT$_{1A}$ and 1,600 nM for 5-HT$_2$ receptors. It was inactive (>10,000 nM) against D_2, H_1, α_1, GABA$_A$, and BZD receptors. In whole animal studies, 8-OH-DPAT was a potent serotonergic agent, producing signs of neurotoxicity (serotonin syndrome) in rats in doses as low as 1 mg/kg, IP. As has been reported, 8-OH-DPAT is not as potent in mice, requiring approximately 10 mg/kg to produce a similar effect. Anticonvulsant properties of 8-OH-DPAT were determined in mice against clonic convulsions induced by pentylenetetrazol (80 mg/kg, SC) and against tonic seizures induced by maximal corneal electroshock (34 mA, 60 Hz, 2 msec pulse width for 200 msec). Unlike the benzodiazepines, but like buspirone, 8-OH-DPAT (up to 10 mg/kg, IP) was without anticonvulsant activity.

Anxiolytic testing in rats was assessed using the Vogel procedure (Vogel et al., Psychopharmacologia 21:1, 1971) and using the Geller and Seifter conflict test (Geller and Seifter, Psychopharmacologia 1: 482, 1960). In the Vogel procedure, 8-OH-DPAT was ineffective in increasing the number of shocks delivered to the rat in doses of 0.0625 to 1.0 mg/kg, IP. This is similar to the lack of effect of buspirone reported previously in the Vogel procedure (Porter and Johnson, Neuroscience Abs., 11:426,1985). However, when 8-OH-DPAT and buspirone were administered concomitantly, a significant increase in shocks delivered was seen.

In drug-experienced rats (Geller & Seifter conflict test), 8-OH-DPAT was more potent in producing the serotonin syndrome. Doses of 0.125 and 0.25 mg/kg, IP, produced the characteristic stereotypy and abolished, or significantly reduced, lever pressing for food reward during conflict and reward sessions. Still lower doses (0.0125 to 0.0625 mg/kg, IP) were without effect in altering response rates. Oral administration of 8-OH-DPAT (0.1 to 10 mg/kg) similarly failed to indicate any anxiolytic activity. Unlike the results in the Vogel procedure, concomitant administration of 8-OH-DPAT and buspirone did not result in an increase in punished responding in the conflict test.

Thus, in animal models, the 5-HT$_{1A}$ agonist, 8-OH-DPAT, does not show anxiolytic activity.

127.14 NEUROCHEMICAL PROPERTIES OF THE NOVEL ANXIOLYTIC ICI 190,622. B.A. Meiners, R.A. Keith*, V. Justice*, T. Bare*, and A.I. Salama. Department of Pharmacology, Stuart Pharmaceuticals, A Division of ICI Americas Inc., Wilmington, DE 19897.

ICI 190,622 (N-allyl-4-aminopyrazolo[3,4-b]pyridine-5-carboxamide) is a non-benzodiazepine which may have anxiolytic activity (see Patel et al., Neuroscience Abst., 1987). The present studies sought to characterize the interaction of ICI 190,622 with the GABA/benzodiazepine receptor complex and with other receptors. ICI 190,622 competitively inhibited [^{3}H]flunitrazepam (FLU) binding in cerebral cortex (CTX) with an IC$_{50}$ of 81 nM and was 4.3-fold more potent in the cerebellum (CB) (IC$_{50}$ = 19 nM). In contrast, diazepam (DZP) showed similar affinities in both regions (CTZ = 7 nM and CB = 9 nM). GABA caused a 1.2-fold increase in the affinity of ICI 190,622 for the benzodiazepine binding site (GABA shift), which was a typical effect with BZ agonists. However, the GABA shift of DZP was larger (1.6). Photoaffinity labeling receptors with FLU causes a decrease in affinity for agonists but not for antagonists (photo shift). As expected, there was a decrease in the affinity of ICI 190,622 (2.0-fold). Consistent with the GABA shift, photo-labeling caused a greater change in the affinity of DZP (34-fold). It has been reported that benzodiazepine agonists increase the binding of [^{3}H]GABA, and antagonists can block this effect (Meiners and Salama, Eur. J. Pharmacol. 119:61-65, 1985). ICI 190,622 (500 µM) enhanced GABA binding (30%), and this enhancement was blocked by the antagonists RO 15-1788 and ethyl-beta-carboline carboxylate. Ex vivo experiments were conducted to estimate the bioavailability and duration of ICI 190,622. Following oral administration, ICI 190,622 displaced [^{3}H]FLU from cerebellum more potently than DZP (ED$_{50}$ = 3 and 6 mg/kg, respectively, 1 hour after administration). Other ex vivo experiments suggested that the duration of action of ICI 190,622 and DZP were similar. In vivo, as well as in vitro, ICI 190,622 was more potent in cerebellum than in cortex. ICI 190,622 (10 mg/kg, 1 hour) did not alter the steady-state levels of norepinephrine, dopamine, 5-hydroxytryptamine or 5-hydroxyindoleacetic acid in whole brain as measured by HPLC with electrochemical detection. This may suggest that the compound and its metabolites are not monoamine oxidase inhibitors. At 10 µM, ICI 190,622 had no significant effects on the responses of alpha-1, alpha-2, beta-1, beta-2, histamine-1, histamine-2, 5HT-2, and muscarinic receptors as measured in a variety of isolated tissue preparations. In conclusion, ICI 190,622 is a potent agonist at the benzodiazepine receptor and in contrast to DZP is selective for the type 1 (cerebellar) benzodiazepine binding site both in vitro and in vivo. Finally, ICI 190,622 has little activity at sites other than the benzodiazepine binding site.

127.15 ICI 190,622: NEUROPHARMACOLOGICAL PROFILE OF A NOVEL POTENTIAL NON-BENZODIAZEPINE ANTIANXIETY AGENT. J.B. Patel and J.B. Malick. Stuart Pharmaceuticals, A Division of ICI Americas Inc., Wilmington, DE 19897.

ICI 190,622, a pyrazolopyridine carboxamide, demonstrated potent anxiolytic activity in rats, squirrel monkeys, and dogs. In the modified Vogel conflict test in rats, ICI 190,622 (MED = 0.8 mg/kg, p.o.) was at least five times as potent as diazepam (MED = 5.0 mg/kg, p.o.). The duration of anxiolytic activity of ICI 190,622 and diazepam was comparable in this test. In the squirrel monkey and beagle dog conflict tests, ICI 190,622 also exhibited an anxiolytic profile, and it was shown to be approximately one-half as potent as diazepam. Like benzodiazepines (BZP), tolerance did not develop to its anticonflict and anticonvulsant activities following 12 consecutive days of oral treatment. Furthermore, ICI 190,622 demonstrated potent activity as an antagonist of metrazole-induced (ED$_{50}$ = 1.0 mg/kg, p.o.) and bicuculline-induced (ED$_{50}$ = 1.4 mg/kg, p.o.) seizures in rats.

In rats, the sedative liability index (index = rotorod (ataxia) ED$_{50}$/anticonflict-MED) for diazepam is 2.0 whereas it was 31.4 for ICI 190,622; thus, ICI 190,622 should possess a wide safety margin in man. Unlike BZP, ICI 190,622's interaction with ethanol is significantly less pronounced compared to that of diazepam. Neurochemically, ICI 190,622 is a potent (Cortex-IC$_{50}$ = 81 nM and cerebellum IC$_{50}$ = 19 nM) displacer of ^{3}H-flunitrazepam from its binding sites in brain (Meiners et al., this meeting).

In summary, ICI 190,622 is a novel anxiolytic agent that offers significant advantages over BZP.

127.16 SM-3997: A NON-BENZODIAZEPINE ANXIOLYTIC WITH POTENT AND SELECTIVE EFFECTS ON SEROTONERGIC NEUROTRANSMISSION. J. Heym, E. E. Mena and P. A. Seymour, Central Research Division, Pfizer Inc., Groton, CT. 06340.

Buspirone is a clinically efficacious anxiolytic which does not act by a conventional benzodiazepine mechanism of action. In fact, buspirone appears to exert its most impressive effects by a direct action at brain serotonin receptors. However, in addition to its serotonergic actions, buspirone has been shown to possess moderate affinity for D2 dopamine receptors and to produce pronounced increases in forebrain levels of the dopamine metabolites HVA and DOPAC. Efforts to develop second generation agents of this type have focussed on compounds that retain the serotonergic properties of buspirone but reduce the dopaminergic effects.

SM-3997 [3aα, 4α ,5 ,7α, 7aα - hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1H-isoindole-1,3-(2H)-dione] is one of a new group of non-benzodiazepine compounds which are characterized by potent and specific effects on brain serotonergic systems. This compound shows anticonflict activity in a modified Vogel paradigm with a minimal effective i.p. dose of 1 mg/kg. SM-3997 binds with high affinity to 5HT1A receptors (Ki = 30 nM) and possesses no substantial affinity (Ki > 1 µM) for 5HT1B, 5HT2, α adrenergic, D2 or benzodiazepine binding sites. Serotonergic neuron firing is potently inhibited by SM-3997 (ID50 = 4 µg/kg i.v.) indicating that it is a very good agonist for somatic 5HT autoreceptors located on serotonergic soma in the dorsal raphe nucleus. In contrast, the behavioral effects elicited in rats by the 5HT1A agonist 8-OH-DPAT are not fully reproduced by SM-3997 and pretreatment with 3.2 mg/kg s.c. of SM-3997 antagonizes the recipricol forepaw treading elicited by a subsequent 8-OH-DPAT challenge. Finally, in contrast to the large increases in dopamine metabolite levels produced by buspirone (200 - 300%), equivalent doses of SM-3997 have only a small effect on these biochemical measures.

These data support the contention that SM-3997 should possess anxiolytic efficacy without producing the side effects associated with benzodiazepine use. It is hypothesized that the anxiolytic profile of this compound stems from its action on serotonergic neurotransmission in the brain. The relatively weak dopaminergic effects of SM-3997 predict that clinical manifestations of altered dopamine function will not occur with this new drug.

127.17 THE EFFECTS OF CHLORDIAZEPOXIDE ON COMPARABLE RATES OF PUNISHED AND UNPUNISHED RESPONDING. S.I. Dworkin, M. J. Blake and S. Izenwasser. Departments of Psychiatry and Pharmacology, Louisiana State University School of Medicine, Shreveport, LA 71130.

Drugs with anxiolytic properties generally increase punished responding. Drug related increases in low rates of behavior not suppressed by punishment have also been reported after the administration of anxiolytic compounds. Thus, these drugs are similar to other behaviorally active agents in that baseline rates of responding can be an important determinant of the behavioral effects of a drug. Therefore, it is important to control for the baseline rate of responding in studies investigating punishment-specific effects of pharmacological agents. Several other variables including temporal patterns of responding and reinforcement density can also alter the behavioral effects of a drug. Procedures developed to determine specific effects of drugs should attempt to control for the potential influences of these factors.

Littermate pairs of male F-344 rats responded under a procedure which generated comparable rates of punished and unpunished responding. One subject of each pair was maintained on a random-ratio (5-100) schedule of food presentation. The interreinforcement intervals from this subject were used to generate a yoked variable-interval schedule of reinforcement for a littermate. A random-ratio (25-200)schedule of electric footshock was then added to the random-ratio schedule of food presentation. Thus, the subject on the ratio schedule received both food and shock, while the rat on the yoked interval schedule received only food presentations. The addition of the punishment contingency resulted in similar rates and patterns of responding by both the punished and yoked unpunished rats. The effects of chlordiazepoxide (3.0-30 mg/kg, i.p.) were determined after stable baselines of responding had been obtained.

Chlordiazepoxide increased punished responding with maximal effects occurring at the 5.6 mg/kg dose. There was, however, little effect of the drug on unpunished responding. These findings suggest the rate increasing effects of chlordiazepoxide can be punishment-specific. Supported by NIH 2 S07 RR05822.

127.18 PUNISHMENT-SPECIFIC CHANGES IN REGIONAL BENZODIAZEPINE RECEPTOR POPULATIONS. M. J. Blake, S. Izenwasser, M. McNulty*, N. E. Goeders and S. I. Dworkin. Departments of Psychiatry and Pharmacology, Louisiana State University School of Medicine, Shreveport, LA 71130.

Benzodiazepines have been shown to increase low rates of responding maintained by several operant schedules of reinforcement. Additionally, these drugs can attenuate the effects of punishment resulting in significant increases in punished responding. Chlordiazepoxide increases punished responding at doses that have little or no effect on similar low rates of unpunished responding. This specific effect on punished responding is most likely the result of alterations in central brain mechanisms that mediate the effects of punished responding. Since the pharmacological effects of benzodiazepines are suggested to result from the binding of these drugs to stereospecific binding sites, changes in these sites may be involved in the punishment-specific effects of these drugs.

Littermate groups of three male F-344 rats responded under a procedure which generated comparable rates of punished and unpunished responding. One subject of each group was maintained on a random-ratio (5-100) schedule of food presentation. The interreinforcement intervals from this subject were used to generate a yoked variable interval schedule of reinforcement for one of its littermates. A random-ratio (25-200) schedule of electric footshock was then added to the random-ratio schedule of food presentation. The addition of the punishment contingency resulted in similar rates and patterns of responding by both punished and yoked unpunished rats. The third animal of each group received noncontingent food and shock yoked to the random-ratio animals. Animals were subjected to the respective treatments for 30 sessions followed by decapitation and dissection of selected brain regions.

Saturation studies were carried out with [^{3}H]Ro15-1788 to characterize changes in benzodiazepine receptors (ie., K_d and B_{max} values) resulting from the treatment conditions. Benzodiazepine receptor binding was significantly increased in the striatum in both the punished and yoked-shock animals as compared to the unpunished controls. Furthermore, the yoked-shock animals had significantly higher binding than the punished animals. In contrast, there were no significant differences in B_{max} in the frontal cortex or hippocampus, nor were there differences in K_d values in any brain region examined. The site-specific alterations of these receptors may underlie the differential effects of benzodiazepines on punished and unpunished responding. Supported by NIH 2 S07 RR05822.

127.19 EFFECTS OF INTRAVENOUS BUSPIRONE AND 8-OH-DPAT ON THE ACTIVITY OF SEROTONERGIC DORSAL RAPHE NEURONS IN FREELY MOVING CATS. C.A. Fornal, L.O. Wilkinson, and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544

The therapeutic efficacy of non-benzodiazpine anxiolytic agents such as buspirone may involve an action on brain serotonergic neurons. These agents bind to the serotonin-1A receptor and inhibit the discharge of serotonergic dorsal raphe nucleus (DRN) neurons recorded in vitro and in anesthetized animals. Recently, we have extended this observation to serotonergic DRN neurons recorded in freely moving cats (Wilkinson et al. Eur. J. Pharmacol., in press). In the present study, we compare the ability of i.v. buspirone to inhibit the spontaneous activity of serotonergic DRN neurons to that of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a specific agonist at the serotonin-1A receptor. Single-unit activity was recorded using movable bundles of 32 and 64 μm diameter nichrome wire electrodes. Serotonergic neurons were identified on the basis of the following established criteria: a) slow and highly regular discharge activity during wakefulness (1-4 spikes/sec); b) long duration action potentials (∿ 2 msec); c) complete suppression of activity during REM sleep and in response to the serotonin agonist 5-methoxy-N,N-dimethytryptamine (250 μg/kg, i.m.); d) histological localization to the DRN. Buspirone hydrochloride and 8-OH-DPAT hydrobromide were dissolved in normal saline and administered remotely via an indwelling jugular catheter. The discharge rate for the one min period beginning 15 sec after drug injection was compared to the one min period immediately preceding the injection. Buspirone and 8-OH-DPAT both produced a dose-dependent inhibition of serotonergic DRN unit activity, however 8-OH-DPAT was approximately 5 times more potent than buspirone. For example, 5 μg/kg buspirone produced a 20-45% suppression of serotonergic unit activity, whereas the same dose of 8-OH-DPAT consistently produced 100% suppression for approximately 3-5 min. Complete suppression of serotonergic unit activity was observed for buspirone at doses of 20-25 μg/kg. These findings are consistent with the greater affinity of 8-OH-DPAT for the serotonin-1A receptor, which may represent the somatic autoreceptor. In addition, in two preliminary experiments, we also examined the responsiveness of DRN neurons to buspirone and 8-OH-DPAT during chronic buspirone treatment (0.5 mg/kg, i.p., 3 times/day, 5-10 days). This schedule was selected to approximate a clinical treatment regimen. In contrast to previous studies, we found no consistent evidence of desensitization following chronic buspirone administration. These data indicate that the delayed clinical efficacy of buspirone is probably not attributable to a desensitization of the serotonin autoreceptor.

127.20 EFFECTS OF SEROTONERGIC COMPOUNDS ON POTENTIATED STARTLE RESPONDING IN THE RAT. R.S. Mansbach* and M.A. Geyer. Department of Psychiatry, University of California, San Diego, La Jolla CA 92093.

The potentiated startle procedure has been suggested as a possible model for the evaluation of anxiolytic compounds. Briefly, this procedure involves the pairing of a noxious stimulus with a neutral light stimulus. When later presented contiguously with an intense acoustic tone, the light induces a respondently conditioned augmentation of the startle response to the tone. This procedure was employed in evaluating the suggested involvement of 5HT1A receptor activation in the behavioral effects of novel, non-benzodiazepine anxiolytic compounds. On two consecutive days, rats were subjected to conditioning sessions in which a light was paired 10 times (mean intertrial interval = 3 min) with a brief 1 mA electric foot shock. Forty-eight hours following the second session, subjects were presented with acoustic startle trials (118 dB(A)), some of which were preceded by presentation of the light stimulus (LT trials) and some of which were not (T-alone trials). Occasional additional trials presented the light alone or no stimulus. In saline-treated animals, the LT trials induced a mean startle 81 percent above that of the T-alone trials. Administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a prototypic 5HT1A ligand, in doses of 0.125 and 0.50 mg/kg s.c. reduced the potentiation of startle in the LT condition to 17 and 7 percent above the T-alone startle, respectively, despite inducing a moderate increase in T-alone startle. Doses of 0.25 and 1.0 mg/kg of the putative 5HT1B ligand, 1-[3-chlorophenyl] piperazine (mCPP), however, did not markedly change the relative potentiation of startle responding in LT trials (83 and 67 percent above T-alone, respectively), despite a substantial decrease in T-alone startle. The non-startle trial presentations produced very low stabilimeter readings under all conditions. These findings support receptor binding and behavioral studies suggesting that novel non-benzodiazepine anxiolytics such as buspirone and isapirone produce their effects via an interaction with the 5HT1A receptor, and demonstrate, in general, the value of the classically conditioned potentiated startle effect in the evaluation of anxiolytic compounds.

128.1 BMY 20661: A POTENTIAL ANTIPSYCHOTIC AGENT THAT DOES NOT BIND TO D-2 DOPAMINE SITES. Duncan P. Taylor, James S. New*, Michael S. Eison, Deborah K. Hyslop, Rhett Butler*, Lloyd E. Allen*, Frank D. Yocca, Cam P. VanderMaelen, and Joseph P. Yevich. Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT. 06492-7660.

BMY 20661, known chemically as 4-{4-[4-(4-furo[3,2-c] pyridinyl)-1-piperazinyl]butyl}-3,5-morpholinedione, was identified as an antipsychotic candidate by its ability to inhibit the conditioned avoidance response in rats with a potency greater than clozapine (ED_{50} values of 11 mg/kg vs 24 mg/kg, p.o., respectively) and its ability to block apomorphine-induced stereotypy (ED_{50} value 34 mg/kg, p.o., compared to 24 for clozapine) and climbing. Moreover, BMY 20661 was active in the Sidman avoidance paradigm at a minimal effective dose of 25 mg/kg, p.o., displaying a clozapine-like profile. Unlike currently-available antipsychotics, BMY 20661 did not produce catalepsy; in fact, it potently (ED_{50} value of 2 mg/kg, p.o.) reversed catalepsy induced by trifluoperazine. Furthermore, whereas chronic administration of haloperidol produced increases in D-2 dopamine binding, administration of BMY 20661 did not. These findings suggest that BMY 20661 should not possess extrapyramidal side effects in clinical use. However, it was not anticholinergic either in vivo or in in vitro binding assays. BMY 20661 appears to have a moderate sedative component as it decreased spontaneous motor activity (ED_{50} value of 9 mg/kg, p.o.), induced rotarod impairment (ED_{50} value of 18 mg/kg, p.o.), and potentiated the hypnotic effects of ethanol (ED_{50} value of 7 mg/kg, p.o.). This may result from its ability to block α_1-adrenergic receptors in vivo: it prevented norepinephrine-induced lethality with an ED_{50} value of 11 mg/kg, p.o. The in vitro receptor binding profile of BMY 20661 is very different from typical antipsychotics: It had no affinity for D-1, D-2, or σ sites, nor did it bind to α_2-adrenergic, opiate, or desipramine sites (IC_{50} values >1000 nM). It displayed very weak affinity for the H_1 histamine site (IC_{50}=660 nM), and it most potently bound at type 1 and type 2 serotonergic sites and α_1-adrenergic sites (respective IC_{50} values of 34, 55, and 41 nM). In contrast to typical antipsychotics, BMY 20661 did not significantly affect the firing of A9 or A10 dopamine neurons. However, BMY 20661 increased the firing rate of noradrenergic neurons in the locus coeruleus, an effect also seen with haloperidol. The pharmacologic profile of BMY 20661 will be compared to those for clozapine and typical antipsychotics. This profile suggests that BMY 20661 possesses antipsychotic efficacy which results from an unusual mechanism, and it appears to be safer than currently available agents.

128.2 THE BINDING OF (+)-[^{3}H]3-PPP AND (+)-[^{3}H]NAN TO HALOPERIDOL-SENSITIVE SIGMA SITES IS INHIBITED BY THE POTENTIAL ANTIPSYCHOTIC BMY 14802. Jennifer Dekleva* and Duncan P. Taylor. CNS Biology, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT 06492-7660

BMY 14802 has been identified as a potential antipsychotic agent. This is based on behavioral testing: (1) It blocks the conditioned avoidance response in rats with a potency similar to that of clozapine, (2) exhibits a clozapine-like profile in the Sidman Avoidance Test (Taylor et al., Soc. Neurosci. Abstr. 11: 114, 1985), and (3) inhibits apomorphine-induced stereotypy and climbing. Unlike currently-marketed antipsychotic drugs, BMY 14802 does not induce catalepsy, in fact, it reverses the catalepsy induced by trifluoperazine. This suggests that BMY 14802 is far less likely to induce the extrapyramidal movement disorders common to this class of psychotherapeutics. Receptor binding studies have previously shown that BMY 14802 exhibits low affinity for D-2 dopamine binding sites in vitro and in vivo. Moreover, BMY 14802 does not bind to D-1 dopamine binding sites (Taylor and Dekleva, Fedn. Proc. 46: 1304, 1987). It has been observed that some conventional antipsychotic drugs inhibit the binding of (+)-[^{3}H]NAN (N-allylnormetazocine, SKF 10,047) in vitro to the "haloperidol-sensitive sigma" site in guinea pig brain. Further, it has been proposed that selective sigma antagonists, devoid of D-2 dopamine antagonist action, may represent a novel class of psychotherapeutic agents in the treatment of schizophrenia. Recently, we have demonstrated that BMY 14802 potently, selectively, stereo-specifically, and competitively inhibits the binding of this ligand (Taylor and Dekleva, Drug. Dev. Res. 10: in press). Interestingly, (+) BMY 14802 displays low affinity for the site labeled by the phencyclidine analog, [^{3}H]TCP (IC_{50}=10,000 nM). It appears that (+)-[^{3}H]3-(3-hydroxyphenyl)-N-(1-propyl)piperidine{(+)-[^{3}H]3-PPP} is a more selective ligand for this site (Largent et al., PNAS 81: 4983, 1984). Our pharmacologic studies confirm this finding. In addition, we have confirmed our earlier work that showed that BMY 14802 inhibits haloperidol-sensitive sigma binding: with this ligand the dextrorotatory enantiomer exhibited a K_I of 28 nM while the levorotatory isomer was ten-fold less potent (K_I=310 nM). It has been suggested that BMY 14802 blocks the behavioral and electrophysiological effects of dopamine agonists by a nondopaminergic mechanism (Matthews et al., JPET 239: 124, 1986). The affinity shown by BMY 14802 for the haloperidol-sensitive sigma site affords support for this suggestion. If clinically efficacious, BMY 14802 would represent a safer alternative to currently available agents for the treatment of schizophrenia.

128.3 THE AUTORADIOGRAPHIC LOCALIZATION OF (±)-[^{3}H]BMY 14802, A POTENTIAL ANTIPSYCHOTIC AGENT. Sandra L. Moon. CNS Biology, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT 06492-7660.

BMY 14802 is a potential antipsychotic agent. In contrast to other antipsychotic drugs, BMY 14802 has low affinity for D-2 dopamine binding sites, and no affinity for D-1 sites. However, like some antipsychotic compounds, BMY 14802 inhibits the binding of sigma ligands (Dekleva and Taylor, Fedn. Proc. 46: 1304, 1987).

Recently we have obtained (±)-[^{3}H]BMY 14802 with a specific activity of 15 Ci/mmol (custom synthesis by NEN). Preliminary in vivo localization experiments were performed by means of auto-radiography. Rats were injected intravenously with 500-900 μCi (±)-[^{3}H]BMY 14802. Cryostat-cut brain sections (20 μm-thick) were opposed to LKB Ultrofilm for 3-12 weeks. Analysis of the resultant autoradiograms revealed a high concentration of label in the anterior cingulate cortex, hippocampus, and the subthalamic region. There was also a widespread distribution of label among cell groups of the pons and medulla.

Preliminary in vitro incubations showed approximately 70 percent decrease in radioactivity in the presence of excess unlabeled (±)BMY 14802. Thaw-mounted frozen brain sections from rat and guinea pig were incubated and apposed to film for 5 weeks or longer. In the rat and guinea pig, labeling was high in cortical regions, including hippocampus and cerebellar cortex. The thalamus concentrated less radioactivity than the subjacent subthalamic region and hypothalamus. The hypothalamus was especially enhanced in the guinea pig. Other labeled regions in both species included the bed nuclei, periaqueductal grey, and superficial layers of the superior colliculus. Labeling in the lower brainstem was relatively less and more heterogeneous in the in vitro conditions than the in vivo experiments.

The pattern of (±)-[^{3}H]BMY 14802 labeling demonstrated many similarities with previously published data on (+)-[^{3}H]3-PPP binding to sigma sites in rat and guinea pig (Gundlach et al., J. Neurosci., 1986).

128.4 BEFIPERIDE, SEROTONERGIC PROPERTIES OF A NEW NON-DOPAMINOLYTIC PUTATIVE ANTIPSYCHOTIC. J.A.M. van der Heyden*, J. Schipper* and M.Th.M. Tulp* (SPON: P. Bevan). Dept. Pharmacol. Duphar b.v., P.O. Box 2, 1380 AA Weesp, The Netherlands.

Befiperide is a novel potential antipsychotic drug on the basis of its inhibition of conditioned avoidance behaviour and apomorphine-induced stereotypy in rats. Unlike the classic neuroleptics, befiperide does not block dopamine (DA) receptors, as established both in vitro (no effect on DA2 receptor mediated release of ^{3}H-DA or ^{3}H-acetylcholine or DA1 receptor mediated c-AMP formation) and in vivo (no increase in DA synthesis rate and DA turnover). As a result, befiperide does not induce the unwanted effects common to most neuroleptics like catalepsy and supersensitivity of the DA system. Since befiperide was developed on basis of a behavioural profile little was known on its mechanism of action except for a lack of DA receptor blocking activity. The most pronounced in vitro receptor affinity of befiperide is for the serotonin (5-HT) type 1A and 2 receptor (22 nM and 45 nM respectively), followed by an affinity of approximately 200 nM for the α_1-noradrenaline and DA2 receptor and 480 nM for the H1 histamine receptor. On basis of its relatively high affinity for 5-HT receptors we have further studied the effects of befiperide and several reference drugs on the serotonin system.

We found that befiperide at a dose of 10 mg/kg orally strongly attenuated 5-HTP accumulation after decarboxylase inhibition in the rat striatum, frontal cortex, hippocampus and nucleus accumbens to about 60% of control. At an oral dose of 15 mg/kg befiperide also lowered 5-HIAA levels in cortex and striatum to about 75% of control. Such effects were not observed for the neuroleptics haloperidol and clozapine. The serotonin agonists TFMPP and 8-OHDPAT produced similar effects in the cortex and striatum whereas no effects were observed with serotonin antagonists such as ketanserin and metergoline. Befiperide did not affect the K^+-stimulated release of 5-HT from rat cortex slices in vitro up to a concentration of 10^{-5}M. TFMPP shows a clear decrease of the K^+-stimulated release, whereas the 5-HT1A agonist 8-OHDPAT showed no effect.

When studying the effects of drugs on the conditioned avoidance behaviour we found that the well known 5-HT1A and 5-HT2 receptor ligands such as 8-OHDPAT, buspirone, quipazine and ketanserin did not inhibit this behaviour at centrally active doses. Like befiperide TFMPP, a 5-HT1B agonist attenuated this behaviour.

In conclusion, the data obtained so far strongly suggest that a 5-HT agonistic activity is present in befiperide, the importance of which for the overall profile remains to be established.

128.5 DOPAMINE AGONIST ACTIVITY OF U-66444B AND ITS ENANTIOMERS: DISCOVERY AND EARLY EVALUATION OF FUNCTIONAL, BIOCHEMICAL AND PHARMACOKINETIC PROPERTIES. P.F.VonVoigtlander, J.S.Althaus*, M.Camacho Ochoa, G.L.Neff* and J.Szmuszkovicz*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Investigation of a novel series of rigid heterocyclic compounds revealed that U-66444B causes hypothermia in mice which is blocked by haloperidol but not by yohimbine. Inhibition of locomotor activity by U-66444B was also blocked by haloperidol and d-amphetamine stimulated motor activity was antagonized by U-66444B. These results suggested that this compound might be a dopamine agonist acting at presynaptic receptors to inhibit the release of dopamine. Evaluation of dopamine release by the measurement of 3-methoxytyramine levels in the corpus striatum revealed that U-66444B does decrease apparent dopamine release. Regarding the involvement of presynaptic receptors in the action of this compound, the ability of U-66444B to reduce the rate of synthesis (DOPA accumulation) and metabolism (DOPAC and HVA) of dopamine in several brain regions was enhanced by gamma butyrolactone and not markedly altered by prior kainic acid lesions. Thus feedback pathways from postsynaptic dopamine receptors did not seem to be principally involved. The measurement of U-66444B brain levels at various time intervals after sc or po administration of the drug to mice revealed that the drug was available by either route and persisted in brain for up to 2 hours. Replication of the above studies with the enantiomers of U-66444B demonstrated that the (+) enantiomer (U-68553B) was by far the more potent and that this was not related to pharmacokinetics as both enantiomers generated comparable brain levels of drug. U-68553B appears to be a structurally novel dopamine agonist that has effects at presynaptic dopamine receptors that may be therapeutically useful.

128.6 **U-66444B AND U-685553B ARE POTENT AUTORECEPTOR AGONISTS AT DOPAMINERGIC CELL BODIES AND TERMINALS.** M.F.Piercey, P.A.Broderick, W.E.Hoffmann* and G.D.Vogelsang. CNS Research, The Upjohn Company, Kalamazoo, MI. 49001 and Pharmacology Dept., CUNY Medical School, NY, NY 10031.

U-66444B and its biologically active stereoisomer, U-68553B, were evaluated for presynaptic and postsynaptic effects in dopaminergic (DA) cell body and nerve terminal regions of chloral hydrate anesthetized rats. To test effects on cell bodies, spikes from DA neurons, identified by long duration (>2.5 msec) action potentials and slow firing rates, were recorded by microelectrodes whose tips were histologically verified as located within cell body regions of the substantia nigra pars compacta (SNPC) or ventral tegmental area (VTA). U-66444B depressed DA neurons in SNPC and VTA in a dose-related manner. ED50 doses depressing firing rates by 50% averaged 3 ug/kg compared to 9 ug/kg for apomorphine and 100 ug/kg for (-)3-PPP. With sufficient dose, all cells were completely silenced. Biological activity for U-66444B resided principally in its (+) stereoisomer, U-68553B, with which it was approximately equipotent. The effects were mediated by DA receptors, since they were promptly reversed by 0.1 mg/kg haloperidol, a DA antagonist. Following 2 weeks of 0.6 mg/kg/day U-66444B, the potency for depressing DA cells was only slightly depressed (ED50=6.6 ug/kg). DA autoreceptor function at nerve terminals was evaluated by in vivo voltammetry. DA release was measured by semi-derivative voltammograms, which were generated every 10 minutes at the tips of stearate graphite electrodes placed directly in the caudate and n. accumbens in vivo. In caudate, 100 ug/kg U-68553B produced a dramatic and prolonged depression of DA release (greater than one hour) compared to weaker and shorter effects of 500 ug/kg apomorphine (less than 20 minutes). In n. accumbens, a similar action occurred following an initial transient (<10 min) increase in DA release. On DA postsynaptic receptors, iontophoretically applied U-66444B and apomorphine were approximately equipotent in depressing caudate neuron firing. It is concluded that U-66444B and its active enantiomer, U-68553B, are more potent, longer acting, and possibly more selective as DA autoreceptor agonists than apomorphine. Moreover, the propensity to produce tolerance is weak.

128.7 **DOPAMINE AUTORECEPTOR AGONIST PROPERTIES OF THE PROPOSED ANTIPSYCHOTIC COMPOUNDS, U-66444B AND ITS (+) ENANTIOMER, U-68553B.** P.J.K.D.Schreur and N.F.Nichols*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Though traditional antipsychotic agents greatly inhibit the symptoms of schizophrenia, their use is often limited by severe or unpleasant side effects which may be an indirect consequence of blockade of the postsynaptic dopamine (DA) receptor. Dopaminergic function may also be decreased presynaptically by a DA autoreceptor (AR) agonist which would decrease the release of DA; thus it may avoid these side effects. U-66444B and U-68553B are potent and selective DA AR agonists with antipsychotic potential.

Exploratory Behavior in Naive Rats. In Omnitech Digiscan Monitors in the dark, DA AR agonists decreased the total distance travelled while paradoxically increasing the number of discrete movements. In contrast, neuroleptics, while decreasing the total distance travelled, also decreased the number of movements (Pharmacol. Biochem. Behav. 25:255-261). U-66444B, U-68553B, apomorphine HCl, and 3-PPP (N-n-propyl-3-(3-hydroxyphenyl)-piperidine) and its two enantiomers decreased the total distance and increased the number of movements in this test.

Locomotor Activity in Habituated Rats. In Digiscan Monitors in the light, DA AR agonists antagonized amphetamine-stimulated locomotor behavior (total distance) without antagonizing apomorphine-stimulated behavior (Pharmacol. Biochem. Behav. 25:255-261). U-66444B, U-68553B, and (+)3-PPP antagonized amphetamine but not apomorphine. Low doses of apomorphine antagonized amphetamine, also.

Turning in Rats with Unilateral Substantia Nigra (6-OH-dopamine) Lesions. DA AR agonists antagonized amphetamine-induced turning (scored visually) at a dose which did not itself cause turning. U-66444B (0.01 mg/kg) or apomorphine HCl (0.01-0.03 mg/kg) antagonized the ipsiversive turning caused by 1 mg/kg d-amphetamine SO4 but did not cause turning alone. A higher dose (0.1 mg/kg) of U-66444B caused contraversive turning and a still higher dose (3 mg/kg) caused ipsiversive turning, both of which could be antagonized by haloperidol HCl. Higher doses of apomorphine HCl (0.1 mg/kg or higher) also caused contraversive turning. U-68553B (0.01-0.1 mg/kg) caused contraversive turning; it was more potent than the racemic mixture. The enantiomers of 3-PPP variably caused contraversive turning.

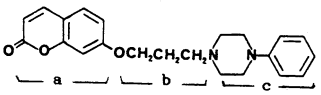
Behavioral Tolerance. After 6 days of continuous U-66444B s.c. infusion (0.3 mg/kg/day) or 14 days of pulsed i.v. infusions (0.6 mg/kg/day), rats showed tolerance to the ability of acute U-66444B (0.1 mg/kg) to antagonize amphetamine-stimulated locomotor activity. This tolerance disappeared by 4 weeks after withdrawal from the continuous infusion.

128.8 OPERANT FORCE-PROPORTIONAL REINFORCEMENT: PIMOZIDE DOES NOT ATTENUATE A RATE-FREE MEASURE OF REINFORCEMENT EFFICACY. M. A. Kirkpatrick and S. C. Fowler. Departments of Psychology and Pharmacology, University of Mississippi, University, MS 38677.

A two step force-proportional reinforcement contingency was used to explore the effects of pimozide (PIM, 0.12, 0.25, and 0.50 mg/kg,ip., injected 4 hr before behavioral tests) on peak force and rate of operant response. Rats (N=4) in the force-proportional group (PRO) learned to press a force-sensing operandum and were rewarded with 8% sucrose solutions for low-force responses (peak forces of 8-23 g) and 24% sucrose for high forces (24 g or more). A second group (N=4) was exposed to an inverse force-proportional procedure (INV); i.e., low forces produced 24% and high forces yielded 8% sucrose solutions, while the third group (N=4) exclusively received 24% sucrose solutions for high forces (NONP). Although the PRO group learned to emit high forces and the INV group learned to make low-force responses, PIM did not reduce the tendency of the PRO group to press harder for more, even though all groups displayed significant dose-related rate decreases ($F[3,27]=9.660$, $p<0.001$). In accord with previous studies, peak force of response was slightly but significantly elevated by PIM ($F[3,27]=2.944$, $p<0.05$). The behavior of groups INV and NONP suggests that the higher concentration of sucrose (24%) did not by itself elicit high-force responding. This observation and the fact that the PRO and INV groups learned to emit forces appropriate for obtaining the sweeter concentration suggest that the PRO group chose the higher concentration because of its relatively greater incentive/motivational value compared to the lower concentration. The failure of PIM to decrease peak force in the PRO group is not consistent with the anhedonia (incentive/motivational) hypothesis of neuroleptics' behavioral effects. These results support the idea that relatively low doses of neuroleptics affect operant responding via motor processes expressed primarily in the temporal domain of behavior.

128.9 7-[3-(4-ARYL-1-PIPERAZINYL)PROPOXY]-2H-1-BENZOPYRAN-2-ONES. A NEW CLASS OF DOPAMINE AUTORECEPTOR AGONISTS. L.D. Wise*, H.A. DeWald*, E.S. Hawkins*, T.G. Heffner, T.A. Pugsley and L.T. Meltzer (SPON: D.K. Boyd). Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105

Dopamine (DA) agonists with selectivity for brain DA autoreceptors have emerged in recent years as a potential therapeutic approach for treatment of cardiovascular and CNS disorders including schizophrenia. PD 116,795, 7-[3-(4-phenyl-1-piperazinyl)propoxy]-2H-1-benzopyran-2-one, is chemically novel as a DA autoreceptor agonist.



PD 116,795

PD 116,795 bound selectively to D_2 DA receptors, attenuated the stimulation of brain DA synthesis induced by gamma-butyrolactone and inhibited firing of substantia nigra DA neurons in rats, all of which indicates that PD 116,795 is a DA agonist. PD 116,795 also inhibited spontaneous locomotor activity in mice after IP administration (ED_{50}: 2.59 mg/kg) and in rats after oral administration (ED_{50}: 2.10 mg/kg). Behavioral stimulation indicative of postsynaptic DA agonist activity was not seen even at doses as high as 100 mg/kg IP in mice. The uniqueness of PD 116,795 led us to examine the structure-activity relationships (SAR) of several related compounds on the basis of their effects on brain DA synthesis. While the benzopyran (a) and propoxy (b) portions of the molecule could be altered significantly without loss of DA agonist activity, the phenylpiperazinyl group (c) was quite sensitive to modifications. For example, addition of chloro or methyl substituents to the phenyl portion of the phenylpiperazine resulted in complete loss of DA agonist activity. Computerized molecular modeling of PD 116,795 and related compounds suggests that the spatial orientation of the phenylpiperazine is quite important in determining DA agonist activity.

128.10 TOLERANCE TO CHRONIC HALOPERIDOL TREATMENT IN THE RAT AS MEASURED BY SPONTANEOUS LOCOMOTOR ACTIVITY. M.A. Notrica*, F.E. Soroko*, B.T. Kenney* and L.E. Boswell* (SPON: A.S. Tadepalli). Department of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Chronic administration of Haloperidol (HAL) to the rat produces an augmented behavioral sensitivity to D_2 agonists and hyperplasia of D_2 receptors. Such epiphenomena may influence the interpretation of studies on prolonged neuroleptic treatment and its subsequent withdrawal in the rat. We assessed the effect of 21 days of HAL (2 mg/kg/day) on spontaneous locomotor activity (SLA) in open-field monitors. Significant decreases in SLA were apparent for the first three days of drug treatment with no statistically significant differences, compared to controls, for the remaining 18 days of the study.

Withdrawal of HAL produces a slight rebound in SLA which persists for a period of 10-14 days after cessation of treatment. During this washout period the effects of other neuroleptic agents was explored. The results of these experiments, along with the effects of chronic chlorpromazine treatment on SLA will be discussed.

128.11 EVALUATION OF THE EFFECTS OF CI-943, A POTENTIAL ANTIPSYCHOTIC AGENT, ON DOPAMINE NEURONAL ACTIVITY. L.T. Meltzer[1], C.L. Christoffersen[1]*, A.S. Freeman[2], and L.A. Chiodo[2]. [1]Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105 and [2]Center for Cell Biology, Sinai Hospital of Detroit, Detroit, MI 48235.

Based on its profile in preclinical behavioral and biochemical tests predictive of antipsychotic activity, CI-943 (8-ethyl-7,-8-dihydro-1,3,5-trimethyl-1H-imidazo[1,2-c]-pyrazolo-[3,4-e]pyrimidine) has been identified as a potential novel antipsychotic agent that does not bind to the dopamine (DA) receptor. The present studies compared the effects of acute and chronic administration of CI-943 and standard antipsychotic agents on the electrophysiological activity of midbrain DA neurons recorded extracellularly in chloral hydrate anesthetized male S-D rats.

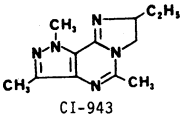
Acute administration of haloperidol (HAL; 0.5 mg/kg, IP) increased the baseline firing rate and antagonized the inhibitory effects of apomorphine (APO) and amphetamine (AMP) on substantia nigra zona compacta (A9) DA neurons. In contrast, CI-943 (20 mg/kg, IP) neither increased the baseline firing rate nor antagonized the effects of APO or AMP.

The effects of acute (PO) and chronic (21 days, drugs administered via drinking water) treatment with HAL (0.5 mg/kg/day), clozapine (20 mg/kg/day; CLZ), and CI-943 (40 mg/kg/day) on the number of spontaneously active DA neurons in A9 and the ventral tegmental area (A10) were evaluated. Acute HAL and CLZ increased the number of active DA neurons in both A9 and A10. In contrast, acute CI-943 did not alter the number of active DA neurons in either region. Chronic HAL decreased the number of active DA neurons in A9 and A10. Chronic CLZ decreased the number of active DA neurons in A10 but increased activity in A9. Chronic CI-943 produced effects that were different than those obtained with HAL or CLZ as it increased the number of active DA neurons in A10 while having no effect in A9.

In this series of electrophysiological studies, CI-943 was differentiated from the typical (HAL) and atypical (CLZ) DA antagonist antipsychotic drugs. These data suggest that the electrophysiological effects of CI-943 are not due to DA receptor antagonism, and are in agreement with in vitro receptor binding data that indicate that CI-943 has no affinity for DA receptors. These data support the hypothesis that CI-943 produces its preclinical antipsychotic-like behavioral effects through non-dopaminergic mechanisms.

128.12 EFFECTS OF THE POTENTIAL ANTIPSYCHOTIC AGENT CI-943 IN PRECLINICAL PREDICTORS OF EFFICACY IN MICE AND RATS. T.G. Heffner, D.A.Downs F.W. Ninteman*, J.N. Wiley*, H.A. DeWald*, L.D. Wise* and F.M. Hershenson*. Departments of Pharmacology & Chemistry, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The efficacy of known antipsychotic drugs is commonly attributed to their blockade of brain dopamine receptors. However, dopamine antagonist antipsychotics are incompletely efficacious in man and produce serious neurological side effects. In order to develop improved drugs for the treatment of schizophrenia, we undertook a program to identify agents which display the behavioral profile of antipsychotic drugs in preclinical tests but which do not interact with brain dopamine receptors. CI-943 (8-ethyl-7,8-dihydro-1,3,5-trimethyl-1H-imidazo[1,2-c]pyrazolo-[3,4-e]pyrimidine) is a novel psychotropic agent, unrelated to any known chemical class of antipsychotics.



CI-943

CI-943 is not a brain dopamine receptor antagonist as assessed by receptor binding assays and by in vivo tests. CI-943 inhibits exploratory locomotion in mice (ED_{50} = 26 mg/kg IP & PO) at lower doses than those which impair motor coordination (ED_{50} > 100 mg/kg IP & PO), a profile shared by available antipsychotics but not seen with a variety of non-antipsychotic sedatives. Despite its lack of affinity for brain dopamine receptors, CI-943 inhibits compulsive cage climbing in mice induced by the dopamine agonist apomorphine (ED_{50} = 41 mg/kg IP). However, the finding that CI-943 does not attenuate locomotor hyperactivity produced by d-amphetamine in mice or rats (ED_{50} > 100 mg/kg IP) but instead augments such effects clearly distinguishes this agent from dopamine antagonists. CI-943 shares with dopamine antagonists the ability to produce inhibition of lateral hypothalamic self-stimulation responding in rats (ED_{50} = 10.8 mg/kg PO) and to reduce time spent by rats on a platform on which they receive rewarding brain stimulation passively. CI-943 also resembles antipsychotics in a one-way conditioned avoidance test in rats, inhibiting avoidance responding (ED_{50} = 7.5 mg/kg PO) at lower doses than those which inhibit escape responding (ED_{50} = 32 mg/kg PO). These results indicate that while it differs from dopamine antagonists in tests for mechanism of action, CI-943 displays the profile of known antipsychotic drugs in a variety of preclinical models of antipsychotic efficacy.

128.13 EFFECTS OF THE POTENTIAL ANTIPSYCHOTIC AGENT CI-943 IN PRECLINICAL PREDICTORS OF EFFICACY AND NEUROLOGICAL SIDE EFFECTS IN RATS AND MONKEYS. D.A. Downs, T.G. Heffner, D.J. Johnston*, K.L. Sledge*, A.E. Williams* and J.N. Wiley*. Dept. of Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

CI-943 (8-ethyl-7,8-dihydro-1,3,5-trimethyl-1H-imidazo[1,2-c]-pyrazolo[3,4-e]pyrimidine) is a potential antipsychotic agent that lacks affinity for brain dopamine receptors as well as other characteristics of dopamine antagonists in neurochemical, neurophysiological, and endocrinological tests. In order to further characterize this novel agent, we compared CI-943 to dopamine antagonist antipsychotics in preclinical tests for efficacy and neurological side effects. Efficacy tests included inhibition of spontaneous locomotion and inhibition of continuous (Sidman) avoidance responding, tests commonly used as preclinical predictors of antipsychotic activity. Liability for extrapyramidal side effects (EPS) was estimated by determining if a behaviorally active dose of test agent elicits acute dystonias in monkeys that have been sensitized to the dystonic effects of haloperidol by its chronic administration. Liability for tardive dyskinesia was estimated by determining if test agents administered chronically to rats induce supersensitivity to the behavioral effects of the dopamine agonist apomorphine. In addition to CI-943, the dopamine antagonists haloperidol (HPD), chlorpromazine (CPZ), and thioridazine (THZ) were examined. Each of the dopamine antagonists tested inhibited continuous (Sidman) avoidance responding in rats and squirrel monkeys at doses that did not impair the ability to respond. CI-943 shared this profile, inhibiting Sidman avoidance responding selectively in both rats (ED_{50} = 19.8 mg/kg PO) and squirrel monkeys (ED_{50} = 7.2 mg/kg PO) with a duration of action in excess of 6 hours. Each of the dopamine antagonists, but not CI-943, produced dystonias in haloperidol-sensitized squirrel monkeys when tested at doses near their ED_{50}s for inhibiting avoidance responding. Each of the dopamine antagonists also inhibited spontaneous locomotor activity in rats (ED_{50}s, mg/kg PO: HPD, 0.2; CPZ, 5.4; THZ, 13.3) at doses that did not produce ataxia and a similar profile of activity was seen with CI-943 (ED_{50} = 15 mg/kg PO). Rats treated daily for 21 days with HPD, CPZ, or THZ at doses equivalent to 3 times the respective ED_{50} for locomotor inhibition displayed augmented locomotor stimulant responses to apomorphine (0.1 mg/kg SC) administered on day 23. However, rats similarly treated with CI-943 (45 mg/kg PO for 21 days) did not display behavioral supersensitivity to apomorphine. These results indicate that although CI-943 shares the preclinical efficacy profile of available antipsychotics, it does not have the profile of dopamine antagonists in preclinical tests for neurological side effects.

128.14 NEUROCHEMICAL PROPERTIES OF CI-943, A NON-DOPAMINE RECEPTOR ANTAGONIST ANTIPSYCHOTIC CANDIDATE. T.A. Pugsley, L.L. Coughenour*, S.L. Myers*, Y.H. Shih*, G.G. Courtland*, W. Berghoff* and S.F. Stewart*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

CI-943 (8-ethyl-7,8-dihydro-1,3,5-trimethyl-1-H-imidazo [1,2-c]pyrazolo[3,4-e] pyrimidine) is a novel compound which has been shown to have an antipsychotic profile in animal tests with a low propensity to cause extrapyramidal side effects (EPS). The present study examined some in vivo and in vitro neurochemical properties of CI-943.

CI-943 (40 mg/kg p.o.) caused a significant increase in rat striatal and mesolimbic homovanillic acid (HVA) 2 hours after dosing; striatal but not mesolimbic 3,4-dihydroxyphenylacetic acid (DOPAC) levels were also increased. Lower doses (1 and 10 mg/kg p.o.) were ineffective. CI-943 (20 mg/kg i.p.) caused a significant increase in striatal HVA, DOPAC and 3-methoxytyramine levels. A duration study indicated that CI-943 elevated striatal and mesolimbic HVA content by 0.5 hours after dosing with levels declining to those of control by 4 hours. CI-943 was also shown to increase striatal DA synthesis. As dopamine (DA) levels were unchanged, these findings indicated that DA turnover was increased. These effects are not due to DA receptor blockade as CI-943, unlike known antipsychotics, did not exhibit affinity for DA receptors. CI-943 did not affect rat serum basal levels of prolactin nor did it inhibit in vivo DA neuronal reuptake. Amfonelic acid (AFA) enhanced the action of haloperidol in increasing striatal HVA with no effect on that of CI-943 and clozapine. This suggests that CI-943, like the atypical agent clozapine, would be predicted to have a low risk of EPS as compared to typical antipsychotics like haloperidol. Chronic administration of CI-943 (40 mg/kg i.p.) to rats for 28 days did not affect the affinity or number of striatal DA receptors; in comparison, haloperidol (0.5 mg/kg i.p.) caused an increase in number of DA receptors with no change in affinity. The latter finding suggests that CI-943 does not cause supersensitivity of DA receptors as do known antipsychotics such as haloperidol and thus may have a low propensity to cause tardive dyskinesia in patients. Except for the striatum where levels of 5-hydroxyindoleacetic acid were elevated, measures of serotonergic and nordrenergic function were not affected by CI-943. In addition CI-943 did not exhibit any significant affinity for a number of CNS receptors in vitro. The molecular mechanism by which CI-943 increases brain DA turnover is not known at this time but appears to be unique in comparison to known antipsychotic agents.

128.15 **MECHANISMS OF TOLERANCE TO CLOZAPINE DURING OPERANT RESPONDING IN RATS.** H.F. Villanueva* and J.H. Porter. Dept. of Psychology, Virginia Commonwealth Univ., Richmond, VA 23284.

Previous studies have shown that the atypical neuroleptic clozapine suppresses operant behavior in rats, but that response rates recover to control levels within several days during chronic dosing regimens (Kaempf & Porter, 1985; Villanueva & Porter, 1987). The mechanisms for this tolerance to the rate suppressing effects of clozapine are unclear, however. The present study was designed to determine whether the tolerance to clozapine in operant studies is behavioral, physiological or a combination of these two mechanisms.

Male rats (80%BW) were trained to respond according to a multiple random interval (MULT RI) food reinforcement schedule with four 10-min components (RI 10-sec, RI 40-sec, RI 80-sec and RI 160-sec) separated by 5-min time out periods. One group of rats (N = 7) received 10 mg/kg injections (ip) of clozapine one hour presession for 10 consecutive days. A second group (N = 6) received vehicle injections one hour presession and 10 mg/kg clozapine injections one hour post session for 9 consecutive days. On the tenth day the order of injections was switched, and for the next 10 days the rats in Group Two received the clozapine injections presession and the vehicle injections post session.

Presession injections of clozapine (Group One) produced a significant reduction in responding and number of reinforcers initially in all four MULT RI schedules, but the animals recovered to baseline levels before the end of the 10-day chronic dosing period. For Group Two response rates and reinforcers remained at baseline levels for the first 9 days of post session clozapine injections. On the tenth day when clozapine injections were switched to presession, response rates and number of reinforcers were not significantly suppressed for the RI 40-, 80- and 160-sec schedules. For the RI 10-sec schedule, however, responding and the number of reinforcers were signifcantly decreased but not as much as was seen on day one for Group One. Again, response rates and reinforcers recovered to baseline levels within the 10-day chronic dosing regimen (with presession injections). For response duration, there was no significant change on day 10 when the clozapine injection was switched to presession.

These results suggest that the recovery of operant responding during chronic dosing regimens with clozapine can be attributed primarily to the development of tolerance to the physiological effects of the drug. This recovery can probably be attributed to the development of tolerance to the sedative effects of clozapine, as human studies report that tolerance develops to the sedative, but not to the antipsychotic effects of the drug (Pinder et al, 1976).

128.16 EFFECTS OF CHOLECYSTOKININ AND γ-TYPE ENDORPHIN ON HYPER- AND HYPO-LOCOMOTION INDUCED BY APOMORPHINE. Y.Igarashi, R.Igarashi and J.M. van Ree*(SPON:M.Okamoto), Department of Psychiatry, Saitama Medical School, Saitama, Japan, *Rudolf Magnus Institutute for Pharmacology, University of Utrecht, Utrecht, The Netherlands.

It has been reported that caeruletide, cholecystokinin(CCK) related peptide, and γ-type endorphins have therapeutic effects on schizophrenic symptomes. Apomorphine injected into the nucleus accumbens (NcAcc) of the rat, considered to be one of sites of action of antipsychotic drugs having antidopaminergic actions, produce hypo- and hypermotility depending on the dosage. Pretreatment of CCK or γ-type endorphins modulate hypo- and hyperlocomotion induced by apomorphine. Recently we reported inhibitory effect of naloxone, an opioid antagonist, on the blocking action of CCK on hyperlocomotion induced by high dose of apomorphine (1). To elucidate the further evidence of the relationship between CCK and endorphins, the effects of antagonists and specific antisera against these peptides were investigated. Male wistar rats weighing 120-130g were implanted stereotaxically stainless steel canulae into the NcAcc. Antagonists or antisera were injected at 70min, and peptides were applied at 60min prior to the behavioural observation. Apomorphine was administered at 20min prior to the observation. Locomotion, rearing, glooming and sniffing time in the small open field were evaluated for 3min as reported elsewhere(2). Low dose of apomorphine(10ng/each side) significantly decrease the locomotor activity, and this hypolocomotion was blocked by pretreatments of CCK-8 sulfated form(CCK-8S) and nonsulfated form (CCK-8NS) and des-enkephalin-γ-endorphin(DEγE). Naloxone, an opioid antagonist, and anti γ-endorphin serum inhibited the blocking action of CCK-8NS and DEγE. Proglumide, a CCK antagonist, and anti-CCK serum also showed the inhibitory effect on the blocking action of CCK-8NS and DEγE. High dose of apomorphine(10ug) produced significant increase of the locomotor activity. Hyperlocomotion induced by high dose of apomorphine was blocked by pretretments of CCK-8S and γ-endorphin(γE). Naloxone and anti-γE serum inhibited the blocking effects of CCK-8S and γE on hyperlocomotion. Proglumide and anti-CCK serum inhibited the blocking effect of CCK-8S on hyperlocomotion , though the blocking action of γE did not affect by pretreatments of both proglumide and anti-CCK serum. These data suggest that the blocking action of CCK on hyperlocomotion, postulated the effect of apomorphine on postdopaminergic receptor, mediated through the endorphin system in the rat NcAcc. On the other hands, the actions of CCK and γ-endorphin on hypolocomotion, the effect of apomorphine on predopaminergic receptor, were only obtained under the intact condition of both CCK and endorphin systems.

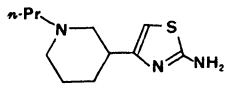
(1) Király,I. and Van Ree, J.M., Neurosci. Lett., 74: 343, 1987.
(2) Van Ree, J.M. et al.,Eur. J. Pharmacol., 93:63, 1983.

128.17 BUSPIRONE, M-CHLOROPHENYLPIPERAZINE (MCPP), 6-CHLORO-2(1-PIPERAZINYL)PYRAZINE (MK212) AND Wy-47,384 DISPLACE HALOPERIDOL-* SENSITIVE SIGMA BINDING IN MOUSE BRAIN IN VIVO. R.P. Tasse, E.A. Muth and T.H. Andree, Wyeth Laboratories, Inc., Division of Experimental Therapeutics, Philadelphia, PA 19101

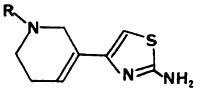
The in vivo binding of 5 μCi/mouse (+)[^{3}H]N-allylnormetazocine (NANM or (+)[^{3}H]SKF 10,047) in brain was examined 15 min following its intravenous injection to vehicle- or drug-pretreated mice. Pretreatment with phencyclidine or one of several sigma receptor ligands displaced the binding of (+)[^{3}H]NANM from that observed following vehicle pretreatment. The putative antipsychotics rimcazole and Wy-47,384, the anxiolytic buspirone, and the arylpiperazine mCPP also inhibited the in vivo binding of (+)[^{3}H]NANM with ID_{50} values of 7.9, 7.3, 4.9 and 4.9 mg/kg i.p., respectively. MK212 produced 40% displacement at 16 mg/kg. Since maximum pharmacologically effective doses of spiperone, (+)SCH23390 and 8-OH-DPAT were each ineffective, the dopamine (D-1 and D-2) and serotonin (5-HT-1A and 5-HT-2) receptor affinities attributed to haloperidol, Wy-47,384 [Andree et al., Soc. Neurosci. Abst. 12:477 (1986)], buspirone and other arylpiperazines fail to account for their in vivo displacement of (+)[^{3}H]NANM. The pretreatment of mice with a maximum, pharmacologically effective dose of the opiate antagonist naloxone, the α_2 adrenoceptor antagonist yohimbine, or the benzodiazepine agonist diazepam also failed to displace the in vivo binding of (+)[^{3}H]NANM. In separate, in vitro receptor binding assays, unlabelled (+)NANM was found to lack affinity at naloxone-sensitive opiate, benzodiazepine, dopamine D-2, cholinergic, noradrenergic (alpha-1, alpha-2 and beta), histamine H-1, and serotonin (5-HT-2 and 5-HT-1A) receptor binding sites. Thus, the displacement of (+)[^{3}H]NANM observed during this investigation appears to have resulted exclusively from the sigma/phencyclidine receptor binding site. These results, combined with the previously reported potential preclinical antipsychotic activity of mCPP and MK212 in conditioned avoidance tests [Moyer and Lucki, Soc. Neurosci. Abstr. 11:1186 (1985)] as well as buspirone's preclinical antipsychotic profile further support a possible role for sigma receptors in antipsychotic drug action.

128.18 DOPAMINE AGONIST PROPERTIES OF AMINOTHIAZOLE BIOISOSTERES OF 3-PPP. J.C. Jaen*, L.D. Wise*, F.M. Hershenson, H. Tecle*, T.G. Heffner, T.A. Pugsley and L.T. Meltzer. Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

3-(1-propyl-3-piperidinyl)phenol (3-PPP) is the prototypical dopamine (DA) autoreceptor agonist. Its limited oral activity and short duration of action prompted us to design PD 118,237, a 4-(1-propyl-3-piperidinyl)-2-thiazolamine, as a bioisosteric replacement for 3-PPP. However, PD 118,237 appeared to be a weak DA agonist based on its low affinity for DA receptors in an in-vitro receptor binding assay and its incomplete antagonism of gamma-butyrolactone-stimulated brain DA synthesis in rats. Molecular modeling studies led to the synthesis of two analogs with improved characteristics: PD 118,440 and PD 120,697.



PD 118,237



R = nPr; PD 118,440
R = allyl; PD 120,697

These 4-(1-alkyl-1,2,5,6-tetrahydro-3-pyridinyl)-2-thiazolamines bound selectively to D_2 versus D_1 DA receptors, inhibited the firing of substantia nigra DA neurons in rats and reversed the increase of brain DA synthesis induced by gamma-butyrolactone in rats. This profile of activity indicates that PD 118,440 and PD 120,697 are DA agonists. Both PD 118,440 and PD 120,697 reduced spontaneous exploratory locomotor activity in mice (ED_{50}: 2.9 and 3.0 mg/kg IP, respectively). In rats, both compounds decreased locomotor activity when administered orally (3 mg/kg) but increased locomotor activity at higher doses (30 mg/kg), suggesting both pre and postsynaptic DA agonist activity. In addition, both compounds stimulated locomotion when administered to rats pretreated with reserpine to produce super- sensitivity of postsynaptic DA receptors. Collectively, these results demonstrate that PD 118,440 and PD 120,697 are brain DA agonists with good oral activity. The data also indicate that PD 118440 is more selective than PD 120,697 for DA autoreceptors but that both compounds are less selective for DA autoreceptors than is ±3-PPP.

128.19 EFFECT OF NEUROLEPTIC WITHDRAWAL ON PLASMA PROLACTIN IN SCHIZOPHRENIC PATIENTS. K. Maeda*, B. Kirkpatrick*, G.K. Thaker, D. Jauch*, T.N. Chase and C.A. Tamminga. NINCDS, NIH, Bethesda, MD 20892, and Maryland Psychiatric Research Center, Univ. of Maryland Sch. of Med., Baltimore, MD 21228.

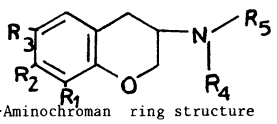
Abrupt withdrawal of treatment with neuroleptics in schizophrenic patients is associated with various withdrawal manifestations. Tardive dyskinesia is the best known example of such a phenomenon. Those manifestations are generally considered to be ascribed to dopamine receptor supersensitivity. However, there has been little direct study in the living human to contribute to our understanding of the mechanism(s) of neuroleptic withdrawal symptoms. Six male patients between the ages of 20 and 30 (mean 25.2) with a DSM III diagnosis of chronic schizophrenia gave a written consent to participate in this study. The patients had received various antipsychotic drugs for at least 12 months. Patients were treated with 0.1 mg/kg haloperidol for 4 weeks. Thereafter, they were abruptly withdrawn from their medication in a placebo-controlled, double-blind design. Clinical assesments of mental status and plasma prolactin determination were done before and thoughout the withdrawal periods. On the morning of the blood sampling, a #19 butterfly needle was inserted into a forearm vein. From a three way stop-cock attached to the butterfly needle, three samples of blood were taken 45 min after insertion of the needle 5 min apart. After all three samples were collected, they were immediately centrifuged and the plasma were stored at -70 C. Plasma prolactin was measured by radioimmunoassay. Mean value for the 3 samples was used for a single prolactin value. The modified BPRS ratings was made by two trained psychiatrist who individually rate the patients' mental status and then develop a concensus rating. As shown in table, marked reduction in plasma prolactin was observed during the first week of drug withdrawal. The prolactin values increased during the later withdrawal phase and the 3rd/4th drug free week as compared to intial withdrawal week. A wide variety of patterns of BPRS changes were observed after neuroleptic withdrawal. These data indicate that dopamine receptor supersensisitivity occurs at the pituitary lactotrophs, and is temporally discordant with any changes in psychosis.

week	0*	1	2	3-4
Prl (ng/ml)	12.8 ± 5.1	3.8 ± 1.0**	5.0 ± 0.8	5.0 ± 1.0

* after 4 weeks of continous treatment with 0.1 mg/kg haloperidol.
** $p \lesssim 0.032$, different from 0, 2 and 3-4 week time points using the Friedman and the Wilcoxson sign rank test.

128.20 DOPAMINERGIC ACTIVITY OF SELECTED 3-AMINOCHROMANS. B.S. Glaeser, J. C. Berry,* W. C. Boyar, R. A. Lovell, and A. J. Hutchinson.* Neuroscience/Cardiovascular Research Department., Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, New Jersey, 07901.

The dopaminergic properties of several 3-Aminochromans were studied using the gamma-butyrolactone model for dopamine autoreceptors (Walters and Roth, Naunyn-Schmiedeberg's Arch. Pharmacol. 296:5, 1976). The structures and initial results of these compounds in the GBL model are summarized in the table below. Compound 3 (CGS 14948A) has been previously described as a dopamine autoreceptor agonist (Berry et al., Fed. Proc. Abstract #2807, 1985) with oral activity in the GBL model and a duration of action of 30 to 60 min post ip administration. Compound 6 (CGS 15397A) had also been shown to have properties characteristic of dopamine autoreceptor agonists. Compound 6 (10 and 30 mg/kg, po) was shown to have oral activity in the GBL model, by reversing the GBL-induced accumulation of DOPA after 30 and 60 min. pretreatment. The duration of action of Compound 6 was estimated by the measurement of dopamine metabolites at various time points after a single injection. At a dose of 5 mg/kg ip, Compound 6 significantly reduced striatal concentrations of DOPAC and HVA 30 and 60 min post administration. Also striatal dopamine levels were elevated 60 and 120 min post administration. In summary minimal dopaminergic activity was observed with compounds possessing only a free amino group versus molecules having a nitrogen substituted with methyl or propyl groups. Maximal dopaminergic activity was observed in molecules having a di-propyl substitution on the nitrogen and with hydroxyl groups at positions corresponding to R_1, R_2, or R_3.



3-Aminochroman ring structure

Compound	R1	R2	R3	N<R5 R4	GBL Model ED_{50} (ip) or % Reversal (mg/kg ip)
1	H	H	H	$N(CH_3)_2$	>20.0
2	H	H	OH	$N(CH_3)_2$	100% (30)
3	H	H	OH	$N(C_3H_7)_2$	0.92
4	H	OH	OH	$N(C_3H_7)_2$	1.68
5	H	OH	H	$N(C_3H_7)_2$	100% (30)
6	OH	H	H	$N(C_3H_7)_2$	0.07
7	OCH_3	H	H	$N(C_3H_7)_2$	100% (30)
8	H	H	H	$N(C_3H_7)_2$	100% (30)
9	H	OH	H	NH_2	23% (1.0)
10	H	H	OH	NH_2	10% (1.0)

129.1 INGESTION-ASSOCIATED CHANGES IN BRAINSTEM SENSORIMOTOR ACTIVITY AS REVEALED IN COMPOSITE [14-C] 2-DEOXYGLUCOSE AUTORADIOGRAPHS FROM 6-DAY-OLD RAT PUPS. C.B. Phifer, L.M. Terry and W.G. Hall (SPON: R.P. Erickson). Department of Psychology, Duke University, Durham, NC 27706.

When infant rat pups are given oral infusions of a liquid diet, the pups exhibit avid ingestion, including vigorous lapping, mouthing and swallowing. Intake by this form of ingestion is usually deprivation-dependent, but when the infusion cannula is appropriately placed in the back of the oral cavity, pups exhibit reflexive swallowing, which is not dependent on deprivation. Using [14-C] 2-deoxyglucose autoradiography (2-DG), we compared changes in relative neural metabolic activity of brainstem sensorimotor systems associated with deprivation-dependent and deprivation-independent ingestion.

Pups used in this experiment were 6 days of age and had been deprived of all nutrient for 24 hr or left nondeprived. Injections of 2-DG (20uCi/100g, s.c.) were given and a 1 hr incorporation period was allowed. During this period, both deprived and nondeprived pups were fed milk through oral cannulas; for comparison purposes, another group of nondeprived pups were not fed. Pups were then sacrificed and their brains were removed, sectioned and autoradiographic images were prepared. Autoradiographic images corresponding to brain sections at the level of the hypoglossal nucleus were selected by matching to the Nissl-stained tissue. Utilizing image averaging techniques, composite images were made from autoradiographs of 6 pups in each of the three conditions. Differences in activity between treatment groups were revealed by subtracting one averaged image from another.

Relative to non-ingesting pups, the brains of ingesting pups, both deprived and nondeprived, showed increased activity in the hypoglossal complex and nucleus tractus solitarius, as well as reticular regions lateral to hypoglossal (parvocellular). There were also clear differences in activity between pups that were deprived and ingesting and pups that were nondeprived and ingesting. The pups exhibiting deprivation-dependent ingestion showed greater activity in the hypoglossal complex, more widely distributed activity in nucleus tractus solitarius, and greater, more-widely distributed activity in reticular regions (particularly in very lateral and ventral areas). Deprived and ingesting pups also showed enhanced activity in the interpolaris trigeminal nucleus (particularly dorsal region) than nondeprived ingesting pups. These results reinforce earlier findings on ingestion-associated activity in sensory and motor nuclei and also reveal corresponding activity changes in known dendritic fields of the brainstem. (Supported by NICHD Grant HD-17458.)

129.2 INTRADUODENAL MEDIUM-CHAIN FATS DECREASE FOOD INTAKE, BUT INTRAPORTAL MEDIUM-CHAIN FATS DO NOT. D. Greenberg, P.A. Foelsch*, P.M. Perez*, and G.P. Smith. Dept. of Psychiatry, Eating Disorders Institute and Bourne Behavioral Research Laboratory, New York Hospital-Cornell Medical Center, White Plains, NY 10605.

Intraduodenal (id) infusions of long-chain triglycerides (LCT, Intralipid, Kabi Vitrum Inc., CA) at physiological rates inhibited feeding and elicited behaviors typical of satiety in rats, but intraportal (iport) infusions did not (Greenberg et al., Abstr. Soc. Neurosci. 10:531, 1984). To determine if this differential satiating potency also occurred with infusions of medium-chain triglycerides (MCT) which are normally absorbed via the hepatic portal system, we investigated the satiating potency of id and iport infusions of the MCT, trycaprylin.

Method: Male Sprague Dawley rats (400-450g) were equipped with chronic gastric cannulas for sham feeding and with chronic Silastic catheters in the duodenum or in the portal vein for tricaprylin infusions. After a 17h overnight food deprivation, rats were permitted to sham feed a high-carbohydrate liquid food (BioServ 40% v:v). All infusions began 12 min after the start of sham feeding and lasted 26 min. Id infusions were 10 ml of tricaprylin (1.25, 1.825 or 2.5 kcal) or 10 ml of 0.15M NaCl. Iport infusions were 1 ml of tricaprylin (1.25 or 2.5 kcal) or 1 ml of 0.15M NaCl. Intakes were measured every 5 min for 60 min.

Results: Id infusions of tricaprylin decreased intake in a dose-related manner, but intraportal infusions did not (see table).

	Percent Inhibition of Intake	
Caloric Load (kcal)	Duodenal Infusion	Portal Infusion
1.25	22.8 ± 8.35	-14.6 ± 10.4
1.825	49.7 ± 8.42	-----
2.5	74.0 ± 6.2	-19.0 ± 20.9

Data are mean ± SE from 5 rats for portal and 7 rats for duodenal infusions. Negative % inhibition indicates larger intake when trycaprylin was infused.

These results demonstrate the same differential satiating potency for tricaprylin (MCT) as we previously reported for Intralipid (98.5% LCT) and support the hypothesis that the major site of the satiating effect of MCT and LCT is preabsorptive.

Supported by St. Luke's Roosevelt Inst. for Health Sci. 6-21435 and NIH NRSA AM07591 (DG); NIMH MH15455, MH40010 and RSA MH00149 (GPS).

129.3 RATS SHAM FEED CORN OIL AND MINERAL OIL, BUT PREFER CORN OIL. S. Mindell* and G.P. Smith. Dept. of Psychiatry, Eating Disorders Institute and Bourne Laboratory, New York Hospital-Cornell Medical Center, White Plains, New York 10605.

Rats prefer fatty foods. It is not clear whether this is due to the orosensory stimuli or post-ingestive effects of fats. To determine if the orosensory stimuli are reinforcing in the absence of postingestive effects, we investigated the effect of corn oil or mineral oil on sham feeding in chronic gastric fistula rats.

Rats were maintained on Purina Rat Chow and were tested after an 18-hour, overnight food deprivation. Group A (N=4) got pure corn oil and group B (N=6) got pure mineral oil to sham feed for 30 min each day. Within 8 days both groups sham fed at stable rates with no difference in acquisition time of sham feeding. After 12 days the mean intake (ml) for 2 tests for group A was 25.4±1.3 and for group B was 23.8±3.9. Group A then received mineral oil and group B received corn oil. Within 3 days both groups had relatively stable intakes; the mean intake for 2 tests for group A was 19.5±2.8 and for group B was 31.3±3.5 ($p<.05$).

Both groups were then given a 30-minute, 2-bottle preference test for two days with both oils available. All rats sham fed more corn oil than mineral oil (corn oil $\bar{X}$=31.5±3.1; mineral oil $\bar{X}$=1.3±0.7). Group A then received corn oil and group B received mineral oil for 3 days (mean intake for 2 tests: group A=21.5±3.0; group B=24.2±4.2). The two bottle preference test was then repeated with very similar results (corn oil $\bar{X}$=27.4±3.0; mineral oil $\bar{X}$=0.2±0.1).

Thus, although rats sham feed both corn oil and mineral oil in 1-bottle tests, they have a marked preference for corn oil in 2-bottle tests. These results demonstrate that in the absence of significant postingestive effects, the orosensory stimuli of corn oil and mineral oil are positively reinforcing because they maintained sham feeding in these rats over a period of at least 8 weeks without evidence of extinction.

[Supported by NIMH MH15455 (GPS).]

129.4 SHORT-TERM FOOD INTAKE AFTER REPEATED INTRADUODENAL GLUCOSE INFUSIONS IN MEAL-FED RABBITS. P.J. Geiselman, A. Acevedo-Cruz*, and L. O'Farrell. Dept. Psychol. and Neuroscience Program, UCLA, Los Angeles, CA 90024.

It has been hypothesized that glucose is satiating when it arrives in the duodenum slowly and is absorbed slowly. Conversely, glucose may stimulate hunger when it arrives in the duodenum quickly and is absorbed rapidly. Compared with free-feeding animals, meal feeders absorb glucose more rapidly and clear glucose from their circulatory systems faster. These responses led to the hypothesis that patterns of meal-taking may determine whether glucose produces satiation or hunger. In the present study, meal-fed rabbits were given duodenal glucose infusions at either a slow or a rapid rate, which had been shown to produce satiety and to stimulate hunger, respectively, in free-feeding rabbits.

Female New Zealand rabbits were chronically implanted with duodenal cannulae and were adapted to a schedule in which chow was available for only 4 hrs daily. Rabbits were infused intraduodenally (10 ml/3 kg BW) with 0.3M glucose, 0.15M NaCl, or were given a mock procedure. Infusion rates were either 1 ml/min or 3 ml/min. Glucose, saline, and mock procedures were repeated for three trials each.

As reported in free-feeding rabbits, meal-feeders also increased their food intake at .5, 1, 2, 3, and 4 hrs following rapid glucose infusion into the duodenum. Interestingly, meal-fed rabbits that received slow intraduodenal glucose infusions also showed an increase in food intake at .5, 1, 2, 3, and 4 hrs postinfusion. These latter results are in contrast to the satiating effect of slow intraduodenal glucose infusion found in free-feeding rabbits. Furthermore, after having received their first slow infusion of glucose into the duodenum, meal-fed rabbits ingested more food in subsequent tests in the saline and the mock conditions. A similar trend was observed following the first fast infusion of glucose. This trend was most apparent in subsequent fast saline conditions. These data suggest that conditioning may have taken place.

These results suggest that the daily pattern of meal-taking can determine whether glucose is satiating or hunger stimulating.

Supported by National Science Foundation under grant no. BNS 87-17377 to PJG.

129.5 FOOD INTAKE AND PLASMA GLUCOSE LEVEL SHIFTS FOLLOWING GLUCOPRIVIC CHALLENGE IN A DESERT-DWELLING RODENT, THE SPINY MOUSE (ACOMYS CAHIRINUS). D.A. Czech, M.M. Ishii,* L.M. Gehrke* and M.A. LaBar*
Dept. of Psychology, Marquette University, Milwaukee, WI 53233.

We previously reported (Czech & Schrank, Soc. Neurosci. Abstr. 12:1294, 1986) that the spiny mouse, like the hamster, gerbil and several murid rodent species, does not exhibit feeding behavior in response to the glucose antimetabolite 2-deoxy-D-glucose (2-DG) (250, 500 & 1000 mg/kg i.p.). In contrast, 2-DG is a potent feeding stimulus in a wide variety of other mammals. In the present study, we extended these observations, investigating Acomys' feeding response to insulin, as well as the time course of shifts in plasma glucose levels following glucoprivic challenge by both 2-DG and insulin.

In the first experiment, male spiny mice were injected s.c. with 4 doses of regular insulin (Iletin) (1, 3, 10 & 30 U/kg) and normal saline in a within-subject design, and powdered food intake to the nearest 0.1 g was measured at 1, 2, 4, 6 & 24 hours. Water was available ad lib. Tests were separated by one week, and began approximately 2 hr into the light period. Cumulative intakes were evaluated with ANOVA and Dunnett's procedures. Significance level was set at $p<0.05$. By the end of the second hour, all doses of insulin, with the exception of the 30 U/kg dose, led to significantly greater food consumption when compared to baseline (vehicle injection) intake. The 30 U/kg dose reached significance at 4 hours. These data are in sharp contrast to the previously observed lack of feeding in response to 2-DG in this species.

In the second experiment, the effect of several doses of regular insulin (1 & 3 U/kg s.c.), 2-DG (250 & 500 mg/kg i.p.) or normal saline on plasma glucose levels was determined in male spiny mice. Blood samples were drawn by retro-orbital puncture technique under brief ether anesthesia, and plasma glucose concentration was determined at 0 (immediately prior to injection), 0.5, 1, 2, 4 & 6 hr (within-subjects), using the glucose oxidase method (Sigma Chemical kit 510-A). Water, but no food, was available ad lib during the sampling period. Plasma glucose concentration was evaluated at each sampling point with ANOVA and Duncan's procedures. Rejection level was set at $p<0.05$. All treatments produced significant hyperglycemic (2-DG) or hypoglycemic (insulin) responses. Following 2-DG, mean glucose peaked by one hour, with the 250 & 500 mg/kg groups reaching 309 & 303 mg/dl, respectively. Plasma glucose in the 1 U/kg insulin group dropped to a low mean level of 52 mg/dl, also within the first hour, while the 3 U/kg group was lowest (18 mg/dl) at the 2 hr sampling point. Only the 3 U/kg insulin group remained significantly different from the vehicle group at the end of 6 hr. Mean baseline plasma glucose (all animals) was 157 ($\pm$ 5 = 1 s.e.m.) mg/dl.

129.6 CARBOHYDRATE RESTRICTED DIETS EFFECT ON BLOOD GLUCOSE, INSULIN, PYRIDOXAL 5'-PHOSPHATE, DOPAMINE-β-HYDROXYLASE AND AMINO ACIDS IN NORMAL AND IMMOBILIZED CAT. L. Thibault* and A.G. Roberge.
Lab. de Nutrition et de Neurochimie, F.S.A.A., Univ. Laval, Québec, Canada, G1K 7P4.

Adult cats were progressively adapted to hypoglucidic semi-purified diets containing casein or soya as protein source to study the effects of a 2 h immobilization period. Body weight of cats fed hypoglucidic diets was significantly decreased. The control casein group showed higher serum dopamine-β-hydroxylase activity and methionine level but lower pyridoxal 5'-phosphate and ammonia content than control soya group. In cats fed hypoglucidic casein diet, serum glucose, insulin, pyridoxal 5'-phosphate and ammonia levels were increased and methionine decreased whereas in cats fed hypoglucidic soya diet, pyridoxal 5'-phosphate content was decreased and dopamine-β-hydroxylase activity increased, when data were compared to their respective control groups. A 2 h immobilization period induced hyperglycemia in all groups whereas in all cats fed soya diets, serum insulin level and dopamine-β-hydroxylase activity were significantly increased and pyridoxal 5'-phosphate content significantly decreased. Immobilization also induced increased serum leucine, isoleucine, valine, phenylalanine, tyrosine and free tryprophan levels in cats fed control casein diet, unchanged serum amino acids in cats fed hypoglucidic casein diet but decreased serum valine, leucine, isoleucine, methionine, phenylalanine and tyrosine levels resulting in increased free tryptophan and tyrosine: neutral amino acids ratios, in cats fed control and hypoglucidic soya diets. These results demonstrate that dietary casein and soya protein might be differentiated on a physiological basis and immobilization emphasized the biochemical disturbances observed between the groups thus suggesting a more acute response to stress in soya groups.

129.7 SUGAR, FATS AND POLYSACHARIDES INHIBIT DISTRESS VOCALIZATIONS AND ELEVATE PAIN THRESHOLDS IN INFANT RATS. D. Shide*, E.M. Blass. (SPON: R.J. Nelson)
Department of Psychology, Johns Hopkins University, Baltimore, Maryland 21218

The discoveries that: 1) neonatal altricial rats can taste and respond to a variety of substances and, 2) endogenous opioid systems appear to be behaviorally functional in 10-day-old rats led us to evaluate interactions between pleasure, psychological and physiological stress. In one experiment, either 7.5% sucrose, corn oil, 2% or 32% polycose was infused through jaw cannulae into the mouths of Day 10 pups that were separated from their mothers but group-housed as a litter. Paw-lift latencies (PLL) from a 48 C hot-plate were determined either 0, 1, 3 or 5 min after termination of sucrose infusions. In a second experiment, individually housed rats received through the indwelling jaw cannula infusions of the same substances. The number of distress vocalizations (DVs) caused by the isolation was recorded on a minute-by-minute basis for experimental and control isolated groups.

Each of these treatments caused a 30-40% decrease in the number of distress vocalizations emitted by Day 10 rats and a 20-100% increase in paw lift latency. The most potent analgesic was 32% polycose which elevated PLL from 19.5 sec (operated control) to 31.2 sec at the end of the polycose infusion. Analgesia remained elevated in all cases even 5 min after infusion termination. These and other behavioral manifestations parallel those induced by intercerebral injections of morphine. They suggest that the positive hedonic affect and the blunting of negative affect by certain substances reflect the taste-release of central endogenous opioids in infant rats.

Supported by Research Grant DK 18560 and Research Scientist Award MH 00524 to EMB.

129.8 FEEDING RESPONSES TO A TRYPTOPHAN DEVOID DIET: CORRELATION WITH BEHAVIOR BUT NOT PLASMA TRYPTOPHAN OR BRAIN SEROTONIN LEVELS IN THE TERRESTRIAL SLUG, LIMAX MAXIMUS. D.W. Gietzen, A.S. Harris*, A. Caprile*, J.M. Larson*, P.M.B. Leung, and Q.R. Rogers. Depts of Physio Sci, Sch Vet Med, Psychiatry, Sch Med, and Food Intake Lab, UC Davis, CA 95616.

Slugs have been shown to depress intake of amino acid deficient diets, as do other species. Since serotonin (5HT) is recognized as a neurotransmitter, and is dependent on the amino acid, tryptophan (TRP), as its precursor, we fed a TRP devoid diet to specimens of Limax maximus and measured terminal brain 5HT and plasma TRP. Cumulative food intake was depressed 50% in a 3 week feeding trial (complete diet group [COM] = 13.3 $\pm$ 1.5 g [mean $\pm$ SE] cumulative intake, devoid diet group [DEV] = 6.7 $\pm$ 0.9 g), in groups of 7 animals with equal body weights at the beginning of feeding. After the 3 week trial, the COM animals weighed 9.6 $\pm$ 1.3g and the DEV animals weighed 6.3 $\pm$ 1.1g. Over 2 weeks, cumulative food intake was again depressed in DEV, to 66% of control (12.3 $\pm$ 1.6 for COM vs 8.4 $\pm$ 1.7g for DEV), with a trend to depressed body weights (57% of COM). It was also observed that the DEV animals seemed to move away more readily during the measurement of body weight. A subjective grading of activity levels on a scale of 1-5 suggested that DEV animals were more active than COM (COM = 2.0 $\pm$ 0.2; DEV = 3.1 $\pm$ 0.5). This experiment was repeated with an additional control group, pair fed to DEV (PAIR). In this trial, activity, as measured by the number of animals with tentacles extended or retracted, was not different between groups. However, time to righting in DEV animals, as measured by the time to touch the whole mantle border to the cage bottom after being placed on its side, was shorter in DEV than either COM or PAIR (χ^2 = 6.14, $p < 0.05$ for probability of time greater than the median time). Thus, the increased mobility was not due to the food deprivation in DEV. At the completion of the second diet trial, perchloric acid extracts of both plasma and brain were analyzed for 5HT and TRP by HPLC with electrochemical detection. There was no measurable TRP in brain, and no measurable 5HT in plasma. There were no differences in either 5HT or TRP between DEV and COM. Values were: Brain 5HT: COM = 67.3 $\pm$ 4.5 ng/mg protein, N = 8; DEV 66.1 $\pm$ 6.3 ng/mg, N = 5; plasma TRP: COM = 92.9 $\pm$ 11.9 ng/ml, N = 6; DEV = 160.9 $\pm$ 32.3 ng/ml, N = 5. Refractive indices for protein in the plasma samples also did not differ. Thus, although we observed 2 behavioral variables, which may be influenced by 5HT, to be altered in the expected direction in the DEV animals, neither 5HT nor TRP concentrations were different between the 2 groups. The inclusion of the PAIR group suggests that the decreases in both food intake and time to righting were not due to the reduction in food intake. The slug may be able to utilize body stores of free amino acids or metabolize endogenous protein in order to maintain the TRP and 5HT levels, but it is clear that the depression in intake of a TRP deficient diet is not due to changes in brain 5HT or plasma TRP concentrations that could be measured after 2 weeks on a TRP deficient diet. (Supported by NIH Grant AM07557).

129.9 VARIATIONS IN SUCROSE CONCENTRATION AND ITS EFFECT ON FOOD INTAKE IN THE DOMESTIC CAT. I.W Castonguay, I.C. Giles*, J.F. Harrison*, and Q.R Rogers. Food Intake Laboratory and Nutrition Department, University of California - Davis Davis CA 95616

Domestic cats do not prefer sucrose, glucose, maltose, or polycose to water (Harrison et al., 1986). When given access to 10% (w/v) solutions of any of these sugars as supplements to a complete maintenance diet, cats fail to adjust their intake of diet and supplement, so as to overconsume on a caloric basis. The present study was conducted to observe the effects of increased concentrations of sucrose on intake and weight gain. It had already been reported that high concentrations of sucrose promote diuresis and diarrhea in cats. The question asked in the present experiment was: "Does the cat alter its consummatory habits in response to increases in sucrose concentration?" Twelve 3 month old cats (6 female and 6 male) weighing an average of 2145 and 2520 g respectively at the start of the experiment were housed individually in stainless steel metabolism cages. After a preliminary baseline period the cats were tested through four cycles of a 5 day sucrose access period followed by a 2 day recovery period during which only water was available as drinking fluid. Water and maintenance diet were available ad libitum throughout the experiment. An ascending series of sucrose concentrations was used (5%, 10%, 15%, 20%). The positions of the water and sucrose solutions were alternated from left to right daily so as to counterbalance for position preference. The experiment was stopped after the 20% sucrose condition due to significant diuresis and diarrhea in most of the animals. Results from the 5 day test periods reveal that neither male nor female cats adjust their intake of food and sucrose supplement so as to maintain baseline caloric intakes. Rather, both sexes overate in response to the 15% and 20% sucrose solutions. Male cats ate significantly more during these periods than did female cats. With access to the 5% concentration, both males and females consumed approximately 5% of their total calories from sucrose. With access to 10% sucrose, the animals ate approximately 8%; with access to 15%, approximately 13%; and with 20% sucrose, approximately 20% total calories from sucrose. No differences in proportion of calories from sucrose between sexes were noted. Consumption of solid diet remained uniform throughout the four sucrose access period, averaging 110 to 120 g/day in males and 80 to 100 g/day in females. These results suggest that cats are not sensitive to the postingestive effects of consuming significant quantities of sucrose. These results also suggest that the cat may be limited in its sensitivity to the caloric content of its diet. These traits may predispose the animal to some forms of dietary obesity.
(Supported in part by NIH Grant AM 07355)

129.10 METHOD OF COMPUTERIZED MONITORING OF MEAL PATTERNS OF ANIMALS ON MULTIPLE DIETS. C. Ian*, G. Shor-Posner*, and S. F. Leibowitz (Sponsor: N. Miller) The Rockefeller Univ. New York, N.Y. 10021

The microstructure of meal patterns in undisturbed rats maintained on 3 diets (protein, carbohydrate and fat), was automatically recorded by computer, at 30 sec intervals, for 24 hrs. This method employed economical, electronic scales, a PC XT computer, data acquisition hardware and simple, custom software.

Sprague-Dawley, male rats were housed individually in modified 9" by 17", wire mesh, stainless steel cages, under a 12:12 hr light-dark cycle at constant temperature. Pure macronutrient diets in glass food jars rested on OHaus, Port-O-Gram C501 electronic scales (500 g capacity and 0.07 g precision) mounted on adjustable platforms. The jars protruded through the bottom of clear, Plexiglas food module boxes attached to the front of each cage. A horizontal shelf with a round hole directly above the jar provided the animal direct access to the food, with minimal effort, and without applying direct pressure on the jar. The animal reached the food through a rectangular hole in the front of the cage. A spillage tray was positioned under the jar to collect and weigh spilled food. This avoided the use of a more restrictive device that might interfere with normal feeding behavior. The scales, calibrated and tested before each test cycle, sent 16 characters of data with each reading, including a question mark indicating an unstable platform and erroneous reading. Data acquisition was facilitated by use of a Stargate OC 8000, 8 channel, expansion board which merged six serial cables into one serial port on the computer housed in a separate room. This allowed continuous data collection with minimal disturbance to the animals. A program in BASICA collected data as often as every second into a DOS text file on a hard disk.

This simple, computerized technique of data collection permitted a precise, continuous recording of small changes in meal taking events in animals on multiple diets. This allowed subsequent analysis of the microstructure of meal patterns over the course of the entire light-dark cycle.

In initial experiments, rats were adapted for 2 weeks, with fresh food and water provided daily, 6 hours prior to dark onset. Food jars were rotated daily to prevent position preference.

A clear and detailed description of rat macronutrient patterns showed a significant circadian distribution of food intake, with peaks of feeding activity occurring at the beginning and end of the dark period. Additional analysis, as described by Shor-Posner et al., revealed specific phases of the nocturnal period are characterized by identifiable and predictable shifts in macronutrient preference and meal size. This useful procedure has multiple applications in feeding and drinking related research and provides remote analysis of multiple diets as well as single or even liquid diets in undisturbed animals.

129.11 NOCTURNAL PATTERNS OF MACRONUTRIENT INGESTION. G. Shor-Posner*, C. Ian*, C. Guerra* and S. F. Leibowitz. (SPON: I. Abramov). Rockefeller University, New York, NY 10021.

Using automated procedures, natural patterns of food intake and macronutrient selection were examined continuously throughout the course of the dark (active) cycle in undisturbed animals. This computerized-technique permitted us to characterize the full sequence of meal-taking events in animals maintained on multiple diets.

Sprague-Dawley adult male rats were provided with pure nutrient diets (protein, carbohydrate and fat) ad libitum and housed in special food module cages under a 12:12 hr light-dark regime. Their patterns of feeding behavior were recorded via computer-interfaced top loading balances (see C. Ian at this meeting).

Analyses of nocturnal feeding behavior indicated that: 1) Rats initiated feeding within 10 minutes after dark onset and consumed between 10-12 discrete meals during the night. The last meal of the cycle was taken approximately 30 min prior to light onset. 2) Meals at the beginning (hrs 1,2) and end (hrs 11,12) of the night were significantly larger than those ingested during the mid-dark phase (hrs 6,7), reflecting a bimodal distribution of feeding activity. 3) Meal frequency tended to remain stable over the course of the nocturnal cycle, although a trend towards increased number of meals during the first two hours was apparent. 4) Specific alterations in patterns of diet selection occurred throughout the dark cycle. At the beginning of the dark period, meals consisted predominantly of carbohydrate (69%). This strong carbohydrate preference was not apparent during the mid-dark hours when no specific dietary pattern was detected. During the late dark hours, in contrast, a very different selection pattern was observed with meals composed primarily of protein and fat. 5) In addition to this dietary shift from the early to late period, we also observed a meal to meal shift of macronutrient intake with alternating carbohydrate and protein meals. These findings of fluctuating macronutrient intake suggest that feeding over the course of the nocturnal period may be differentially regulated and that composition of a specific meal may affect subsequent intake.

129.12 2-BUTEN-4-OLIDE, TUMOR NECROSIS FACTOR AND INTERLEUKIN-1ß ACTING AS FEEDING SUPPRESSANTS. Y. Oomura, C.R. Plata-Salamán*, S. Nemoto*, A. Niijima and I. Matsumoto*. Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka 812; Natl. Inst. for Physiol. Sci., Okazaki 444, Japan.

Regulation of feeding behavior and control of body weight involve neuronal and humoral factors. We investigated the effects of 2-buten-4-olide (2-B40, a new endogenous sugar acid at 3 µM concentration in blood that increases to 15 µM after 48 hr of food deprivation) recombinant human tumor necrosis factor (rhTNF) and recombinant human interleukin-1ß (rhIL-1ß) on food intake in the rat.

The 2-B40 after intracerebroventricular (1.2 to 5.0 µmole/rat), intraperitoneal (0.4 to 1.7 mmole/kg) or intragastric administration decreased food intake dose-dependently by reducing meal frequency, meal size and eating rate, and prolonging meal duration and post-prandial intermeal intervals. Drinking patterns and locomotor activity were not significantly affected. Electrophysiological studies show that 2-B40 suppressed the activity by hyperpolarization of glucose-sensitive neurons in the lateral hypothalamic area (LHA) and facilitated the activity by depolarization of glucoreceptor neurons in the ventromedial hypothalamic nucleus (VMH). This effect was the same as glucose. In other studies, intravenous administration of 100 pmole of 2-B40 increased the activity of several sympathetic nerves including the adrenal pancreatic, hepatic and renal nerves efferents, whereas the vagal efferent nerve activity decreased. This evidence suggests that 2-B40 produces a sympathetic activation, since blood glucose also increased and insulin level decreased. In other experiments, 2-B40 suppressed gastric acid secretion.

Intracerebroventricular microinfusion of rhTNF (50 to 500 ng/rat) or rhIL-1ß (1.0 to 13 ng/rat) suppressed short- and long-term food intake in a dose-dependent manner. The central infusion of heat-inactivated rhTNF or rhIL-1ß had no such effect. Intraperitoneal administration of rhTNF and rhIL-1ß in similar or higher doses than the central administration had no effect. Short-term rectum temperature was slightly increased by rhTNF and rhIL-1ß. Since TNF and IL-1ß are released by monocytes during inflammatory processes, these results suggest that TNF and IL-1ß play a role in the feeding suppression observed during illness by direct action at the central nervous system. The effects on the LHA and VMH are now being investigated.

These findings suggest that these three endogenous substances, 2-B40, TNF and IL-1ß act as feeding suppressants by action at the level of the nervous system.

129.13 FUNCTIONAL ORGANIZATION OF LATERAL HYPOTHALAMIC GLUCOSE-SENSITIVE (GS) AND GLUCOSE-INSENSITIVE (GIS) NEURONS IN THE MONKEY. Z. Karádi*, Y. Oomura, H. Nishino*, S. Aou* and I. Fujita* (SPON: K. Uchizono) Natl. Inst. Physiol. Sci., Okazaki 444, Japan.

GS- and GIS-neurons in the lateral hypothalamic area (LHA) show characteristic neuronal responses during operant bar-press feeding tasks; i.e. the GS-neurons decrease activity during bar-press and reward phases, while the GIS-neurons increase firing to cue light and cue tone. To elucidate these neurons' input-output organization as well as their specific function in the olfactory and gustatory information processing, LHA single unit responses to electric brain stimulations as well as to various odorants and taste solutions were studied.

Electric shocks to the dorsolateral prefrontal and premotor cortices were followed by similar activity changes of the GS- and GIS-neurons. On the other hand, the GS-neurons responded more often to electric stimulation of the orbitofrontal cortex than did the GIS-neurons, while stimulation of the motor cortex evoked firing changes in more GIS- than GS-neurons.

Odor stimuli elicited excitatory and/or inhibitory responses in almost all of the GS-neurons; however, only less than 50% of the GIS-neurons responded to smells. Manyfold olfactory responsiveness, for two or more odors, of the GS-neurons was observed, whereas the GIS-neurons responded predominantly to one specific smell only.

Gustatory stimulations resulted in firing changes of the most GS-neurons, whereas fewer responses of the GIS-neurons were seen. The GS-neurons responded prevailingly to two or more taste qualities, while the GIS-neurons did mainly to only one. The overwhelming majority of the GS-neurons showed "bimodal" responsiveness to both, gustatory and olfactory stimulations, in contrast with the GIS-neurons that responded predominantly to either taste or odor.

On the basis of the present data different functional organization of the GS- and GIS-neurons in the LHA is suggested. The GS-neurons, having strong orbitofrontal innervation, seem to integrate multimodal, mainly "internal" sensory information in regulating different mechanisms related to food intake. On the other hand, the GIS-neurons, with marked motor cortex connections, distinguish specific "external" sensory cues to modulate selection and acquisition of foods.

129.14 HEAT-INDUCED REDUCTION OF FOOD INTAKE IS ATTENUATED IN CAPSAICIN PRETREATED AND VAGOTOMIZED RATS. E.H. South and R.C. Ritter. WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843 and Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Rats and other mammals decrease their food intake when subjected to high ambient temperatures. This heat-induced suppression of intake may depend on thermosensitive neurons in the skin, viscera and/or brain. Systemic pretreatment of rats with capsaicin, a neurotoxin that selectively damages some unmyelinated primary sensory neurons in both somatic and visceral nerves, has been reported to attenuate heat-induced suppression of daily food intake in the rat (Cormareche-Leydier, 1981). In an initial effort to assess the thermosensitive substrates damaged by capsaicin we compared heat-induced suppression of 24 hr rat chow intake and the 45 min intake of highly preferred food (cookies) in rats treated with systemic capsaicin or vehicle only.

FOOD INTAKE AT TWO AMBIENT TEMPERATURES

TREATMENT	N	CHOW(24h)	COOKIES(45m)	CHOW(24h)	COOKIES(45m)
Capsaicin	8	20.6±.6g	4.6±.9g	16.3±4g*	4.5±.7g*
Vehicle	8	20.3±.4g	4.3±.6g	13.8±.5g	2.7±.3g

(* significantly different from vehicle intake)

To further investigate capsaicin's effect on thermosensitive mechanisms, we measured rectal temperature and short term cookie intake in hot ambient temperature in rats treated with 175mg/kg capsaicin (HDC), 25mg/kg capsaicin (LDC) or vehicle. Additionally, we evaluated the contribution of vagal damage to alterations of heat-induced suppression of 45 min cookie intake in rats with subdiaphragmatic or sham vagotomy. We found that HDC and LDC rats ate significantly more cookies in hot temperatures than vehicle rats; however, the HDC rats also had significantly elevated rectal temperatures (39.4 ±.2°C) when compared to either LDC (38.4±.2°C) or vehicle (38.0±.2°C) rats. Vagotomized rats, however, not only failed to reduce their intake in the heat, but actually ate 50% more cookies at 32°C than at 24°C. These data indicate that capsaicin damages the substrate(s) responsible for suppressing food intake at high ambient temperatures. Furthermore it appears that dissociation of capsaicin-induced damage to thermoregulatory mechanisms and mechanisms involved in heat-induced suppression of food intake may be possible by varying capsaicin dose. The data also suggest that afferents in the vagus nerve may be involved in suppression of food intake at high ambient temperatures. Such vagal afferents may represent part of the capsaicin sensitive neural substrate mediating heat-induced suppression of food intake. Additional experiments are underway to test this hypothesis. Supported by NIH grants R01 NS20561 and R01 NS21805.

129.15 SUPPRESSION OF SHAM FEEDING BY INTRAINTESTINAL NUTRIENTS IS ATTENUATED FOLLOWING FOURTH VENTRICULAR CAPSAICIN. D. Fear*, D. Yox, and R.C. Ritter (SPON: M. Hyde). Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional program in Veterinary Medical Education, University of Idaho, Moscow, Idaho 83843.

Information concerning nutrient arrival in the small intestine is carried to the caudal hindbrain by vagal sensory fibers (Ewart and Wingate, 1985). Most of the vagal sensory fibers from the gastrointestinal tract are small and unmyelinated. Previously, we have demonstrated that intraperitoneal pretreatment of rats with capsaicin, a neurotoxin that destroys a subpopulation of unmyelinated sensory fibers in the vagus nerve and elsewhere, attenuates suppression of sham feeding by intraintestinal infusion of certain nutrients (Yox and Ritter, 1986). In order to determine whether this effect of IP capsaicin is due to damage of vagosensory afferents, we applied the toxin via the fourth ventricle near the central terminals of vagal sensory fibers. Rats were pretreated with 300 µg of capsaicin or injection vehicle (40% DMSO in 0.9% NaCl) via the fourth cerebral ventricle. The rats were subsequently implanted with stainless steel gastric cannulae and Silastic duodenal catheters. Rats sham ingested 15% sucrose while intraintestinal infusions of maltose, sodium oleate or casein hydrolysate were made. The nutrient solutions were all 300mOsm/L with caloric concentrations of 0.13kcal/ml. Percent suppression of intake by the various nutrient solutions was calculated relative to the intake following infusion of NaCl (300mOsm/L). Our results, shown in the table, demonstrate that fourth ventricular capsaicin treatment attenuates suppression of sham feeding by intraintestinal oleate and maltose but not casein hydrolysate.

% Suppression of Intake (Mean ± S.E.)

Infusate	Capsaicin	Vehicle
Sodium oleate (C18)	54.9 ± 8.9	78.1 ± 4.4
Maltose	7.2 ± 6.0	27.8 ± 6.0
Casein hydrolysate	15.4 ± 3.7	15.2 ± 4.1

The effect of fourth ventricular capsaicin is similar to that of IP capsaicin. The proximity of the capsaicin application to the vagal afferent terminals in the nucleus of the solitary tract suggests that the ingestive effect of IP capsaicin is due, at least in part, to its action on unmyelinated vagal afferents. The data are also compatible with the proposition that some nutrients that suppress feeding do so by activating a substrate which is not destroyed by capsaicin. Supported by NIH grants R01 NS20561 and R01 NS21805.

129.16 LATERAL HYPOTHALAMIC LESIONS DO NOT ALTER THE ANOREXIA INDUCED BY CENTRAL INJECTIONS OF ETHANOLAMINE-O-SULFATE (EOS). D.V. Coscina, J. Snow* and J.N. Nobrega. Sect. of Biopsychology, Clarke Insti.-Psychiatry, Univ. of Toronto, Toronto, Ontario, Canada M5T 1R8.

EOS is a drug which inhibits the catabolism of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). We have previously shown that intracisternal (i.c.) injections of relatively low doses of EOS produce normal dose-dependent anorexia in three models of chronic overeating plus block the acute overeating induced by systemic injection of insulin or 2-deoxy-glucose (2DG). Given this effectiveness of EOS to reverse or prevent overeating, an understanding of its mechanism of action could prove useful in (a) improving our basic knowledge of the CNS controls over food intake, and (b) perhaps leading to more successful treatments in the prevention of excessive food consumption. Previous work from other laboratories has implicated the lateral hypothalamus (LH) as one brain site which might mediate GABA-induced anorexia. If that is true, one might expect that lesions of the LH would prevent or minimize the anorexia which follows i.c. EOS. To test that possibility, 10 adult female rats received bilateral radiofrequency heat lesions of the LH and 10 other rats received anesthetization as a control treatment. All animals had free access to Purina Lab Chow pellets (4% fat) and tap water at all times during these studies. Following 2 weeks of post-operative recovery, all rats were tested at separate times for their recovery of normal daily feeding and drinking after 0, 100 and 200 ug EOS in 20 ul deionized water injected i.c. under methoxyflurane anesthesia. As in previous work, EOS induced anorexia which increased in magnitude and duration as dosage increased. However, lesioned rats showed the same magnitude and duration of anorexia as controls after each treatment. To determine the functional effectiveness of LH lesions to produce long-term intake deficits, separate tests were conducted of acute feeding in response to intraperitoneal (i.p.) challenge with 2DG (400 mg/kg) or acute drinking in response to i.p. challenge with hypertonic saline (1M NaCl; 1% free-feeding body weight). In both tests, LH-lesioned rats showed only 50% of the intakes shown by controls. These latter data make it unlikely that the lack of differential anorectic response to i.c. EOS was due to insufficient injury to the LH brain axis. Therefore, our data imply that neither the intrinsic cell population of the lateral hypothalamus nor the fibers of passage which normally traverse it represent likely neural substrates upon which EOS acts to induce anorexia.

129.17 MEAL INITIATION FOLLOWING INDUCTION OF TRANSIENT DECLINES IN BLOOD GLUCOSE: EVIDENCE FOR CAUSALITY. L. A. Campfield and F. J. Smith
Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110

We have recently shown that a brief (3-5 min) rise in plasma insulin (I) (45%) preceded the transient declines (-10%) in blood glucose (BG) which occurred approximately 13 min prior to meal initiation (MI) in free-feeding rats. These studies, together with others from our lab, suggest that transient declines in BG are causally related to MI. This hypothesis was tested using an acetylcholine analog (Ach A) known to cause a brief rise in I with a similar magnitude and time course. Ach A was infused IV in an attempt to induce transient declines in BG and the latency to MI was determined. Sixteen female Wistar rats (220 gms) were implanted with both chronic cardiac (blood withdrawal) and femoral vein (infusion) cannulas. Following a 7 day recovery period and return of food intake and body weight to pre-op values, 21 experiments were performed during long intermeal intervals of the light phase. Fresh water and powdered chow were available ad lib. Following IV heparin (100-300 U), BG and meal pattern were continuously monitored for up to two hours in 15 experiments. In 6 other experiments, meal pattern only was monitored to allow comparison without blood withdrawal. Random sequences of up to 4 doses (45-350 µg/kg BW) of Ach A (carbamyl-β-methylcholine chloride) were infused at least 20 min apart until a fall in blood glucose, if any, was observed. In 9 experiments, Ach A infusion (227±30 µg/kg BW) was followed by a transient decline in BG (-9.4 ± 1.3% at 9.4 ± 1.4 min) with a shape similar to that observed prior to meals and MI occurred 17.1±3.2 min after the beginning of the decline in BG. The average meal size was 1.1±0.3 gm. In contrast, in 6 other experiments BG did not significantly change (<5%) following a smaller average dose of Ach A (168±21 µg/kg BW)and no feeding occurred. In 4 of the 6 experiments without BG monitoring, MI occurred 19.6±3.9 min after Ach A infusion (274±19 µg/kg BW). The frequency of induced feeding was greater in the late (79%) than in the early (28%) light phase. Therefore, infusion of Ach A induced transient declines in BG followed by MI with an average latency of 17 min, while smaller doses of Ach A did not result in significant declines in BG nor feeding. These studies demonstrate the experimental induction of MI during the long intermeal intervals of the light phase in non-deprived rats but only following a brief fall and rise of BG. The latency of MI following Ach A corresponds to the temporal relationship between a rise in I and MI in free-feeding rats. These studies provide strong support for a causal relationship between transient declines in BG and MI under free-feeding conditions. We conclude that transient declines in BG are among the endogenous factors controlling meal initiation.

129.18 SHORT TERM EFFECTS OF FRUCTOSE ON MEAL INITIATION AND BLOOD GLUCOSE DYNAMICS. F.J. Smith, D. M. Whyte* and L. A. Campfield
Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110

We have previously shown that small (-11%) transient (~18 min) declines in blood glucose (BG) which precede feeding are causally related to meal initiation (MI) under free-feeding conditions. We have also shown that IV infusions of glucose (0.2 ml of 10%) that blunt these declines can delay MI. Since oral and IV fructose (F) have been reported to cause transient hypoglycemia, we used F infusions to test the specificity of glucose to delay MI and to mimic the transient declines in BG. Chronic cardiac and femoral cannulas were implanted in 13 female Wistar rats (250 gm). Following a 7 day recovery period and return of food intake and body weight to pre-op values, continuous monitoring of BG and meal pattern was performed in the light (n = 10) and dark phases (n = 5). Fresh water and powdered chow were available ad lib. Following IV heparin (100-300 U), blood withdrawal (25 µl/min) was begun and continued for up to 2.5 hours. When F (0.2 ml of 10%) was infused IV during a transient decline in BG, MI occurred with a normal latency. Random sequences of F (dose range: 0.05-0.3 ml of 10%), separated by at least 30 min, were then infused IV during intermeal intervals. During the early dark phase, IV F was followed by a slight decrease or increase in BG (-4 to 10% at 6 min). In the light phase, however, three types of dose-dependent declines in BG were observed: a fall to a suppressed level (-6%) for at least 30 min (n = 4); a transient fall (-10% at 8 min) and a delayed return to baseline at 28 min (n = 4); and a transient fall (-9% at 8 min) and return to baseline at 17 min similar to that seen prior to MI (n=3). In these 3 experiments, MI occurred with a latency within the normal range (14.5 ± 1.5 min). No feeding was observed following the other F infusions. Similar effects on BG were observed following oral administration of a range of F doses (0.5-1.5 ml of 50%) in 2-hour fasted rats. These results demonstrate that F was not able to uncouple BG from MI and that both oral and IV F were followed by small, rapid declines in BG in the light but not in the dark. Since the perturbations in BG following F were similar in magnitude to the transient declines in BG prior to meals, these studies suggest that they were detected by peripheral and central glucose receptive elements. Thus, the conclusion that fructose infusions have no effect on the CNS or access to glucose receptive elements in the CNS may have to be re-evaluated.

129.19 EFFECT OF FORCE FEEDING ON WEIGHT AND SUBSEQUENT FOOD INTAKE OF WEIGHT-GAIN "RECOVERED" AREA POSTREMA-LESIONED RATS. * Nancy J. Kenney, Jon N. Kott, C. Shawn Kreig, A. Susan Ruiz and Naomi Tomoyasu. Dept. Psych. Univ. of Washington Seattle WA 98195.

Ablation of the area postrema and subjacent medial commissural nucleus of the solitary tract (APX) of rats results in transient hypophagia and permanent weight loss. We have reported that force feeding APX rats immediately following the ablation (the period of maximum hypophagia and weight loss) results in weight gain similar to that of intact rats. When force-feeding ceases ad lib intakes of the previously force-fed APX rats remain similar to those of intact rats. No weight loss ensues. In this study, effect of force feeding begun one month after APX is examined.

Twelve rats with thermal AP/cmNTS lesions (APX) and 14 sham-lesioned (SHAMX) rats were studied. Five APX and 6 SHAMX rats were fitted with gastric catheters for force-feeding. All surgery was performed under pentobarbital anesthesia. Rats were fed a milk diet throughout the experiment. Force feeding of catheterized rats began 1 month after APX or SHAMX and continued for 14 days. Non-catheterized rats continued to ingest milk ad lib. All rats were returned to ad lib feeding for the last 8 days of the study.

Force-Feed Period: Force-fed APX gained more weight than ad-lib-fed APX or SHAMX rats or force-fed SHAMX rats. Average weight change from the day of lesion to the end of force feeding was significantly greater for force-fed APX rats than for ad lib-fed APX rats ($p< 01$) but did not differ from that of either group of SHAMX rats. Average weight gains and food intakes of ad lib-fed APX and SHAMX rats did not differ over this period.

Period After Force Feeding: Return to ad lib feeding had no effect on intakes or weight gains of previously force-fed SHAMX rats. The two SHAMX groups did not differ in daily intake or weight change over these 8 days. Upon return to ad lib feeding, intakes of the previously force-fed APX rats were reduced compared to those of APX rats fed ad lib throughout the study ($p<.01$). The previously force-fed APX rats lost weight rapidly. At the end of the study, weight change from the day of lesion of previously force-fed APX rats did not differ from that of APX rats fed ad lib throughout.

These results show that APX rats do gain weight in response to elevated caloric intakes. While our previous data indicate that force feeding immediately after APX induces permanent increases of weight, the current findings show that later force feeding has only transient effects on the body weights of APX rats.

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129.20 INTRAPERITONEAL BUT NOT INTRAGASTRIC PHENYLALANINE SUPPRESSES SHORT-TERM FOOD INTAKE IN MALE RATS. R.J. Bialik, E.T.S. Li* and G. H. Anderson, Dept. of Nutritional Sciences, University of Toronto, Toronto, Canada M5S 1A8.

Intraperitoneal (ip) administration of amino acids, such as phenylalanine (PHE), suppress short-term food intake (FI) in rats. This effect is generally considered to be the consequence of an increase in the concentration of the amino acids in the plasma and/or brain. If this is the case, other routes of administering PHE should also cause FI suppression when similar increases in plasma and brain levels of PHE are achieved. The present study compared the effects of intragastric (ig) and ip administration of PHE on short-term FI and PHE levels in plasma and brain of male rats. Rats, adapted to a 12 h (1900-0700 h) feeding schedule (food was available only during the dark), received on different days either PHE (as the methyl ester) or an equamolar dose of alanine methyl ester (ALA) 30 min prior to food presentation. ALA, which did not affect FI, served as a control for osmolarity and nitrogen content of the treatment solutions. It was found that 90 mg/kg was the lowest dose that suppressed FI during the first 2 h of feeding when PHE was given by ip injection (mean difference score = 2.0±0.6 grams, $P<0.02$). FI was not affected by ig administration of PHE at any of the doses tested (90,180,360,540 or 720 mg/kg). At the time that food was presented (1900 h), levels of PHE were similar in plasma and brain (approx. 200% and 125%, as % Baseline: Baseline = 7.49 µmol/dl plasma and 52.3 nmol/g brain) following ip and ig administration (90 mg/kg). However, the peak levels of PHE in plasma and brain were higher, and occurred earlier, following ip (411% at 5 min and 157% at 10 min) than following ig administration (188% and 126% at 15 min). The failure of ig PHE to suppress FI was not due to lower plasma or brain levels of PHE. At a dose of 720 mg/kg ig, PHE did not affect FI although, at the time of food presentation, plasma (860%) and brain (215%) PHE concentrations were much higher than were observed following a threshold dose of PHE given by ip injection. We conclude that FI suppression following PHE administration is not determined simply by the peak plasma or brain concentration of this amino acid.

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130.1 PREFERENTIAL MOTONEURONAL DENDRITIC GROWTH INTO SYNAPTOGENIC FIELDS: A COMPUTER ASSISTED QUANTITATIVE STUDY OF GOLGI IMPREGNATED DEVELOPING MOUSE SPINAL CORD. J. E. Vaughn and R. P. Barber.* Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010

The branching of neuronal dendrites may be influenced by initial synaptic relationships formed by afferent axons with dendritic growth cone filopodia. This synaptotropic hypothesis of dendritic branching predicts that dendritic growth will be biased into regions containing numerous prospective presynaptic elements. The developing mouse spinal cord provides a natural experiment to test this prediction because synapses are only found within the marginal zones bordering the motor columns during the early period of synaptogenesis (embryonic days 11-14, E11-14). Therefore, during this time, most motor dendritic growth would be expected to be directed externally into the marginal zones, whereas internally directed growth should become more prevalent later when the formation of synaptic junctions begins to take place within the intermediate zone, i.e., the motor columns proper.

A computer assisted three dimensional reconstruction system has been used to test these predictions in Golgi impregnated preparations of developing mouse (C57BL/6J) spinal cords ranging in age from E13 through postnatal day 1 (P1). Mean dendritic lengths and branch densities are significantly greater ($P < 0.001$ and < 0.01, respectively) for marginal zone dendrites than for intermediate zone dendrites at early ages (E13-14), but there are no significant differences in these measures at later stages of development (P0,1). These results suggest that motor dendritic growth is initially biased into the marginal zone by synaptogenic afferents, but that this preferential distribution is progressively lost as synapses develop within the intermediate zone to attract or stabilize internally directed dendritic growth. Thus, the findings of this study are consistent with predictions of the synaptotropic hypothesis of dendritic branching. Supported by NSF grant BNS-8219831.

130.2 COMPUTER SIMULATIONS OF NERVE FIBRE OUTGROWTH. S.M. Bunt and G. Rowe*. Departments of Anatomy and *Mathematics, University of Dundee, DD1 4HN, U.K.

We have produced a computer simulation of nerve fibre growth in a defined pathway based on the fibres detecting the concentrations of an activator and inhibitor in the environment through which they grow. Manipulations of this simulation have identified the values of certain parameters which cause the model to produce fibre outgrowth in a pattern similar to that observed in vivo.

We have used an adaptation of Gierer and Meinhardt's reaction-diffusion model to set up a two dimensional gradient of activator and inhibitor over which the "nerve fibres" extend, sampling varying areas of the environment as they grow. In this model the activator catalyses both its own production and that of the inhibitor while the inhibitor suppresses the production of activator. The activator is assumed to be a large molecule which can only diffuse slowly and therefore causes short range interactions while the inhibitor is assumed to be a smaller, faster diffusing molecule and therefore causes long range interactions (Gierer, A. and Meinhardt, H., Kybernetik, 12:30, 1972).

We have varied the size of the area sampled by the simulated "growth cone" and the irregularities in the underlying gradients. The ability of the fibres to find their way across a field increases with the size of the area sampled by the growing fibre and decreases as the irregularities of the underlying gradients are increased. We have also varied the speed of growth of the "axon" and the timing of the setting up of the gradients. One of the more interesting results of this procedure has been the observation of moving waves in the underlying gradients which can cause fibres to stop in different parts of the field depending on their time and speed of outgrowth, thus setting up a pattern without any copy of the pattern ever existing in either the underlying gradients or the labelling of the fibres.

This work is supported by the M.R.C. and the Nuffield Foundation.

130.3 PREFERENTIAL REGENERATION OF SPINAL AXONS THROUGH THE SCAR IN HEMISECTED LAMPREY SPINAL CORD. D.I. Lurie* and M.E. Selzer. David Mahoney Institute of Neurological Sciences and Dept. of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Spinal axons of the large larval sea lamprey (4-5 years old) regenerate selectively in their normal rostrocaudal direction and on the correct side of the cord (Yin et al, Science 224:894-896, 1984). The scar in the lamprey, unlike that in mammals, is not an absolute impediment to axonal regeneration and directional specificity is maintained by neurites growing distal to it. We wished to determine whether the scar provides active support for regeneration in the sense that axons would grow preferentially through the scar rather than around it. Therefore, spinal hemisections were made at the level of the 3rd gill in 15 larvae and the animals allowed to recover for 10 weeks. The large reticulospinal neurons (Muller and Mauthner cells) or their giant axons were impaled with HRP-containing mircoelectrodes and injected with label. Wholemounts of brain and spinal cord were stained with the Hanker-Yates reagent and the regenerating neurites visualized. Forty-one of 74 regenerating neurites (55%) grew beyond the level of hemisection. Of these, 33 (80.5%) grew through the scar to remain on the correct side while only 8 (19.5%) crossed the midline growing around the scar.

It is possible that this tendency for axons to regenerate through the scar reflects the greater availability of empty spaces on the hemisected side. In order to rule this out, 14 animals received double simultaneous hemisections at the level of the 3rd gill on the left and the 7th gill on the right. In this case, both hemicords contained large numbers of degenerating axons and potential empty spaces, both rostral and caudal to the scars. Nevertheless, axons grew through their ipsilateral scar rather than around it. Seventy-nine of 132 neurites (60%) regenerated beyond the site of the ipsilateral hemisection. Of these, all grew through the scar and none crossed to the contralateral side.

We conclude that in order to regenerate on their appropriate side, lamprey spinal axons grow preferentially through a scar rather than around it. This suggests that the scar may play a positive role in supporting axonal regeneration. (NIH Grant NS 14837).

130.4 EARLY CROSS-STRIATION FORMATION IN TWITCHING XENOPUS MYOCYTES. Y.Kidokoro and M.Saito*. Jerry Lewis Center,UCLA School of Medicine,Los Angeles,CA 90024.

Growing neurites release acetylcholine (ACh) even before contacting target muscle cells in culture (Young and Poo, Nature, 305:634, 1983; Hume et al., Nature, 305:632, 1983). Physiological significance of this phenomenon is yet to be discovered. In Xenopus myocytes we found that spontaneous release of ACh from growing neurites caused twitches and that these twitching myocytes developed cross-striation earlier than those without nerve contact or those with nerve contact but without twitching in the same culture. This effect was blocked by 0.1 μM α-bungarotoxin.

Nerve-muscle co-cultures were prepared from Xenopus embryos at stage 14 shortly after completion of gastrulation (Nieuwkoop and Faber, Normal Table of Xenopus laevis (Daudin), Elsevier, Amsterdam, 1967). They were plated on the collagen coated cover glass in culture medium (60 % strength of DMEM supplemented with 5 % horse serum). After one day of incubation at room temperature (21-22 °C) many cells attached to the substrate. A great majority of them were myocytes and some of them had a neuron adhered on the surface. About two third of nerve-contacted myocytes twitched spontaneously. These twitches were blocked by α-bungarotoxin indicating that they were caused by spontaneous release of ACh from neurons.

Cross-striation of non-nerve-contacted myocytes first developed at about 23 hours after plating. 44 hours after plating virtually all myocytes were cross-striated. The time for 50 % of non-nerve-contacted myocytes to acquire cross-striation was about 29 hours. Nerve-contacted and twitching myocytes developed cross-striation much earlier. At 23 hours a majority of twitching myocytes showed clear cross-striation. Nerve-contacted but non-twitching myocytes did not develop striation earlier than non-nerve-contacted myocytes.

Iontophoretic application of ACh caused twitches in non-nerve-contacted myocytes. After a few hours of twitching at a rate between 0.1 and 1 Hz these myocytes developed cross-striation. The myofibrils may self-organize to exhibit cross-striation when they are activated to contract.

It is plausible in the animal that successful innervation by motor nerve promotes differentiation of the target muscle cell.

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130.5 A POSSIBLE ROLE FOR SOMATOPLEURAL TISSUE IN SPECIFIC MOTONEURON QUIDANCE IN THE EMBRYONIC CHICK HINDLIMB. M. Dias* and C. Lance-Jones, Dept. of Neurobiology, Anatomy and Cell Science, Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261

Although the formation of motoneuron projections to individual muscles in the embryonic chick limb has been shown to involve the specific recognition of environmental cues, the source of these cues is unknown. Experiments carried out in our laboratory and that of Keynes suggest that the lumbosacral (LS) somites or their derivatives, the limb muscle cells, are not the primary source. Another candidate is the somatopleural mesoderm that lies lateral to the LS somites and gives rise to all limb connective tissues. To examine this hypothesis, the somatopleure (somatopleural mesoderm + overlying ectoderm) adjacent to the LS somites was removed from one side of stage 14-15 chick embryos and shifted anteriorly to replace the last 1-3 segments of thoracic (T) somatopleure. Since muscle cell migration does not begin until stage 16, only prospective connective tissues within the limb are thought to be shifted.

Embryos were incubated until stages 28-36 when limb nerve patterns were examined. Thirteen embryos had limbs which were clearly shifted 1 (n=7), 2 (n=5), and 3 (n=1) segments anteriorly. Within these limbs nerve trunks were positioned appropriately but received spinal nerve contributions from more anterior segments than normal. Crural trunks that normally receive contributions from LS1-3 received contributions from T7-LS2, T6-LS1, or T5-T7. Sciatic trunks that normally receive contributions from LS3 and LS4-8 received contributions from LS2-3, LS1-3 or T7-LS3 and a variable number of more posterior spinal nerves. In every limb 1-3 novel muscle nerves were found coursing from sciatic trunks to anterior muscles that are normally innervated by crural nerves. As the sciatic trunks contained axons that would normally course within crural trunks, an interpretation of these findings is that specific course corrections have been made by mischannelled axons in response to guidance cues from the somatopleure. Two pieces of evidence support this idea. First, in two embryos we have identified motoneuron projections to anterior muscles via horseradish peroxidase labelling and found them to be correct with respect to segment of origin. Second, we find a clear relationship between the extent of the shift and the specific novel muscle nerves present. For example, novel nerves to the femorotibialis muscle were most common. This muscle is normally innervated by axons in LS2 and LS3 or those axons most commonly channelled into the sciatic. While preliminary, these data suggest that axon pathway choices may be made on the basis of cues arising from the somatopleure or its derivatives. Supported by NS-8518864.

130.6 GROWTH CONE INTERACTIONS WITH ANTERIOR AND POSTERIOR SCLEROTOME. K. W. Tosney. Department of Biology, University of Michigan, Ann Arbor, MI, 48109

The somites have long been regarded as important to the development of spinal nerve segmentation. Keynes and Stern (1984, *Nature* 310:786) elegantly showed that axons prefer the anterior of each somite for outgrowth. Axons extend through the ventral portion of the somite, the sclerotome, which is essential for segmentation (Tosney, 1986, *Soc. Neurosci. Abs.* 12:174). I examined anterior (A) and posterior (P) sclerotome populations with scanning electron microscopy to determine if tissue-level differences could be important for segmental outgrowth. I found that differences are of insufficient magnitude or develop too late to explain the absence of outgrowth into P sclerotome. The important population differences may, therefore, lie at the cellular level and be accessible *in vitro*.

Growth cones may avoid growing into P sclerotome because they cease motile activity (become "contact paralyzed") upon contacting P cells. To test this hypothesis, I videotaped interactions between motoneurons and A or P cells on laminin substrata in culture. Motoneurons were labeled by injecting spinal nerves with DiI, a non-toxic fluorescent dye (Honig, and Hume 1986, *J.Cell Biol*, 103:171) 5-8 hours before explanting small pieces of the ventrolateral spinal cord. A or P cells were labeled with rhodamine.

I found that motoneuron growth cones were not contact paralyzed by either population: they continued to extend processes after contact. However, preliminary results show intriguing differences in the interactions. Motoneuron growth cones often extended onto and over the surface of A cells. In contrast, when they encountered a P cell, they generally remained on the substratum. In addition, their forward progress was often delayed. They continued to extend filipodia but remained in place, moved around the side of the P cell or advanced only after the cell moved away. If these preliminary results hold up, they will suggest that motoneuron growth cones prefer anterior over posterior sclerotome cells as a substratum and that their preference may, at least in part, cause their segmental pattern of outgrowth.

#Supported by NIH grant NS 21308 and a Rackham Grant to Augment International Partnerships. I obtained preliminary results using video in the laboratory of Dr. J. Raper, Max-Planck-Institut für Entwicklungsbiologie, Tübignen, West Germany. I am grateful to him, Dr. F. Bonhoeffer and Dr. S. Chang for their kind assistance.

130.7 MONOCLONAL ANTIBODIES RECOGNIZE SEVERAL DEVELOPMENTALLY REGULATED PROTEINS IN THE HAMSTER PYRAMIDAL TRACT. K. Kalil and M. Perdew* Dept. of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706

The hamster pyramidal tract continues to grow into the spinal cord until two weeks postnatal. We were interested in proteins whose expression might be correlated with the late development of the pyramidal tract. We made a series of monoclonal antibodies directed against infant pyramidal tract or a growth cone preparation of young hamster brains, and found that at least three of these recognized developmentally regulated proteins correlated with the late outgrowth of the pyramidal tract.

The first of these was obtained from a fusion in which immunization was carried out using a homogenate of lightly fixed medullary pyramidal tract from 3-day-old hamster brains. The antibody recognized the 200 kDa subunit of the neurofilament protein, as determined by immunoblotting with isolated neurofilament proteins. Indirect immunofluorescence with ammonium precipitated ascites was used to localize the antigen on cryostat sections of the brain and spinal cord of 1 to 23-day-old hamsters. At birth the long tracts, as well as the local fiber systems of the brainstem and spinal cord, were brightly fluorescent. In contrast, the pyramidal tract was almost completely unstained at 2 weeks, and by 23 days contained only sparse punctate labeling. If the 200 kDa subunit serves a cross-bridging function in the neuronal cytoskeleton, then the late appearance of this element of the neurofilament protein may confer upon these fibers an extended period of plasticity in which regrowth after injury can occur.

Additional monoclonal antibodies were obtained from fusions in which mice were immunized with growth cone membranes prepared from 3-day-old hamster brains by subcellular fractionation. Two of these, 3-11G and 6-4E, recognized proteins that appear to be developmentally regulated, since immunofluorescence revealed ubiquitous staining on brain and spinal cord sections up to 1 week postnatal but at 9 days showed restriction of fluorescence in the spinal cord to the pyramidal tract. Within the cerebellum, labeling was restricted to a region of the molecular layer just dorsal to the Purkinje cells. Thus, both antigens appear in pathways that continue to develop relatively late postnatally. The 3-11G antibody recognizes a 30 kDa polypeptide whose developmental decline was quantified by ^{125}I-streptavidin immunoblotting of a crude synaptosomal preparation of hamster brain separated by SDS PAGE. Preliminary results suggest that 3-11G recognizes a membrane associated protein that, in agreement with the immunohistochemistry, shows the steepest decline during the first postnatal week.

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130.8 ESTABLISHMENT OF A SENSORY PATHWAY IN THE AMPHIBIAN TAIL SPINAL CORD. R.H. Nordlander. Dept. of Oral Biology, Case Western Reserve University, Cleveland, OH 44106.

Dorsal roots are absent in the tails of Xenopus larvae. Instead, central axons of sensory ganglion (SG) neurons of the tail enter the spinal cord via ventral roots (Awwiller and Nordlander, these proceedings, 1985). Once in the cord, these axons take a diagonal path rostrally and dorsally through the substance of the cord to reach the dorsolateral (DL) tract in which they travel to the hindbrain. The purpose of the present study was to characterize the initial ingrowth of central SG axons and, in particular, to describe the morphology and behavior of their growth cones as they find their way across the cord.

Central SG axons and their growth cones were filled with horseradish peroxidase (HRP) applied to the peripheral processes or cell bodies of SG neurons at the level of the 20th myotome in embryos and larvae (stages 32-48). After appropriate diffusion times (20 to 120 min), spinal cords were processed with conventional DAB methods and cleared with glycerine for viewing as wholemounts.

Sensory ganglia of the tail first appeared during late embryonic stages (35-38) as small clusters of fusiform cells sitting on ventral roots at the point of their bifurcation into dorsal and ventral rami. The earliest diagonal central SG fibers were seen in the tail cords several stages later. These early axons had very small diameters, but, later some thicker axons were also observed. SG axons from a single segment traveled in a closely packed arrangement within the ventral root. Upon entering the cord they spread out and traversed the cord individually, recoalescing when they joined the DL tract.

Growth cones at the lead of SG axons were large and showed complex configurations as they navigated from the ventral root entry point to the DL tract. These growth cones frequently extended filopodia rostrally or caudally into longitudinal axon bundles of the lateral marginal zone. Veils commonly appeared at dorsal and rostral borders of the growth cones, sometimes conforming to the contours of axons within the longitudinal bundles they were crossing. Yet, despite these evidences of diversion of dalliance by the growth cones, the axon trailing behind each followed a clear diagonal path as described above. Growth cones retained some complexity as they first entered the DL tract, but became more streamlined and simple at progressively more rostral levels.

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130.9 TRANSIENT AXONAL GLYCOPROTEINS DEFINE PATHWAY SELECTION BY DEVELOPING SPINAL CORD AXONS. J. Dodd, S.B. Morton*, D. Karagogeos* and T.M. Jessell. Center for Neurobiology and Howard Hughes Medical Institute, Columbia University, New York, NY 10032.

An initial step in the establishment of specific neural connections is the selection of distinct substrate pathways by developing growth cones and axons. To determine mechanisms that contribute to the guidance of axons in mammalian CNS we have analyzed pathway selection by spinal cord projection neurons. Two major classes of supraspinal projection neurons differentiate in the dorsal region of embryonic rat spinal cord. Association (A) neurons project radially and ipsilaterally in the transverse plane before turning rostrally to form the lateral funiculus. Commisural (C) neurons project ventrally and medially in the transverse plane, cross the midline at the floor plate (FP) and then fasciculate in a rostro-caudal plane to form the contralateral ventral funiculus. Extensive overlap in the spatial location of neuronal cell bodies and in the timing of axon extension of A and C neurons suggests that they respond initially to distinct guidance cues. C axons approach the midline bilaterally, yet project rostrally only after crossing the FP. However, the bilateral symmetry of C axon projections implies that equivalent cues should exist on both sides of the midline. Guidance could be achieved by modification of the surface properties of growth cones and axons during passage across FP cells. Growth cones and axons may, therefore, be capable of responding to guidance cues only after such modification.

To analyze cell surface changes during C neuron pathway selection, we generated monoclonal antibodies (MAbs) that identify antigens expressed selectively by C axons, and FP cells. C axons were identified by expression of a series of cell surface glycoprotein antigens (C-TAGs) with MW's in the range 110-160 Kd. FP cells were identified by expression of keel antigens K1 and K2. C-TAGs are expressed on C neurons as they differentiate adjacent to the roof plate of the spinal cord in E11 embryos and on C axons as they project ventro-medially in the transverse plane. The expression of C-TAGs decreases on C axons after crossing the midline and C axons established in the ventral funiculus do not express C-TAGs. By E15, when all C neurons have projected across the midline, expression of C-TAGs has terminated. The NILE/L1 glycoprotein exhibits a converse distribution; it is not present on C axons projecting in the transverse plane but appears after fibers have passed across the FP. Cell surface glycoprotein expression by C axons is, therefore, modified during passage across FP cells. Such modification may confer or reflect the ability of C axons to respond to guidance cues only after crossing the midline.

Midline guidance cues may involve differential cell adhesion. NCAM is located selectively on FP cells and C axons but not on adjacent neuroepithelial cells in E11 rat spinal cord. The extension of C axons along the contralateral edge of FP cells may involve NCAM-mediated adhesion between C growth cones and FP cells.

130.10 EXPRESSION OF NEURONAL ADHESION MOLECULES ON CULTURED NEURONS. A.N. van den Pol, M. Ellisman#, M. Manthorpe¶, K. Anderson#, W. Stallcup+. Sect. Neurosurgery, Yale Univ. Med. Sch., New Haven, CT 06510; #Dept. Neurosci. and ¶Biology, UCSD, La Jolla, CA 92093; +La Jolla Cancer Res. Ctr., La Jolla, CA 92037.

To examine the distribution of several plasmalemmal molecules involved in adhesion of neurons, we used colloidal gold immunocytochemistry on primary cultures of CNS neurons from rat and chicken. Antisera against rat NILE (Stallcup et al., J. Neurosci. 5:1090, 1985) and against the chicken homologue of rodent L1 (Rathjen et al., J. Cell Biol. 104:343, 1987; gift of Dr. U. Rutishauser) were used. Cells from the rat hypothalamus (E18) or cerebellum (P6), and from the chicken forebrain (E8) were dissociated and grown on polyornithine or laminin/polyornithine treated plastic or glass substrates for light and electron microscopic thin section evaluation. Additionally, cells were grown on formvar coated gold EM grids, stabilized with carbon, and treated with polyornithine and laminin. After several days in culture, cells were fixed with paraformaldehyde and immunolabeled with 5, 10, or 15nm colloidal gold adsorbed to secondary antisera. Following immunolabeling, whole mounts of cells grown on EM grids were further stabilized with a light platinum rotary shadowing which facilitated visualization of plasmalemmal membranes, but did not obscure the gold particles (Ellisman et al., JEMT, in press).

Chicken forebrain cultures contained predominantly neurons as determined with tetanus toxin staining and morphology. These cells grew short neurites with extensive growth cones and filopodia radiating out from the perikaryon. Gold immunolabeling of the L1-homologous antigen was found on neurites, perikarya, and on growth cones including their lamellipodia and filopodia, but was absent from control cells of meningeal origin. Cultures from rat brain contained a heterogeneous population of neurons and glia; long neurites extended from neurons. With both fluorescent microscopy and with ultrastructural analysis of NILE immunolabeling, the high density of colloidal gold varied between neurites but was minimal on underlying astrocytes. Additional experiments using cells grown on grids and labeled with antisera against chick or rodent NCAM confirmed our previous ultrathin section studies (van den Pol et al., J. Cell Biol. 102:2281, 1986) demonstrating the presence of this molecule on neuritic growth cones.

The presence of these antigens (similar or identical to L1, NILE, NgCAM) and NCAM on growth cones and associated filopodia is consistent with hypotheses suggesting that these molecules may contribute to axonal adherence and guidance.

130.11 INTRINSIC DIFFERENCES IN THE GROWTH RATES OF EARLY EMBRYONIC SENSORY NERVE FIBERS Alun M. Davies Dept. Anatomy, St. George's Hospital Medical School, Tooting, London SW17 ORE, U.K.

The distance axons have to grow to reach their targets in development varies form one population of neurons to another. To investigate whether there are intrinsic differences in the growth rates of axons from different populations of neurons at this early stage of development, and if so, whether growth rate is related to the distance the axons have to grow to their targets in vivo, a comparative study of the growth of early embryonic cranial sensory neurons in culture was carried out. The trigeminal (maxillo-mandibular lobe), geniculate, petrosal and nodose ganglia were dissected from chick embryos at the stage of development during which the earliest neurons of these ganglia are being born and the earliest nerve fibers are growing to their targets (at embyronic days 3 and 4). These neurons were chosen because the distance between their peripheral and central targets increases in the order: trigeminal<geniculate<petrosal <nodose. The neurons were grown in low-density, dissociated culture on a laminin substratum in Fl4 medium supplemented with fetal calf and horse sera. These early neurons exhibited a characteristic bipolar morphology with fine, straight, unbranched neurites extending in opposite directions. In each culture the neurite lengths of over 50 individual neurons were measured serially at 2 hourly intervals for the first 18 hours in culture. These measurements showed that there are significant differences in neurite growth rates between these populations of sensory neurons, the rate being slowest for trigeminal neurons and increasing in the order: trigeminal<geniculate< petrosal<nodose. The growth rate of nodose neurons was between 2 and 3 fold greater than trigeminal neurons.

These findings indicate that there are intrinsic differences in the growth rates of nerve fibers from pre-target-encounter sensory neurons, and raise the possibility that these differences are related to the distance the corresponding axons have to grow to reach their targets in vivo (i.e., those that have further to go grow faster).

131.1 REPRESENTATION OF VESTIBULAR AFFERENTS IN FIFTH SOMATIC SENSORY AREA (SV) IN THE CAT. A.Mori, T.Yoshida*, H.Hiraba* and R.Sumino*. Nihon University School of Dentistry, Department of Physiology, 1-8-13 Kanda-surugadai, Chiyoda-ku, Tokyo 101, Japan.

By using the unit technique, we have found that a new and complete somatic sensory area of the body surface of the cat is located in the rostro-caudal part of the medial bank of the anterior suprasylvian sulcus (ASSS). We have designated this area as the Fifth Somatic Sensory Area (Mori et al. Neurosci. Abstr.15, 1985). In the present study we examined projections from the vestibular organ to the SV.

Under ketamine anesthesia a chamber was installed over the skull surrounding the SV. A tungsten in glass microelectrode was inserted into the SV and the receptive fields of neurons were examined by using natural somatic stimulation. The spikes were monitored on the oscilloscope through a window discriminator. A bipolar electrode, which used electrical stimulation, was placed on the round window. The sites of penetration were recorded on photographic prints of the cortical surface, taken through a dissecting microscope. The electrode tracks were reconstructed using the lesions made during the experiments, and analyzed with respect to this region, using the criteria defined by Hassler and Muhs-Clement (1964).

The activity of 278 neurons was recorded through 47 electrode penetrations made in the SV. 82% (228) of the responsive neurons were activated only by somatic sensory stimulation of the contralateral side. 18% (50) of the responsive neurons were activated by both vestibular and somatic stimuli of the contralateral side. Many of these multimodal vestibular neurons were found to be located in the rostral area of the medial bank of the ASSS. However, unmultimodal vestibular activated neurons were not found in the SV. Although multimodal vestibular activated neurons received inputs from a wide area of the face and/or neck and neck and/or trunk of the contralateral side, the forelimb and hindlimb areas of the SV did not converge from the vestibular afferents. In conclusion, these findings suggest that neurons of the SV have an integrational function of inputs of somatic and vestibular afferents.

131.2 ANALYSIS OF THE PERIODICITIES IN SOMATOSENSORY CORTICAL ACTIVITY PATTERNS. M. Tommerdahl,* B.L. Whitsel, E.G. Cox,* M. Diamond, and D.G. Kelly.* Depts. of Physiology and Mathematics, UNC, Chapel Hill, NC 27514

The highly periodic nature of stimulus-evoked C14-2-deoxyglucose (2DG) somatosensory cortical activity patterns suggested the use of spectral image analysis techniques. An image analysis system was developed for this purpose, allowing digitization, storage, display and quantitative analysis of 2DG autoradiographic data. In addition, software was developed to generate high resolution 2- and 3-dimensional reconstructions of the data series provided by sequences of serial autoradiographs. Two types of analyses have been carried out to date. In the first, we sought to quantify the global spatial distribution of labeling within extensive (typically greater than 5mm^2) cortical sectors. In the second, we used spectral analysis techniques to assess the periodicities in the labeling pattern within relatively small tangential (350um) or radial (1500um) sectors of individual high resolution autoradiographs. These two approaches will subsequently be referred to as "low" and "high" resolution analyses, respectively. The low resolution analyses provided new information about the spatial distribution of stimulus-evoked 2DG labeling in the S-I cortex of cats and monkeys. Of special interest is the finding that periodic, low frequency spatial variations (<3 cycles/mm) in C14 uptake occur in two locations: along the long axis of the elongated strips of label which characterize 2DG patterns, and in the cortex adjacent to the strips. This result is seen as consistent with the view that the intrinsic circuitry of the cortex enhances (via cooperative and competitive lateral interactions) local discrepancies in the pattern of afferent input to adjacent cortical columns. The high resolution analyses revealed prominent high spatial frequencies (5 - 40 cycles/mm) in the labeling that exists within individual patches. This is viewed as evidence that the population of neuronal elements within the column-shaped territory occupied by a single patch is activated non-uniformly. Comparisons of the periodicities within 2DG labeled patches with those existing in the same regions in Nissl stained sections suggest that the within-patch 2DG activation pattern is modular (i.e., is made up of active and inactive minicolumns). Other observations show that the within-patch labeling pattern changes systematically with increasing distance from the patch center. It is anticipated that spectral analyses of 2DG data can lead to a detailed 3D view of the spatiointensive patterns of activity that a somatic stimulus evokes within a single cortical column. Supported by NIH grants DE07509 and DE02668.

131.3 PATTERNS OF CORTICO-CORTICAL CONNECTIVITY CAN PREDICT PATCHES OF STIMULUS-EVOKED METABOLIC ACTIVITY IN MONKEY SOMATOSENSORY CORTEX. S.L. Juliano, D.P. Friedman, and D. Eslin*. USUHS, NIDA and Lab. Neuropsych. NIMH, Bethesda, MD.

It has been clearly established that stimulus-evoked metabolic activity in the somatosensory cortex occurs in the form of intermittent, column-like patches. Similar appearing patches revealed by axonal transport are produced by the type of cortico-cortical projection which terminates most heavily in layer IV and appears to sequentially relay sensory information from earlier to later stages in sensory information processing. The relation of the patches elicited metabolically to those formed by cortico-cortical connections, however, is not clear. If the layer IV projection system does indeed transmit sensory information in a sequential manner, it may directly contribute to the definition and location of the individual metabolic "columns". To test this possibility, experiments were conducted in which cortical regions in area 1 of Macaca fasicularis monkeys were defined electrophysiologically. After characterizing the best neuronal response, iontophoretic injections of WGA-HRP were made in the identified cortical sites. The injection sites were 500 - 750 um in diameter and designed to correspond in size to the width of one metabolic "column". Two days later the animals underwent a 2DG experiment, during which they received the somatic stimulus previously determined to best activate the neurons isolated at the injection site. The region of skin stimulated was small (a few mms in diameter) and identical in location and modality to the stimuli which elicited responses in the recording session. Evaluation of both forms of labeling on adjacent sections demonstrated that within areas 1 and 2, but outside the injection sites, the patches of anterogradely labeled HRP terminals were precisely aligned with the stimulus-evoked 2DG patches. However, in area 3b, which contained mainly retrogradely labeled patches of HRP filled cells, the match between the 2DG and HRP distributions was less precise. Control studies in which the stimulus site used during the 2DG experiment did not match the neuronal response properties identified at the site injected during the recording session revealed that the stimulus-evoked metabolic patches were not in alignment with either the retrogradely or anterogradely transported HRP label. Since the relationship between the 2DG and the HRP patches in areas 1 and 2 was stronger than that observed in area 3b, our findings are consistent with the hypothesis that the metabolically activated patches are at least in part defined by cortico-cortical connections that relay information serially. Supported by NS-24014.

131.4 EVIDENCE FOR DISCONTINUOUS BODY SURFACE REPRESENTATION IN SI. O. Favorov*, M. Diamond, and B. Whitsel (SPON. J. Greenspan). Dept. of Physiology, The University of North Carolina, Chapel Hill, NC 27514

The two cornerstones of current views of somatosensory cortical topography -- i) uniformity of the receptive fields (RFs) of the neurons in a cortical column, and ii) continuous representation of the skin within most cortical sectors -- are reexamined. Near-radial microelectrode penetrations were inserted into area 3b of cat and area 1 of Macaca fascicularis monkey. Two RF mapping methods were used: i) single neuron maximal RF mapping in the absence of general anesthesia, and ii) multi-unit minimal RF mapping in the deeply anesthetized subject.

At least 10 maximal RFs were mapped in each penetration and were found to vary markedly in size and locus. In spite of the prominent RF variations, in many penetrations the maximal RFs of all sampled neurons shared in common a very small skin area. In the rest of the penetrations, RFs mapped consecutively in the penetration had in common some small skin area only until a point, after which all the subsequent RFs shared a new skin area, nonoverlapping the first one. The data suggest that: i) all neurons comprising a narrow cell column in SI include in their RFs a common small skin area, and ii) such columns are separated by abrupt boundaries. Statistical analysis supported this view. When the electrode was advanced across such a column (termed a "segregate") no systematic shift in RF position on the skin was detectable. In contrast, the crossing of a segregate boundary was associated with a shift of RFs to a new skin location.

In several penetrations, after the electrode reached the white matter, deep general anesthesia was induced and minimal RFs were mapped as the electrode was withdrawn in 150 um steps. Comparison of maximal and minimal RF data collected in the same penetrations revealed that within a segregate, all minimal RFs mapped essentially the same position on the skin and, furthermore, this position matched closely the skin area common to all the maximal RFs. In addition, crossing of a segregate boundary was marked by a rapid shift in minimal RFs from one position to another. Thus, both RF mapping methods reveal the same plan of topographic organization: a plan in which the body surface is represented by discrete units -- segregates. Supported by NIH grants DE 07509 and DE 02668.

131.5 THE BODY SURFACE IS REPRESENTED IN SI BY A MOSAIC OF SEGREGATES. M. Diamond, O. Favorov*, and B. Whitsel. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514

Receptive field (RF) analyses (see preceding abstract by Favorov et al.) have led us to conclude that in SI of cat and Macaca fascicularis monkey the body surface is represented by discrete columnar modules, termed segregates. The questions remain "What is the size and shape of segregates?" and "What is their overall configuration?" We have used two different mapping methods, single neuron maximal RF and multi-unit minimal RF, to arrive at an answer.

Based on the RFs of single neurons in SI of both cat and monkey, collected in the absence of general anesthesia, segregate boundaries often meet at acute angles. This finding eliminated the possibility that segregates are elongated parallel strips. Two shapes remained plausible for segregates: i) complex strips (analogous to the cortical ocular dominance columns), or ii) tiles in a mosaic (analogous to the barrels in rodents).

To choose between the strip and mosaic arrangements, we used the minimal RF method in cats to map all the segregate RF centers and boundaries within a small cortical field of area 3b. Using arrays of closely spaced penetrations, we find in a typical experiment that one segregate (the "central" segregate) is bounded by six other segregates. The data indicate that the central segregate has the shape of an irregular hexagon of approximately 400 um diameter. From the results of these experiments, we conclude that segregates are arranged in the cortex in a mosaic pattern (each segregate a tile). The relation among the RF centers of neighboring segregates suggests a rough somatotopy, but the overall topography of the segregate map is characterized by many reversals.

Several parallels exist between segregates of cat and monkey SI and barrel-based columns of rodent SI: 1) just as every neuron in a segregate responds to a common skin area, every neuron in a barrel-based column responds to a principal whisker; 2) just as the boundaries of segregates are abrupt, so are the boundaries of barrels, 3) no systematic topographic gradient exists within segregates or barrel-based columns, and 4) both segregates and barrels are arranged in a mosaic pattern. Since a similar unit has been identified in SI of cat, monkey, and rodent we propose that representation of the body surface by a mosaic of discrete columns may be a general principle of SI. Supported by NIH grants DE 07509 and DE 02668

131.6 THE MORPHOLOGY OF SINGLE PHYSIOLOGICALLY IDENTIFIED THALAMOCORTICAL AXONS INNERVATING SOMATOSENSORY CORTEX IN MACAQUE MONKEYS. P.E. Garraghty and M. Sur. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

We have studied the morphology of thalamocortical terminal arbors in area 3b of postcentral somatosensory cortex. The structure of thalamocortical arbors presumably underlies many features of somatosensory cortical organization that have been previously described, including the modular segregation of neurons related to rapidly adapting (RA) and slowly adapting (SA) input, and plasticity in cortical maps following alterations in peripheral input.

We recorded from axons in the white matter below the representation of the hand in postcentral cortex in two monkeys (Macaca fascicularis) using micropipettes filled with horseradish peroxidase (HRP). When an axon was recorded, we delineated its receptive field, determined its modality (deep, joint, or cutaneous), and, if cutaneous, its submodality (SA or RA). We then impaled the axon and injected HRP iontophoretically into it.

We report here on the morphology of eight axons that responded to cutaneous stimulation. Axonal arbors within area 3b ramify in cortical layers 3 and 4, and, in many instances, in layer 6 as well. Individual arbor widths range from about 350 to 500 um. We have as yet detected no difference between the SA and RA fibers we have recovered. Seven axons which were injected superficially in the white matter, close to their terminal arbor, arose from parent trunks which we traced to a depth of 1 mm or so in the white matter. One RA axon, injected about 7 mm deep in the white matter very close to what appears to be its most proximal branch point, showed an extensive branching pattern and terminal arbor. This axon's terminal field was composed of four separate zones in area 3b spanning about 2 mm of cortex along its mediolateral axis. The individual arbors, however, were still about 350-500 um in width and were separated from each other by spaces of roughly the same width. Since the branches that give rise to this extended arbor occurred far below the region of termination, it is possible that many thalamocortical axons have quite extensive terminations which would be revealed if their trunks were injected deeper in the white matter.

In any event, individual terminal zones 350-500 um wide are consistent with the hypothesis that they underlie the columnar parcellation of somatosensory cortex. The presence of arbors spanning several such columns suggests that all regions within the arbor may not be equally effective in driving cortical cells under normal conditions, and such arbors may provide the substrate for a cortical response to changes in the pattern of input.

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131.7 CORTICAL-STRIATAL PROJECTIONS FOLLOWING INJECTION OF PHA-L INTO A PHYSIOLOGICALLY IDENTIFIED CORTICAL MOTOR OUTPUT REGION IN THE SECOND SOMATO-SENSORY CORTEX (SII) OF CAT. R.S. Waters, E.J. Johnson*, K. Park*. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Sch. of Med., Memphis, TN 38163.

We previously reported that intracortical microstimulation (ICMS) as low as 2-3μA delivered to a physiologically identified cortical motor output region (CMOR) in the second somatosensory cortex (SII) of cat produced contraction of facial muscles. A subsequent investigation of the motor output pathway from SII using anterograde neuroanatomical tracers (tritiated amino acids and WGA-HRP) failed to show terminal labeling in the facial nucleus. However, terminal fields were found in a number of intermediate brainstem nuclei as well as in the neostriatum. In this report we describe the results of our recent investigation of the SII projection to the neostriatum using a more sensitive immunocytochemical tracing technique.

Placing cats under inhalation anesthesia, the dura over the ectosylvian gyrus was removed and the exposed cortical surface was covered with warm mineral oil. The gas anesthesia was then turned off, and the cat was maintained on Nembutal and Ketamine throughout the mapping and injection portions of the experiment.. A tungsten-in-glass microelectrode was inserted into the ectosylvian gyrus and used to deliver a train of cathodal pulses (12-13 pulses/train, 0.2ms duration, 300Hz, 40μA maximum intensity) to the underlying cortical tissue. With this technique, a restricted region of cortex was identified where stimulating currents of 10μA or less were effective in evoking contraction of the facial muslces. Once this zone was clearly delineated, a 2.5% solution of phaseolus vulgaris leucoagglutinin (PHA-L) was iontophoresed into the center of this region with anodal pulsed current of 5 μA for a period of 20-40 minutes. Following a survival time between 14-21 days, the animals were anesthetized with Nembutal and perfused through the aorta with buffered acetate and borate fixatives. The brain was removed and sectioned at 50 micron thickness on a vibratome. The tissue was processed in accordance with the method Gerfen and Sawchenko (84).

All injections sites were localized to the deep layers of SII cortex. Following the injection of PHA-L into SII, labeled axons and terminal processes were found in the caudate, putamen, and claustrum. Within the caudate, long cruciform-like axons were most often seen running in a dorsal-lateral direction in the most lateral portion of the caudate. Axons were observed to branch extensively and most often showed a beaded appearance suggestive of boutons en passant. Discrete clusters or patches of terminals in the caudate were not obvious. Long axonal terminals were also found in the putamen and nearby in the claustrum. These results suggest that cell bodies in SII cortex exert influence over a large portion of the striatum and may play an important role in controlling or modulating motor output to the facial muscles.

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131.8 AN ANTEROGRADE TRACING STUDY OF THE PROJECTION FROM A PHYSIOLOGICALLY IDENTIFIED LOW THRESHOLD REGION IN THE SECOND SOMATOSENSORY (SII) CORTEX TO THE FOURTH SOMATOSENSORY CORTEX (SIV) IN CAT USING PHA-L. E.J. Johnson*, R.S. Waters, K. Park* (SPON: A. GRANATA). Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Sch. of Med., Memphis, TN 38163.

Cortical motor output regions (CMORs) have been identified in the primary motor, second somatosensory (SII), third somatosensory (SIII), and fourth somatosensory cortex (SIV) where low threshold intracortical microstimulation is effective in producing contraction of the facial muscles controlling eyeblink, ear movement, and vibrissae deflection. The results of studies where tritiated leucine/proline or WGA/HRP was injected into each CMOR suggest that these regions are highly interconnected. However, the details of these projections, particularly the pattern of axonal terminations, were not previously available using the earlier anterograde tracing techniques. In this report, we describe the results of our examination of the cortico-cortical projection from a physiologically identified low threshold site in SII, to SIV using the recently developed phaseolus vulgaris leucoagglutinin (PHA-L) tracing technique. Iontphoretic injections of a 2.5% solution of PHA-L were delivered through a micropipette (tip diameter 40-60 microns) into the center of a physiologically mapped region in SII cortex where currents of 10 mA or less were effective in producing contractions of the facial muscles. Anodal constant current was pulsed (5uA, 7s duration) through the micropipette for a period of 20-40 minutes. With these parameters the central core of the injection varied between 200-1000 μm in diameter. Following survival times of 14-20 days, cats were sacrificed and the PHA-L visualized by the avidin-biotin immunoperoxidase procedure of Gerfen and Sawchenko (84).

PHA-L injections were localized to the deep layers of SII cortex where the lectin was incorporated into the cell bodies and dendrites of a central core of neurons. Labeled cells were also observed in the immediate vicinity of the injection site but outside the central core. A few labeled neurons were found at distances greater than 5 mm from the injection site suggesting retrograde transport by damaged axons at the injection site.

Cortico-cortical fibers in SII show two distinct pattens of projection to SIV. The majority of the axons leave the injection site but remain within the gray matter to enter the superficial layers of SIV. These axons branch extensively in the upper layers and are characterized by a beaded appearance suggestive of wide spread terminal fields. In the second pattern, fibers leave the injection site and enter the white matter where they travel several millimeters before reentering SIV in a distincitive columnar type arrangement. These axons ascend to the more superficial layers of cortex, branch extensively, show characteristic beading at their terminal portions, and do not appear to overlap with axons originating in the gray matter. It remains to be determined whether the projection from SII to SIV serves to link together cortical areas sharing similiar motor control over the facial muscles.

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131.9 ORGANIZATION OF CORTICOSPINAL PROJECTIONS IN THE MACAQUE: EXTENT OF PROJECTIONS FROM THE PARIETAL LOBE. R.P. Dum and P.L. Strick. V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-HSC at Syracuse, Syracuse, NY 13210.

We have examined the origin of corticospinal projections from the parietal lobe using retrograde transport of HRP. In 2 monkeys (Macaca nemestrina), tracer was placed in the dorsolateral funiculus at C2. The distribution of labeled neurons in the parietal lobe was reconstructed from serial sections.

All of the cytoarchitectonic fields of primary somatosensory cortex (areas 3a, 3b, 1 and 2) contribute to the corticospinal tract. However, the density of projections from individual areas varies. The highest peak density of labeled neurons is in parts of areas 1 and 2 (140 neurons/mm^2) and the lowest peak density is in parts of areas 3a and 3b (76 neurons/mm^2).

Except for area 3a, most corticospinal neurons are not uniformly distributed within a single area. For example, the density of corticospinal projections is particularly low in the lateral portion of areas 3b, 1 and 2 near the end of the intraparietal sulcus. Based on current maps of somatosensory cortex, this region appears to correspond to the representation of the forelimb digits. In contrast, the highest density of corticospinal projections originates from the portions of areas 1, 2 and 5 which are adjacent to the postcentral sulcus. This region is bordered medially and laterally by regions with sparse corticospinal projections. The densely labeled region at the postcentral sulcus appears to correspond to the rostrocaudal strip in areas 1, 2 and 5 which contains a sequential cutaneous representation of the arm (Pons et al., J. Comp. Neurol. '85).

Corticospinal projections also originate from dorsal and lateral portions of area 5 and from multiple areas in and around the lateral sulcus (area 7b, SII, retroinsular and granular insular cortex). These projections all appear to originate from cortical regions which are responsive to peripheral afferent input (e.g., Robinson and Burton, J. Comp. Neurol. '80).

These observations indicate that the parietal lobe contribution to the corticospinal tract originates from multiple and diverse cytoarchitectonic areas. These cortical areas are thought to be at different levels in the processing of somatosensory information. Furthermore, based on their corticospinal projections, specific regions within some cytoarchitectonic areas appear to be preferentially involved in the processing of sensory information at the spinal level.

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131PO SUBCORTICAL EFFERENT PROJECTIONS FROM CELLS IN THE INFRAGRANULAR LAYERS OF THE RAT BARREL FIELD

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The rat barrel field provides the opportunity to ask fundamental questions about connections between sensory and motor areas of the brain. We studied efferent projections of cells in Layers V and VI by injecting the tracers wheat germ agglutinated HRP, diamidino yellow, or true blue into subcortical target structures and reacting sections through the barrel field cortex to reveal retrograde labeled cells. An alternate series of sections was processed for cytochrome oxidase. Our results confirm and extend the previous report of Wise and Jones (J. Comp. Neurol. 175, 129, 1977). Layer V is readily subdividable into a superficial, cytochrome- oxidase pale sublamina Va, and a deep, darkly staining Vb. Cells in these sublaminae differ strikingly in their efferent targets. Most, probably all pyramidal cells in sublamina Vb project to the pontine nuclei. Double retrograde labelling reveals that the axons of a subset of the cortico-pontine cells in sublamina Vb bifurcate with a branch to the superior colliculus. Another subset of Vb corticopontine cells send a branch to non-relay thalamic nuclei. The major subcortical target of cells in sublayer Va is to the caudate/putamen. Cells in layer VI are labeled only after injections are made which include the ventrobasal thalamus.

Recent physiological studies report that cells in the two sublaminae of layer V are activated at different latencies following vibrissal stimulation (Armstrong-James et al., J. Physiol. in press). Anatomical studies (Keller et al., Brain Research 343, 159, 1985) demonstrate that Vb but not Va of mice receives a direct thalamic afferent projection. Our own results combined with these physiological and anatomical findings suggest that sensory information is processed in parallel by the two sublaminae of layer V. Cells in one sublamina project principally to the cerebellum by way of the pons, the other mainly to the basal ganglia.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS III

132.1 **RESPONSES OF CUTANEOUS MECHANORECEPTORS TO STRIPE PATTERNS ON THE OPTACON.** J.A. Santoro*, C.I. Palmer and E.P. Gardner (SPON: M. Bak). Dept. of Physiology, NYU Sch. Med., New York, NY 10016.

The OPTACON (144 miniature probes arranged in a 6 x 24 matrix) simulates motion across the skin by sequentially strobing adjacent probes. Although widely used as a reading aid for the blind, and in psychophysical studies of tactile discrimination, almost no electrophysiological studies of the neural activity generated by the OPTACON have been published. We now report the responses of the three major classes of cutaneous mechanoreceptors to moving stripe patterns displayed on the OPTACON. Single fiber recordings were made from 98 cutaneous afferents innervating the glabrous skin of the anesthetized monkey's hand. Four ms pulses were applied simultaneously to one or two rows; active rows were shifted in 1.1 mm steps across the OPTACON at 10, 20 or 40 ms interpulse intervals.

Meissner's afferents (RAs) (n=33) were extremely responsive to OPTACON stimulation, firing one or two impulses to stimulation of each row within their receptive field (RF). Most RAs fired one spike/pulse, yielding uniform sensitivity throughout the RF; the remainder fired two spikes, to probe indentation and retraction. RAs responded in an all or none fashion to repetitive activation of 2-5 rows, with identical latencies on each trial. Sequence of receptor activation determined RF boundaries, which stretched one row in the direction of motion. **Pacinian corpuscles** (PCs) (n=12) had larger RFs (5-24 rows), but more irregular responses; intertrial variability was greater than for RAs. Multiple spike bursts were more common; PCs fired up to 4 spikes/pulse. **SA mechanoreceptors** (n=53) responded only to contact of the RF with the tactile array, but none responded to dynamic patterns. Thus SAs contribute only noise to the CNS from skin contact, but provide no useful information concerning spatial patterns.

Firing rates of RAs and PCs were tightly linked to frequency of stimulation at 25-100 Hz; average rates were highest to 100 Hz, and decreased exponentially. Most RAs showed no change in total spikes/sweep over these frequencies. PCs displayed slight decreases in total spike output at high frequency, due to occlusion of multiple spike responses from subsequent stimuli.

Responses to pairs of rows separated by 1-12 mm were similar to those evoked by single rows. RAs did not sum inputs from two rows within their RFs, responding only to the shorter latency row. Individual stripes could be resolved only when their spacing exceeded the diameter of the RF. RAs had better spatial resolution than PCs, due to their smaller RFs, and more regular responses.

Although use of the OPTACON as a reading aid depends crucially upon the ability to resolve spatial characteristics of letters displayed, we have shown that it is ineffective for SA receptors, which most clearly resolve Braille dots. The OPTACON is highly selective for RAs and PCs, which accurately transmit information about temporal frequency of stimulation.

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132.2 **FREQUENCY FILTERING OF SENSORY INFORMATION IN SI CORTEX.** E.P. Gardner, H.A. Hamalainen and S. Warren. Dept. of Physiology, NYU Sch. Med., New York, NY 10016.

Santoro, Gardner and Palmer (*Soc. Neurosci. Abstr. 13:* (this vol.)) have demonstrated that dynamic patterns on the OPTACON (144 miniature probes arranged in a 6 x 24 matrix) excite only RA and PC mechanoreceptors, whose firing rates are tightly linked to the frequency of stimulation over the range 25-100 Hz. Average rates of RAs and PCs were highest to 100 Hz stimuli, decreasing exponentially as a function of frequency. Most RAs showed no change in total spikes per sweep when the frequency was raised from 25 to 100 Hz, while PCs displayed slight decreases at high frequencies. We now report that a remarkable transformation of sensory information occurs between the periphery and cortex, involving enlargement of receptive fields (RFs), and filtering of high frequency responses in SI cortex of alert rhesus monkeys.

Four ms pulses were applied simultaneously to the central four columns of a single row on the OPTACON; the active row was shifted proximally or distally to the immediately adjacent row (1.1 mm), or to rows 2.2 or 4.4 mm away, at intervals of 10, 20, 40, or 80 ms. Single unit responses in areas 3b, 1 and 2 integrated information from at least 16 rows; most cells responded to rows covering an entire digit, or a large fraction of the palm.

Temporal frequency of stimulation seems to be the major determinant of cortical firing patterns. Phase locking of spikes was most apparent in low frequency responses consisting of short highly regular bursts of 3-5 spikes, separated by silent periods. The spike train changed to continuous, low amplitude, frequency modulated activity at high frequencies. Unlike peripheral afferents whose firing rates mimic stimulus frequency, average firing rates of cortical neurons were relatively uniform at all frequencies. Over the range 100-25 Hz, we observed a linear increase in total spikes/sweep of the bar across the RF, due to increased spikes/pulse, and RF expansion along the path of stimulation. Unlike RAs whose responses were constant, cortical average spikes/pulse rose from 0.5 at 100 Hz, to 3 at 25 Hz. No further increases in spikes/pulse or RF dimensions occurred at lower frequencies. High frequency attenuation of firing rates and RF size suggest a time dependent central inhibitory process, which is maximum at 10 ms, and terminated by 40 ms. Thus high frequency stimuli are selectively attenuated by central neuronal processing, which smooths out variations in firing during the period of stimulation.

High frequency stimuli are perceived as moving stimuli by humans and elicit continuous, regular firing by cortical neurons. Low frequency stimuli feel pulsatile or punctate; their cortical responses are burstlike. Thus the difference between punctate and moving sensations may be encoded by the properties of the cortical spike train.

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132.3 SENSORY RESPONSES AND PREMOVEMENT ACTIVITY CHANGES IN PRIMARY SOMATOSENSORY CORTEX DIFFER WHEN MONKEYS MAKE HAND MOVEMENTS IN RESPONSE TO VISUAL VS. VIBRATORY CUES. R. J. Nelson. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

Changes in the sensory responsiveness of primary somatosensory (SI) cortical neurons to peripheral input occur when monkeys make stereotyped wrist movements with the stimulated hand in response to a vibratory "Go-Cue", as compared to when no movement was required (Nelson, Brain Res., 304 [1984]:143-148). This indicates that "motor-set" influences SI neuronal responses to sensory input. As well, premovement activity changes in SI neurons often greatly precede movement, suggesting that these neurons receive centrally generated modulatory inputs (Nelson, Brain Res., 406 [1987]:402-407). The current experiments were designed to determine if premovement activity changes in SI neurons, and those associated with the vibratory Go-Cue onset, are related in general to the Go-Cue itself and not modality specific.

Rhesus monkey made flexion or extension wrist movements with their stimulated hand in response to palmar vibratory stimulation of either 27, 57 or 127 Hz. This was the trigger stimulus for the movements which the animals made at reaction time latencies. It remained on until the animal moved at least 5° from a previously held position in this self-paced task. The animals viewed a visual display of their current wrist position at all times. In requesting visually triggered movements, the display of current position, shown by LEDs, was shifted from the center zone by an amount equal to a 5° wrist movement. Animals learned to move in the direction opposite the lamp shift. Movements of at least 5° in the appropriate direction were rewarded with fruit juice. The animals began the next trial by repositioning the handle in the center zone. The activity of single SI cortical neurons and the wrist position were digitized by conventional means. Reaction times, stimulus related responses, and premovement activity changes were analyzed to compare vibration vs. visually triggered movements.Visual reaction times averaged about 100 ms slower than for vibratory cued trials for the 59 SI neurons analyzed to date. Some 24/59 (41%) responded at short latency (<30ms) to a vibratory Go-Cue, whereas 1/59 (2%) showed any change in discharge with the visual trigger stimulus. The 24 neurons often responded differently to vibration if it was the Go-Cue, as opposed to when the movements were extinguished. Of the sampled neurons, 55/59 (93%) exhibited some premovement suppression or facilitation in firing rate. Of these, 51/59 (86%) had activity changes for vibratory cued trials that led those for visually cued trials; the opposite was true for 4/59 (7%). The magnitude of premovement activity changes also differed under the two conditions. Some 37/59 (63%) neurons had a greater change in activity for vibratory vs. those triggered by visual stimulation; the reverse was true for 17/59 units (29%) and 5/59 cells (8%) showed similar magnitude or no change.

These results indicate that set-dependent changes in sensory responsiveness, occuring at stimulus onset, vary with different behavioral contingencies . They also suggest that premovement, centrally generated modulatory influences differ depending on the modality of the stimuli present. Since vibratory stimulation, under some circumstances, is know to alter muscle spindle responses, perhaps, these differences depend upon whether the present peripheral inputs might be capable of disrupting the monitoring of performance of a required movement.

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132.4 COMPARISON OF HUMAN AND MONKEY ESTIMATES OF ROUGHNESS. R. Sinclair and H. Burton. Department of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, Mo. 63110

Humans and Rhesus Macaques discriminated the "smoother" of pairs of textured surfaces presented on 16cm long rectangular blocks "stroked" in a distal-proximal fashion (Sinclair & Burton, Soc. Neurosci. Abst. 12:1431,1986), with specified force and velocity. Each block contained two 8 cm gratings of nyloprint etched with ridges oriented transverse to the direction of stroking. Gratings varied in spatial period from 650 to 3050 μm, with ridge-groove ratios (RG) of 10, 25 & 50%. All combinations of surfaces were presented as paired comparisons. Humans were instructed to identify the "smoother" of each pair. Monkeys were first trained to make easy discriminations to the 80% criterion level, and then progressively given harder discriminations. Human psychophysical data indicated that perceived roughness increased with increasing spatial period. For comparisons of surfaces with the same RG, monkeys were required to choose the surface with the lower spatial period. The effect of varying RG across spatial period was not as clear. To obtain a less biased estimate of what the monkey considered the "smoother" of two surfaces of differing RG ratio, trials where monkeys were required to make a correct response were interspersed with trials where they were rewarded for either choice. Only easy discriminations were presented for correct response trials. The purpose of these was to keep the animal from adopting a random strategy on the free choice trials. The assumption for the free choice trials was that since monkeys were trained to choose the "smoother" of any two surfaces, they would do so even when not required. Results from these free choice trials were used to calculate the monkey's estimate of roughness for comparisons across RG.

The order of surfaces from smoothest to roughest was the same for both species. To date, monkeys have been able to discriminate at the 75% correct level, two surfaces differing in spatial period by 15%. Other studies indicate that humans can, on average, discriminate a difference of 5%. In both species, surfaces with the same spatial period were identified as smoother as groove size decreased. This is consistent with human psychophysical and with recent electrophysiological studies in somatosensory cortex in which larger groove size resulted in greater response when spatial period was held constant.

This study attempted to minimize methodological differences between the two species studied. Humans and monkeys apparently perceive roughness in a similar fashion. This provides support for generalizing to man, the findings of electrophysiological/ behavioral studies in monkey. (Funds from NS09809)

132.5 MODULATION OF SOMATOSENSORY CORTICAL UNITARY RESPONSES DURING ACTIVE TACTILE DISCRIMINATION IN THE MONKEY. C.E. Chapman. Centre de recherche en sciences neurologiques and Ecole de réadaptation, Université de Montréal, Montréal, CANADA

It has been reported that somatosensory input to primary somatosensory cortex is diminished during movement. Paradoxically, the ability to discriminate differences in texture is reportedly uninfluenced by movement. These two findings could be reconciled if the modulation of afferent input during movement varied with the task. The purpose of this study was to characterize the discharge properties of neurones in primary somatosensory cortex of the awake monkey during the performance of a task requiring the animal to actively explore and discriminate between two different surfaces.

A monkey, macaca mulatta, was trained to perform a scanning movement over two different surfaces. One surface was smooth while the other was initially smooth, becoming textured (embossed Braille characters) over the second half of its length. Following a visual cue, the animal made a scanning movement, monitored with LEDs set into the walls of the groove, over the surface. Following completion of the movement, the monkey signalled the type of surface encountered by pushing (smooth throughout) or pulling (smooth/rough) a lever with the contralateral arm.

Recordings have been made from 38 neurones in the cutaneous hand representation of primary sensory cortex and evidence was obtained to suggest that somatic input to sensory cortex is indeed modulated as a function of the task. 23 of the 38 units were modulated during the scanning movement. Of 18 cells whose discharge increased during the scanning movement, 7 showed no discharge or a decrease in discharge as the digits were moved back over the surface. Such units were not obviously directional when their receptive field was determined. Furthermore, passive movement, in the same direction as the scanning movement, had only weak excitatory effects or no effect in 4 of 5 cells tested. Such observations indicate that somatic input to some cells is enhanced during the scanning movement. The opposite effect, diminished transmission of somatic input to cortex, was also observed. Five units showed a decrease in discharge during the scanning movements yet all showed evidence of an excitatory receptive field on one or more of the digits. Of a further 15 cells, unmodulated during the scanning movement, 5 showed an increased discharge as the monkey resumed the initial position. These results indicate that peripheral input from the hand can be enhanced or diminished during a task requiring the animal to discriminate different surfaces. Supported by the FRSQ and the Canadian MRC.

132.6 A Comparison Between Asleep and Waking Cortical Depth Profiles of Somatosensory Evoked Field Potentials (SEP), Multiple Unit Activities (MUA) and Current Source Densities (CSD) in the Postcentral Gyrus of Behaving Rhesus Monkeys. L.J. Cauller* and A.T. Kulics* (spon: L. Fechter). Dept. of Neurobiology, N.E. Ohio Univs' College of Medicine, Rootstown, OH 44272

Multiple electrode arrays (15 tips; 200 um fixed separation) were constructed to simultaneously record the somatosensory evoked electrical signals from fifteen, equally spaced levels from dura to white matter through cortex. A detachable, screw-type microdrive permitted multiple penetrations through postcentral gyrus in the awake or sleeping monkey. The multi-channel responses to electrocutaneous or mechanical stimulation of the contralateral hand was saved on FM tape for offline digital analysis. Records were high pass filtered (>500Hz) and then integrated (5ms time constant) to determine the profile of MUA. For penetrations that were found to be perpendicular to the cortical surface, depth profiles of SEPs were digitized and processed for CSD analysis. These methods are an elaboration of those reported previously (Kulics and Cauller, Exp. Brain Res. 62:46-60, 1986) and have led to a model of the sleeping cortical process.

The somatosensory cortical response consists of two distinct components, a surface positivity at 12 ms (P1) that is a reliable measure of stimulus strength, and a later suface negativity at about 50 ms (N1) that predicts the monkey's performance on a stimulus strength discrimination task. Both P1 and N1 are associated with peaks of MUA through layers III-V. P1 is thought to reflect the synchronous activation and discharge of middle layer pyramidal cell bodies and N1 is generated by a massive postsynaptic sink into the layer I/II distal apical dendrites of pyramidal cells whose bodies are in layers II-V.

The sleep state was identified when a monkey's eyes closed and the cortical electroencephalograph (EEG) displayed large slow wave activity while wakefulness was defined as low amplitude, high frequency EEG activity with eyes opened. While the P1 component of the SEP, and the associated early MUA was increased during the sleep state, the late MUA that is associated with N1 during the awake state, is replaced by inhibition as indicated by a level of MUA that falls below prestimulus activity. That late inhibition during sleep is associated with a massive late current source in layers II-IV which lasts about 100ms following the P1 excitatory response. Occasionally, a rebound burst of MUA follows the long inhibitory phase at about 125ms post stimulus which is associated a surface positive-negative wavelet. The possibility that the sleeping cortical response could result from a voltage-sensitive potassium conductance (ie. M-current) that is inactivated by cholinergic inputs during wakefulness is considered in the model of the cortical sensory process. Supported by NIMH grant MH38157.

132.7 SEP AND MUA RESPONSES TO MECHANICAL STIMULI IN SI AND SII CORTICAL AREAS OF AWAKE MONKEYS. H. Hämäläinen, M. Sams*, S. Carlson*, A. Pertovaara , K. Reinikainen*, and R. Näätänen*. Dept. of Psychol. and Dept. of Physiol., Univ. of Helsinki, Finland.

We measured simultaneously local early and late somatosensory evoked potentials (SEPs) and multiple unit activity (MUA) elicited by mechanical pulses and vibration. The recordings were obtained from hand projection areas of cortical regions SI and SII. Our aim was to compare the responses of these cortical areas to different stimuli, and to evaluate the relationship between neuronal spike-activity and evoked potentials.

Awake monkeys (Macaca Arctoides) were used in the experiments. The recordings were obtained with glass-coated tungsten macroelectrodes, and the signals were fed to different filters for SEP and MUA measurements (600 ms analysis period). The MUA was converted to trains of 0.4 ms pulses by a window discriminator, and the average firing probabilities were calculated by a computer. The stimuli were single tactile pulses and vibration (duration 400 ms of 24 or 240 Hz frequency.

In SI only responses to stimulation of the contralateral hand were obtained, and largest responses were measured to slow pulses and low-frequency vibration. The main SEP components measured to single pulses were a large negative deflection - often consisting of several peaks - at 40 ms, followed by a slower positivity which returned to the baseline within 200 ms after the stimulus onset. Usually the large negativity was preceded by a short-latency (13 ms) positive deflection, the amplitude of which was dependent on the recording depth. With low-frequency vibratory stimuli similar initial deflections were recorded, but they were followed by oscillation tuned to vibration frequency, and by an off-response at the end of vibration. The SEPs to high-frequency vibration showed the same pattern except that no oscillations tuned to vibration frequency could be seen. The onset of the pulse-elicited MUA responses (duration about 75 ms) corresponded to that of first SEP components. The distinct peaks in MUA often elicited by low-frequency vibration were time-locked to the SEP oscillations.

In SII completely different responses were measured. A basic pattern to all stimulus types consisted of a slow positivity starting immediately at the onset of the stimulus (peaking at 100-200 ms), followed by a large slow negativity (peaking at 270-400 ms). Sometimes also a distinct negative peak (at 70-100 ms) was seen together with the slow positivity. SEPs to ipsilateral hand stimulation were usually weak in SII with all but high-frequency vibration which elicited similar responses to both contra- or ipsilateral stimulation. In contrast to SI responses, the MUA usually increased rather smoothly after stimulus onset, and its decay corresponded to the increasing negativity in the simultaneously measured SEP.

132.8 MOVEMENT CORRELATED MODULATION OF SENSORY TRANSMISSION TO SINGLE UNITS IN THE VPL THALAMUS H.-C. Shin and J.K. Chapin, Hahnemann University, Philadelphia, PA 19102.

We have previously shown that cutaneous sensory transmission to single neurons in the rat somatosensory cortex is reduced a mean 71.2% during locomotion, and is phasically modulated over the step cycle. As part of an effort to determine the level(s) in the somatosensory system at which this modulation occurs, similar studies have now been carried out in the ventroposterolateral (VPL) thalamus. The experiments involved recording single units in the forepaw area of the VPL thalamus in awake, freely moving rats in which stimulating electrodes had been chronically implanted under the skin of the forepaw. This allowed standardized low current stimulation pulses to be applied during different phases of the forepaw step cycle.

Initial studies showed that transmission to single neurons in the VPL was depressed by mean 32% during movement as compared with rest; much lower than the 71% reduction observed at the cortical level. The next step was to determine the degree to which this modulation varied over the step cycle. Using frame-by-frame video tape analysis to define the step cycle phase, "peri-footfall sensory gating histograms" were constructed to graphically illustrate the variation over the step cycle in the neurons' sensory responsiveness to the applied forepaw stimulation. The results showed that while some VPL neurons were subject to a tonic reduction in sensory responsiveness over the step cycle, many cells were phasically modulated. An average of peri-footfall gating histograms from all 30 units in this sample showed a strong sensory inhibition during the 50 msec just following footfall, and a sensory facilitation during the late-stance-to-foot-off phase of the step cycle. The post-footfall inhibition was strongest in the 10 neurons which also responded to the footfall itself, and thus may have resulted partly from an interaction between the test stimulation and the natural stimulation caused by footfall. In the 20 cells which did not respond to footfall, sensory inhibitions were also observed during the entire swing phase of the step cycle. We conclude that phasic somatosensory modulation occurs in the VPL thalamus as well as the cortex, but that the modulations are distinctly different in their temporal patterns, polarities, and magnitudes. Supported by BNS-841979, AA-06965, and KO2-AA00089 to JKC.

132.9 PHARMACOLOGICAL ACTIVATION OF LOCUS COERULEUS NEURONS BY LOCALLY INFUSED CARBACHOL: EFFECT ON CORTICAL NEURON EVOKED ACTIVITY. L.M. Adams and S.L. Foote. Department of Psychiatry, Univ. of California, San Diego, La Jolla, CA 92093.

Previous studies have revealed a modulatory action of iontophoretically applied norepinephrine (NE) on cortical neuron responsiveness, suggesting a role for the cortically projecting NE-containing nucleus locus coeruleus (LC) in modulating cortical function. As an alternate means of determining the effects of NE release in cortical target areas, LC neurons were activated by local infusion of the cholinergic agonist bethanechol (BCHOL) while simultaneously monitoring the spontaneous and sensory-evoked activity of cortical neurons. Utilizing a previously described cannulae-microelectrode assembly (Soc Neurosci Abst. 12:154,1986), BCHOL was infused into the LC of halothane-anesthetized rats and its effect on firing rate monitored by means of a metal microelectrode. A 12-25 ng infusion (50 nl/1 min) produced a 4-fold increase in firing rate with 30-60 second latency to maximum effect and 3 min duration. A second microelectrode was lowered into the hindlimb region of primary somatosensory cortex and the appropriate receptive field activated either electrically or by air-puffs. Post-stimulus-time histograms (PSTHs, 50 sweeps) were computed on-line to determine the effect of LC activation on cortical neuron response profiles. PSTHs were accumulated over 50 sweeps (3 min) before and at 6-min intervals following BCHOL infusion. Infusion of BCHOL produced one of two effects depending on the baseline response properties of the cortical neuron being recorded from. The most typical baseline response consisted of a transient, short-latency (15-20 ms) excitation followed by a prolonged period (50-150 msec) of post-stimulus inhibition (PSI). One minute after BCHOL infusion, the number of short-latency spikes and the duration of PSI were reduced, and there was a corresponding increase in the number of longer latency spikes. Recovery occurred over a period of 6-12 min. The other type of cortical baseline response was a short latency burst not followed by PSI. For these cells, BCHOL infusion reduced both the spontaneous firing rate and the short-latency evoked response. Since the reduction in spontaneous activity occured at lower doses and was slower to recover than the reduction in evoked responding, the ratio of evoked to spontaneous activity was enhanced. The majority of units tested were found in layers IV or Va. Infusion of BCHOL 400-500 µg below the LC failed to alter cortical activity. Intravenous clonidine in a dose (10-20 µg/kg) which completely inhibited LC firing for 30-60 min, antagonized the BCHOL effects on cortical evoked responses. The spatial restriction and pharmacological specificity of BCHOL effects suggest a direct mediation by LC activation. Studies are underway to attempt to further define the relationship between cortical neuronal response types and the effects of LC activation.

132.10 SOMATOSENSORY EVOKED POTENTIALS FOLLOWING INTRATHECAL DYNORPHIN A (1-13) INJECTION IN THE RAT. D.D. Rigamonti, J.B. Long, F.C. Tortella, R.P. Gussio*, A. Martinez-Arizala* and J.W. Holaday. Neuropharmacology Branch, Department of Medical Neurosciences,Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Pharmacological exposure of the rat spinal cord to several endogenous peptides [dynorphin A (DYN), arg-vasopressin, and somatostatin] has been observed to produce neural injury, blood flow reductions, and neurological impairments. Among these peptides, DYN has been specifically implicated as a mediator in spinal cord injury. Cortical somatosensory evoked potentials (SEP) have been routinely used to assess spinal cord integrity and functional status following trauma and ischemia. Therefore, the SEP was employed as an adjunctive test to assess the time course and extent of spinal cord dysfunction following DYN (1-13) exposure. Adult male Sprague-Dawley rats anesthetized with ketamine and xylazine were placed in a stereotaxic frame, and cortical evoked potentials were averaged (N=50-2000) using either forelimb or hindlimb stimulation (Nicolet Pathfinder II). The control SEP altered with changes in strength, frequency, and duration of the stimulus, and location of the cortical recording electrodes. These parameters were adjusted to obtain the optimum cortical response. Following intrathecal injection of DYN (50 nmoles) into the lumbar subarachnoid enlargement, alterations in the SEP were observed. These were mainly in the amplitude and shifts in the latency of major cortical responses. Changes in amplitude varied from slight to total disappearance of the cortical response. The forelimb evoked potential following DYN injection also was generally increased in amplitude. Following recovery from anesthesia, neurological impairments were compared with SEP alterations. This neurophysiological test is a powerful tool to correlate neuropathological and functional changes following peptide injury to the rat spinal cord.

133.1 IDENTIFICATION OF DECUSSATION SITE OF LOCUS COERULEUS (LC) AXONS WHICH AFFECT LHRH NEURONAL ACTIVITY. M. S. Gitler * and C. A. Barraclough. Dept. of Physiology, University of Maryland. Sch. of Medicine, Baltimore, MD 21201.

Electrical stimulation (ES) of LC amplifies luteinizing hormone (LH) release after medial preoptic nucleus (MPN) LHRH neurons have been partially depolarized. We also observed that these stimulatory LC fibers must decussate to reach contralateral LHRH neurons for the LH amplifying effect to occur. The purpose of this study was to determine where the decussation of these LC axons occurs. Estrogen-treated ovariectomized rats were anesthetized with chloral hydrate. One group of control rats received right MPN electrochemical stimulation (ECS) (0.1mA/60 sec d.c.) at 0 time. In a second group, the right MPN was ECS and 30 min later the left LC was electrically stimulated (0.1 mA, 10 Hz, 1 msec duration, 15 sec on/off) for 15 min. Blood was collected at various times and plasma LH levels were measured by RIA. In different experimental groups one of the following brain transections was made with a knife blade: (I) unilateral (left) cut of medial forebrain bundle (MFB) at the level of the posterior hypothalamus. This transection spares the tegmental and posterior commissural decussations of LC fibers; (II) a midline transection in the MPN region which coursed through the anterior commissure (AC) to the top of the third ventricle. This cut should disrupt AC decussation of LC axons; (III) a midline cut in the MPN region which passed through both AC and optic chiasma (OC) to the sphenoid bone. This transection should interrupt both AC and OC decussations by LC axons. In rats receiving cut I (MFB), the transection was placed either 10 min before or 25 min after MPN-ECS. LH responses to MPN-ECS did not differ from controls in either group. In other rats, the MFB cut was placed 25 min after MPN-ECS and 5 min later the LC was ES. The normal amplifying effect produced by LC-ES was completely abolished by this transection. In all of the remaining groups, transections were made 25 min after MPN-ECS. Cut II (AC): after MPN-ECS, LH levels remained slightly elevated from 60-90 min in transected vs. control rats. In other AC-transected rats which received combined MPN+LC stimulation, peak LH concentrations were markedly amplified to the same extent as controls. However, while control LH levels approached baseline values at 180 min, they remained significantly elevated from 60-180 min in the AC transected rats. Cut III (AC+OC): This transection did not affect LH responses to MPN-ECS. In contrast, this cut completely blocked the amplifying effects of LC-ES. We concluded that: (1) stimulatory LC axons cross midline via the supraoptic commissure to affect LHRH neuronal activity in the contralateral MPN; (2) By disrupting neural elements in the AC region we have unmasked an inhibitory component which affects LHRH neuronal responsiveness to LC-ES. Whether this inhibitory effect is exerted via LC input to MPN or is the consequence of our disrupting inhibitory rhinencephalic tracts to MPN remains to be resolved.

133.2 **EXCITATION OF LOCUS COERULEUS FROM PARAGIGANTOCELLULARIS MAY BE MEDIATED BY AN AMINO ACID TRANSMITTER.** M. Ennis and G. Aston-Jones, Dept. Biol., New York University, NY, 10003.

Recent studies reveal that the major afferent to rat locus coeruleus (LC) is nucleus paragigantocellularis (PGi) in the medulla. We have also recently reported that PGi inputs are predominantly excitatory on LC. PGi-induced excitation of LC is resistant to the muscarinic antagonist **scopolamine**, but is abolished by kynurenic acid, an antagonist of excitatory amino acid (EAA) transmission. Here we further specify the transmitter and receptor involved in PGi-induced excitation of LC.

Extracellular recordings were obtained from single LC neurons with micropipettes in anesthetized rats. Subcutaneous 26 g needles were used for electrical footpad stimulation (FS) and 250-uM wires were used for bipolar electrical stimulation of PGi.

The nicotinic antagonist **mecamylamine** (1.0 mg/kg, i.v.) had no effect on PGi-evoked excitatory responses in 5 LC neurons. Similarly, this agent had no effect on responses of LC cells to FS, and did not change the firing rate of LC cells. **Kynurenic acid** (0.53 umols, icv) completely blocked PGi-induced excitation of LC neurons, and this agent also abolished excitation of LC by FS ($p<.01$ for both). **D-glutamylglycine (DGG)**, a preferential NMDA-kainate antagonist, blocked PGi-LC excitation in a dose-dependent manner in all 8 neurons tested, markedly attenuating ($p<.01$) excitation at 0.1 umols and completely blocking excitation at 0.32 umols (icv). Similarly, DGG exerted a dose-dependent antagonism of FS-excitation in LC, reaching significance at 0.18 umols ($p<0.2$). DGG also decreased spontaneous discharge ($p<.05$) for 6 cells tested with 0.32-1.0 umols.

The specific NMDA antagonist **2-amino-7-phosphonoheptanoic acid (AP7)** also attenuated PGi- ($p<.01$) and FS-elicited ($p<.05$) excitation in LC, but at a relatively high dose (0.1 umols; icv). Also, this agent was less effective than kynurenic acid or DGG, failing to completely block PGi or hindpaw responses in any cell.

In contrast to DGG and AP7, the preferential quisqualate antagonist **glutamate diethyl ester (GDEE)** (15 umols, icv) did not block LC responses to PGi or FS stimulation ($p>.1$).

Although additional tests are needed to exclude other possible effects of EAA antagonists (e.g., on peptide transmission), the present results suggest that an EAA mediates the activation LC by its major afferent, PGi. The ability of EAA antagonists to attenuate FS-responses in LC is consistent with our hypothesis that sensory stimuli activate LC through PGi, and implies an EAA in this pathway. However, it is also possible that EAA antagonism of FS responses was effected prior to PGi. DGG and AP7, but not GDEE, antagonism of LC responses implicates kainate or NMDA subtypes. Supported by PHS MH09381, Alz. Dis. Related Dis. Assoc., Am. Fed. Aging Res., and ONR Contract N00014-86-K-0493.

133.3 RESPONSE OF LOCUS COERULEUS NEURONS TO SOMATOSENSORY STIMULI IS NOT BLOCKED BY LESIONS OF TWO MAJOR AFFERENT INPUTS. Kurt Rasmussen and George K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06508.

Stimulation of somatosensory nerves is known to have strong excitatory effects on noradreneragic neurons of the locus coeruleus (LC); indeed their burst-pause response to somatosensory nerve stimulation is an identifying characteristic of these neurons. A recent study (Aston-Jones et al., Sci., 234 (1986) 734) has reported that direct afferents to the LC are almost exclusively restricted to two medullary nuclei, i.e., the paragigantocellularis (PGi) and the prepositus hypoglossi (PrH). These findings raise the possibility that somatosensory input to the LC is relayed through one, or both, of these two medullary nuclei. In order to test this hypothesis, we lesioned each of these nuclei to see if we could block the effects of stimulating the sciatic nerve on the LC. We also lesioned the paraventricular hypothalamic nucleus (PVH), a possible minor afferent to the LC, and the ipsilateral medullary lateral reticular nucleus (LRN), a structure which is contiguous with the PGi and was suggested to be an afferent to the LC in an earlier study (Cedarbaum & Aghajanian, J. Comp. Neurol., 178 (1978) 1).

LC single unit recordings were obtained from chloral hydrate anesthetized male rats. Electrolytic lesions were made with a radiofrequency lesion maker, and the extent of the destruction was evaluated by examining histological sections. The sciatic nerve was stimulated at three different current settings with a bipolar hook electrode. The responses of five LC cells to each current level were examined in each animal. Although the projection to the LC from the PGi is predominantly ipsilateral, we also destroyed the PGi bilaterally in some animals. As compared with sham operated controls, destruction of ipsilateral PGi, bilateral PGi, PrH, ipsilateral LRN, or PVH did not alter the effects of sciatic nerve stimulation on the LC, indicating that afferent information from the sciatic nerve to the LC is not relayed through these nuclei. Alternatively, afferent information to the LC from the sciatic nerve could project directly from the spinal cord. This view is consistent with anatomical evidence for a possible direct projection to the LC from lamina X (Aston-Jones et al., Sci., 234 (1986) 734) and both anatomical and electrophysiological evidence for a direct projection to the LC from lamina I (Cedarbaum & Aghajanian, J. Comp. Neurol., 178 (1978) 1; McMahon & Wall, Brain Res., 333 (1985) 19).

In conclusion this study indicates that the strong excitation of the LC produced by somatosensory nerve stimulation is not mediated through relay nuclei in the medulla (PGi, PrH, LRN) or hypothalamus (PVH), but is probably a result of a significant direct input from the spinal cord.

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133.4 THE ANTERIOR HYPOTHALAMUS PROVIDES STIMULATORY INPUT TO TUBEROINFUNDIBULAR DOPAMINE NEURONS WHICH IS NOT MEDIATED BY PROLACTIN. A.C. Barton, K.T. Demarest and K.E. Moore. Dept. of Pharmacol./Toxicol., Mich. State Univ., E. Lansing, MI 48824.

Tuberoinfundibular dopamine (TIDA) neurons in the medial basal hypothalamus (MBH) receive both stimulatory and inhibitory afferent input. For example, prolactin stimulates the neurosecretory activity of the TIDA neurons which, in turn, release DA into the hypophysial portal blood to inhibit further secretion of prolactin from the anterior pituitary (Endocrinol. 106: 526, 1980; Neuroendocrinol. 38: 467, 1984). The objective of the present study was to determine the role played by putative afferent neuronal connections (1) in maintaining basal neurosecretory activity of TIDA neurons, and (2) in the stimulatory actions of prolactin on these neurons. The neurosecretory activity of TIDA neurons was estimated by measuring the rates of synthesis, turnover, or metabolism of DA in the terminals of these neurons in the median eminence. DA synthesis and turnover were determined by measuring the rate of accumulation of dihydroxyphenylalanine (DOPA), following NSD 1015 (100 mg/kg, i.p.) and by the alpha-methyltyrosine(250 mg/kg free base, i.p.) induced decline in DA, respectively. DA metabolism was estimated by measuring the concentration of the DA metabolite, dihydroxyphenylacetic acid (DOPAC). Complete and retrochiasmatic (RC) deafferentations of the MBH were made to either completely isolate TIDA neurons from the rest of the brain or to interrupt rostral neuronal connections to the TIDA neurons, respectively (Endocrinol. 80: 608, 1967). Complete deafferentation of the MBH reduced the basal rate of DA synthesis, and RC deafferentation decreased the rates of synthesis, turnover and metabolism of DA in the median eminence of female but not male rats. A knife cut 1 mm rostral to the RC cut failed to alter basal TIDA neuronal activity. These results suggest that afferent neuronal inputs originating in or coursing through the caudal portion of the anterior hypothalamus mediate a tonic stimulatory influence on TIDA neurons in the female rat while this influence is either absent or repressed in the male. Since TIDA neurons in the female rat receive tonic stimulation by prolactin, it was investigated whether the RC deafferentation-induced decrease in TIDA neurosecretory activity was a direct consequence of the interruption of afferents mediating the action of prolactin. In intact female rats, intracerebroventricular (ICV) administration of rat prolactin (10 µg/3 µl) or systemic administration of haloperidol (2.5 mg/kg, s.c., which increases circulating levels of prolactin) increased DA synthesis in the median eminence. This stimulatory action of prolactin was not blocked by RC deafferentation. Decreasing circulating levels of prolactin, induced by the administration of bromocriptine (3 mg/kg, s.c.), reduced the rate of DA synthesis in the median eminence of both sham and RC deafferentated rats; in both groups this bromocriptine-induced decrease in DA synthesis was reversed by ICV administration of prolactin. These results suggest that the RC deafferentation-induced decrease in TIDA neurosecretory activity is a consequence of removing stimulatory afferent input to these neurons which is mediated by something other than prolactin. (Supported by NIH Grant NS09174.)

133.5 INTERACTIONS OF DOPAMINE AND ACETYLCHOLINE ON PREFRONTAL CORTEX(PFC) NEURONS AND THE EFFECTS OF GABAERGIC AND ENKEPHALINERGIC STIMULATIONS OF VENTRAL PALLIDUM ON PFC ACTIVITIES. C.R.Yang* and G.J. Mogenson (SPON: W.T.Blume). Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The prefrontal cortex (PFC), innervated directly by the mediodorsal thalamus (MD), receives prominent inputs from mesocortical dopaminergic (DA) neurons and from cholinergic (ACH) neurons of the ventral pallidum/substantia innominata (VP/SI). In turn, efferents from the PFC project to the nucleus accumbens (NAC). From the NAC, GABAergic and enkephalinergic output neurons synapse onto the VP/SI neurons which ascend directly to the PFC, or indirectly via the MD. Hence, PFC is an important site where interactions of the DA and ACH systems occur and where the output from the NAC may be fed back via the NAC-VP/SI-MD-PFC 'loop'. These possibilities were studied using extracellular single-unit recordings in urethane- or ketamine-anaesthetized rats.

145 PFC neurons 2-4mm below the surface of the cortex (layer III-VI) were identified as PFC-NAC neurons by their antidromic responses to NAC stimulation (onset latency=15±2 ms). 36 of these neurons were also inhibited by MD stimulation. Iontophoretic application of ACH (5-80 nA, 40-70s) to 32 of 87 PFC neurons produced a gradual increase in firing which outlasted the application period. When DA or its D-2 receptor agonist (LY171555) was applied iontophoretically onto 27 PFC and 5 PFC-NAC neurons, there was a significant (p 0.001) increase in the firing rate and duration of the response to ACH. The doses of DA or the D-2 agonist used (2-7nA)produced only minimal changes (±20%) of baseline firing of the PFC neurons. Furthermore, following blockade of of the D-1 receptors by SCH23390 (2mg/kg,i.p.) the potentiation of the cholinergic response by the very small amount of D-2 agonist on the PFC neurons persisted suggesting a sensitive and selective D-2 receptor mechanism which potentiates cortical cholinergic transmission.

Muscimol (1-2ug/0.5ul), a GABA agonist, or D-ala-D-met-ehkephalin (lug/0.2ul) microinjected into the VP/SI mimicked the actions of the NAC output. Muscimol increased the firing rate in 11 of 25 PFC-NAC neurons by 70-400%, perhaps by disinhibition. Injection of procaine (40ug/0.2ul) into the MD (to block the transmission of ascending VP-PFC neurons relayed via the MD)further enhanced the discharges in 4 of these PFC neurons but 4 others were inhibited. Met-enkephalin in the VP/SI produced no clear response in the PFC, but the opioid inhibited the spontaneous firing of 10 of 20 MD neurons. Taken together these results suggest GABAergic and enkephalinergic neurons from the NAC may regulate ascending VP/SI neurons which influence the activities of PFC-NAC neurons directly, or indirectly via the MD. The potentiation of the cholinergic transmission by mesocortical DA may be part of a mechanism for cognitive processes involving the ascending VP/SI cholinergic neurons.(Funded by M.R.C. of Canada)

133.6 LACK OF EFFECT OF L-PHENYLALANINE ON PHOTICALLY-INDUCED MYOCLONUS IN THE BABOON, Papio papio. B.S. Meldrum. Department of Neurology, Institute of Psychiatry, London, SE5 8AF, U.K.

Administration of L-phenylalanine (PHE) to rodents can modify reflexly-induced seizure responses in susceptible strains (Truscott, Pharmac. Biochem. Behav. 3: 939, 1975). It is thought that PHE competes with tyrosine for uptake into the brain and decreases the synthesis of catecholamines. Direct evidence for an effect of PHE on seizure threshold in primates is so far lacking. We have studied the effect of the acute oral administration of PHE on photically-induced myoclonic responses and on plasma levels of large neutral amino acids in Papio papio from the Casamance region of Senegal. Four highly photosensitive adolescent baboons each received (in random order, at weekly intervals) PHE 0 (=alkali solution), 50,150 or 450 mg/kg orally. Myoclonic responses to a standardised 5 min period of photic stimulation prior to and at hourly intervals after drug administration were scored according to Meldrum et al, Electro. clin. Neurophys. 29: 333,1970. Similar decreases in seizure score over time were observed after each of the four treatments. Ranking of the decreases in seizure score showed that there was no significant difference in dosage effects (Friedman's test on ranks).

Peak plasma PHE concentrations of 60, 93, 561 and 2155 umoles/l were observed approximately 120 min after administration of PHE, 0,50, 150 and 450 mg/kg, respectively. In contrast plasma tyrosine levels did not exceed 78 umoles/l in any baboon throughout the 4 hour sampling period. Calculation of the plasma PHE: large neutral amino acid ratio (a predictor of the competitive uptake of phenylalanine into brain) revealed a dose-related 2-, 11 and 34-fold increase compared to control. Thus even at extremely high concentrations of plasma PHE, neither pro- nor anticonvulsant effects were observed in baboons.

133.7 DIABETES-INDUCED DECREASES IN BRAIN TYROSINE LEVELS RESULT IN DECREASED SYNTHESIS IN MESOPREFRONTAL CORTICAL DOPAMINE NEURONS. D.H. Karasic, C.W. Bradberry, A.Y. Deutch and R.H. Roth. Yale Univ. Sch. Med., New Haven, CT 06510.

Hydroxylation of the amino acid tyrosine (Tyr) is the rate-limiting step in the synthesis of dopamine (DA). The activity of Tyr hydroxylase is influenced by precursor availability in DA neurons that are activated pharmacologically or physiologically.

It is known that subsets of midbrain DA neurons exhibit different basal firing rates. A large amount of work in our laboratory has been directed towards characterization of the regulatory features of the mesoprefrontal cortical (mPFC) DA neurons. We have recently demonstrated that DA synthesis in these rapidly firing mPFC DA neurons is dependent on Tyr availability, while other mesotelencephalic DA neurons, such as the mesostriatal neurons, under normal conditions do not show such a dependence.

Brain Tyr levels are significantly reduced in the streptozotocin (STZ)-induced model of diabetes in the rat. Using this model, we have examined whether diabetes-induced changes in brain Tyr can result in alterations in in vivo Tyr hydroxylation in mesotelencephalic DA neurons. Diabetes was induced by administration of 65 mg/kg STZ i.v., and confirmed by blood glucose levels of 400 mg% at time of sacrifice three weeks later. As an index of in vivo Tyr hydroxylation, tissue levels of L-DOPA 30 min. following NSD-1015 treatment (100 mg/kg i.p.) were measured in the prefrontal cortex and striatum, as well as in the DA cell body regions of the ventral tegmental area (VTA) and substantia nigra (SN). Cortical and striatal Tyr levels were also determined in the same animals.

A significant decrease in L-DOPA accumulation in the prefrontal cortex but not in the striatum of diabetic animals was observed. In both regions, in vivo Tyr hydroxylation correlated with the levels of endogenous Tyr. A corresponding significant decrease in L-DOPA accumulation was seen in the VTA but not in the SN. This finding is consistant with the fact that projections to the prefrontal cortex arise primarily from the VTA while the SN projects mainly to the striatum. These data suggest that changes in brain Tyr availability may result in significant alterations in DA synthesis in mPFC DA neurons, but not in nigrostriatal DA neurons. Our results are consistant with previous characterizations of metabolic differences between various midbrain DA neuronal populations. The susceptibility of mPFC DA neurons to diabetes-induced alterations in cortical Tyr is of special interest due to the possible role of this subset of DA neurons in cognitive function, and the knowledge that some cognitive deficits may occur in patients with uncontrolled or long-lasting diabetes.

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133.8 CEREBRAL INFARCTION AND CSF MONOAMINE METABOLITES IN THE RAT. R.M. Parikh*, A. Justice, T.H. Moran, J.A. Bowersox*, R.G. Robinson. Dept. of Psychiatry, Johns Hopkins Univ. Baltimore, MD 21205.

As an extension of our investigations in a rat model of stroke, we have studied the effects of unilateral middle cerebral artery (MCA) ligation on cerebrospinal fluid (CSF) monoamine metabolites at different intervals over a 40 day post-operative period.

Male Sprague-Dawley rats were divided into five groups: an unoperated control group (n=9), a right craniotomy group (n=5), a left craniotomy group (n=4), a right MCA ligation group (n=10) and a left MCA ligation group (N=10). CSF (100 ul) was collected percutaneously from the cerebello-medullary cistern on the day of surgery just prior to the operative procedure and at days 5, 20 and 40 following the surgical procedure. The measurements of CSF catecholamine metabolites - MHPG, 5-HIAA and HVA were carried out using a high pressure liquid chromatography (HPLC) assay. At the end of the experiment,brain chemistry measures were obtained using an HPLC assay for NE, DA, 5HT and 5-HIAA in 7 brain regions.

There was significant depletion in CSF MHPG levels in the right lesion group at days 5 ($p<.05$), 20 ($p<.01$) and 40 ($p<.05$) post-operative, when compared with the control group. No such depletion was observed in the left lesion animals. Similarly, there was reduction in CSF 5-HIAA levels at days 5 ($p<.01$) & 20 ($p<.01$) in the right lesion group compared with the left lesion group. There was a significant correlation between CSF 5-HIAA levels at day 40 and brain concentrations of 5HT in the frontal cortex on both the left ($r=.42$, $p<.05$) and right sides ($r=.34$, $p<.05$) across all 38 animals.

This study has demonstrated a time-dependent differential effect of unilateral ischemic stroke on CSF neurochemistry in the rat dependent on the cerebral hemisphere involved. It has also demonstrated a significant relationship between CSF neurochemistry and brain transmitter concentrations. It is hoped that by establishing relationships between CSF metabolite and brain transmitter concentrations in the rat, we will be able to make inferences about the relationship between these measures in human stroke.

133.9 NOREPINEPHRINE RECEPTOR AGONISTS AND ANTAGONISTS INFLUENCE RATE AND MAINTENANCE OF RECOVERY OF FUNCTION AFTER SENSORIMOTOR CORTEX CONTUSION IN RAT. M.S. Weaver*, L.J. Farmer* and D.M. Feeney. Depts. of Psychol. and Physiol., Albuquerque, NM 87131.

A single dose of amphetamine, combined with task relevant experience, produces an enduring acceleration of recovery of locomotor ability after unilateral sensorimotor cortex ablation. Norepinephrine (NE) has been implicated in mediation and maintenance of this effect (CRC Critical Reviews in Neurobiology, 1987, 3: 135-197). We examined effects of NE receptor agonists and antagonists on beam-walking ability after cortical contusion (Br. Res., 1981, 211: 67-77). Rats were given a single drug or saline injection (i.p.) 24h after a 400g/cm focal contusion of the right sensorimotor cortex. Beam-walking tests were conducted 1, 3, 6 and 24 hours postinjection continuing every other day for 15 days. Prazosin (4mg/kg) retarded recovery and a trend toward accelerated recovery was observed for yohimbine (10 mg/kg; p=.13). Propranolol (10 mg/kg) and methoxamine (1, 4 or 8 mg/kg) showed no effects. The lack of an effect of methoxamine may be due to its short half-life. These results indicate a role for NE in recovery after cortical contusion but do not clarify the receptor type mediating this effect. After recovery from this cortical injury, continued NE function may be important for maintaining locomotor ability. Therefore, fully recovered sham or contused rats were injected 30d post injury with either propranolol (10 mg/kg), clonidine (.4 mg/kg), prazosin (4 mg/kg) or phenoxybenzamine (PBZ; 10 mg/kg). Beam-walking tests were conducted 1, 3, 6, 24 and 48 hours postdrug. Clonidine, prazosin and PBZ, but not propranolol, reinstated deficits.

Comparing these results to previous data using ablation of the same cortical area, the contused brain appears less responsive to drugs that promote recovery and more responsive to drugs which slow recovery. This could be due to more severe remote effects of the impact injury because of greater edema and/or shock forces. However, reinstatement of symptoms by drugs affecting alpha- but not beta-NE receptors is identical in the two models of hemiplegia.

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133.10 PROSTAGLANDINS AND NOREPINEPHRINE IN PERIPHERAL NERVE OF EXPERIMENTAL CHRONIC PROGRESSIVE DIABETIC NEUROPATHY. Kim K. Ward*, James D. Schmelzer*, Phillip A. Low Department of Neurology, Mayo Foundation, Rochester, MN 55905

Electrophysiologic studies on motor, sensory and sympathetic nerves of chronic progressive experimental streptozotocin diabetes of 4 months duration were done. Significant slowing of motor, sensory and sympathetic nerve conduction velocities was found associated with a reduction in amplitudes of caudal nerve and sciatic-tibial muscle compound action potentials.

The norepinephrine (NE) levels of superior cervical ganglion and sciatic nerve were significantly reduced in diabetic animals. Sciatic nerve sheath in vitro biosynthesis of 6-keto $PGF_{1\alpha}$ was significantly reduced in diabetic nerves. All studies were done on groups of at least 7 rats.

Table

Blood Glucose, Nerve Norepinephrine and Prostaglandins in Chronic Diabetes

Group	Control	Diabetes
Blood glucose (mg/dl)	120±6[d]	500±27[c]
HbA_{1c} (%)	5.36±0.1	17.8±0.5[c]
6-keto $PGF_{1\alpha}$ (pg/mg)	856±47	521±54[c]
TxB_2 (pg/mg)	640±130	319±50[a]
NE (ng/mg/wt) Cx Sym Gangl	35.6±2.1	24.6±1.5[c]
Sciatic	0.63±0.05	0.48±0.03[b]
Tibial	0.86±0.07	0.74±0.06[d]

a, $p<0.05$; b, $p<0.01$; c, $p<0.001$; d, SEM

We conclude that chronic diabetes results in a neuropathy involving motor, sensory and sympathetic nerve fibers. The findings of altered nerve NE and prostacyclin metabolism suggest that vasoregulation may be perturbed in chronic progressive experimental diabetic neuropathy. (Supported in part by grants from NINCDS (NS-14304; NS-22352) MDA, Mayo and Mogg funds.

133.11 DECREASED SPEED OF AN INTERNAL CLOCK IN PARKINSON'S DISEASE (PD). S. Corkin, A. Gruber, G. Desclos, and J.H. Growdon. Department of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 01239, and Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Maricq and Church (1983) reported that rats given methamphetamine (which enhances release of dopamine, DA, and norepinephrine, NE) showed acceleration of an internal clock, whereas rats given haloperidol (which blocks DA release) showed deceleration of the clock. We therefore predicted that patients with PD, who have a DA deficiency, would show a decrease in clock speed and that this deficit may constitute an objective measure of bradyphrenia (slowness of thought). Sixteen patients with idiopathic PD who were not demented and 13 age-matched, healthy control subjects (HCS) were trained on a time estimation task modeled after that used by Maricq and Church. All PD patients were receiving anti-parkinson medication. During training subjects viewed a series of squares on a computer screen that remained in view for either 1.0 sec. or 4.0 sec. After each square had disappeared, subjects reported whether the square had stayed on the screen for the shorter time or for the longer time. Then the correct response appeared on the screen. In the test phase, squares were presented for 1.0, 1.3, 1.6, 2.0, 2.5, 3.2, and 4.0 sec. A tone preceded each trial as a signal to attend to the screen. Subjects were told to say "short" if the stimulus duration seemed short based upon their training or "long" if the stimulus duration seemed long. The correct response was given for the 1.0 and 4.0 sec. durations but not for the others. The 7 stimulus durations were presented 10 times each for a total of 70 trials. In the results for normal subjects, the mean point of indifference (PI, the duration to which subjects responded "short" and "long" equally often) was at the geometric mean of the two extreme durations, i.e. 2.0 sec. In contrast, the mean PI for the PD group fell at 2.5 sec. The control and PD groups differed significantly at three stimulus durations in mean percentage of "long" responses (1.6 sec. -- HCS = 22.3%, PD = 3.8%; 2.0 sec. -- HCS = 52.3%, PD = 31.8%; 2.5 sec. -- HCS = 82.3%, PD = 56.3%). These results suggest that timing was altered in the PD group such that stimulus durations were consistently underestimated. Our findings support Maricq and Church's view that decreases in levels of DA cause decreases in internal clock speed.

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133.12 NEUROTRANSMITTER METABOLITE LEVELS IN EQUINE AND CANINE CSF: COMPARISON OF CISTERNAL AND LUMBAR CSF. D.M. VAUGHN, G.B. SMYTH*, E. COLEMAN*, B. WHITMER*, S. SIMPSON*, AND C. SATJAWATCHARAPHONG*. Scott-Ritchey Res. Prog., Small and Large Animal Clinics, Coll. of Vet. Med., Auburn Univ., Auburn, Al 36849.

Cerebrospinal fluid (CSF) is often collected from the lumbar region in clinical medicine. Biochemical and cytological evaluation of lumbar CSF is used to help evaluate neuronal status in disease or after drug administration. In clinical veterinary medicine, CSF is primarily obtained from the cisterna magna in dogs, and from the lumbo-sacral region in horses. The present studies were undertaken to determine if there was a significant difference in the concentration of Dopac, HVA, and 5-HIAA in cisternal versus lumbar CSF of dogs and horses.

CSF samples were collected from adult mixed breed dogs and horses within 10 min of induction of general anesthesia. With the dogs in sternal and the horses in lateral recumbancy, separate spinal needles were inserted into the lumbar subarachnoid space and into the cisterna magna. Canine CSF was removed first from the the lumbar then the cisternal region. Equine CSF was removed simultaneously from both regions. CSF was deproteinized by centrifugation over deproteinizing filters at 5°C. Neurotransmitter metabolites were evaluated by HPLC and electrochemical detection.

Equine and canine cisternal CSF contain measurable amounts of Dopac, HVA, and 5-HIAA. Lumbar CSF from dogs contains significantly lower amounts of HVA (cisternal= 108.0 ± 13.7 ng/ml vs. lumbar= 8.8 ± 0.7 ng/ml). In addition, canine lumbar CSF contained 24% less 5-HIAA (cisternal= 46.3 ± 8.1 ng/ml vs. lumbar= 36.6 ± 4.0 ng/ml). Opposite alterations in protein content occured within canine CSF. Lumbar CSF contained a significantly higher protein content (27.2 ± 2.3 mg/dl) than cisternal CSF (16.1 ± 1.8 mg/dl).

Equine cisternal CSF contained an average of 2.4 ± 0.2 ng/ml Dopac, 43.7 ± 3.4 ng/ml HVA, and 48.8 ± 3.7 ng/ml 5-HIAA. Dopac and HVA were not present in equine lumbar CSF. A significant 67.6% decline in 5-HIAA content (15.8 ± 3.7 ng/ml) was present in equine lumbar CSF. These data indicate cisternal CSF is a better indicator of brain neurotransmitter output and that opposite concentration gradients may exist between protein content and neurotransmitter metabolites in CSF.

133.13 GENETIC DIFFERENCES IN BRAIN CATECHOLAMINE LEVELS IN THREE TYPES OF DOMESTIC DOGS AND THEIR F_1 HYBRIDS. C. Arons*, D. Corry*, B.E. Ginsburg*, D. Morse*, and W.J. Shoemaker (SPON: K. Yurko). Dept. of Psychiatry, Univ. of Connecticut Health Center, Farmington, CT 06032

Genetic differences in CNS catecholamine systems have been reported for various strains of inbred mice and other laboratory species. In several instances these differences have been correlated with behavioral differences among the strains. In this study, we extend the brain-behavior correlation to another species, the dog. We have assayed catecholamine levels in 3 types of purebred dogs which have distinct behavioral characteristics and in their F_1 hybrids. The purebred types are: 1) Shar Planinetz, a livestock guarding dog that is non-aggressive toward livestock; 2) Border Collies, a livestock herding dog that displays components of predatory behavior but does not attack livestock; and 3) Siberian Huskies, a northern type dog that shows full predatory behavior towards livestock and other prey. We have used HPLC with electrochemical detection to analyze regional brain extracts for dopamine (DA), norepinephrine (NE), epinephrine (EPI) and serotonin. The results show little variation in EPI, it is found in low levels in all brain regions in all dogs. In contrast, some trends are apparent among the purebreds when key regions of the DA and NE systems are considered. In all major cell body regions of DA-containing neurons, Shars possessed the lowest levels of this transmitter compared to Border Collies and Huskies: substantia nigra, ventral tegmental area, and hypothalamus. In the Shars the NE level in the nucleus locus ceruleus is also the lowest among the 3 purebreds. Shars display low transmitter levels in the terminal regions of these amine systems as well: septal nuclei, hypothalamic regions, hippocampus and amygdala for NE; caudate nucleus, septum and locus ceruleus for DA. However, in two prominent DA regions, the globus pallidus and the nucleus accumbens, transmitter levels are quite high in the Shars. Border Collies show the opposite pattern; high transmitter levels in cell body regions and most terminal regions for both DA and NE. The Huskies show no clear trend in amine levels but rather show a mixture of both high and low levels in both cell body and terminal areas. Among the hydrids, one apparent trend was that the amine levels of the Border Collie x Shar Planinetz hybrids was intermediate between the two parental breeds. While we can suggest that some of the trends in transmitter levels might be correlated with general behavioral characteristics such as the low dopamine levels in Shars and their less active nature compared to Border Collies and Huskies, we cannot, at this time, draw any correlations with specific behavioral differences.

133.14 EFFECTS OF INHIBITION OF MAO SUBTYPES ON PLASMA AND STRIATAL LEVELS OF CATECHOLS AND HOMOVANILLIC ACID. D. Hovevey-Sion,* I.J. Kopin, R.W. Stull*, D.S. Goldstein*, NINCDS and NHLBI, National Institutes of Health, MD.

Deamination by monoamine oxidase (MAO) is a major route of metabolism of endogenous catecholamines. Two types of MAO -- MAO-A and MAO-B -- have been described. In the present study we measured plasma and corpus striatum levels of catechols and homovanillic acid (HVA) during inhibition of MAO-A (clorgyline 2 mg/kg, i.p.), MAO-B (deprenyl 1 mg/kg, i.p.) or both MAO-A and MAO-B (nialamide 75 mg/kg), and peripheral neuronal MAO (debrisoquin 40 mg/kg, i.p.) in pentobarbital-anesthetized rats. Levels of catechols in vena cava plasma and in the left corpus striatum were assayed using liquid chromatography with electrochemical detection. Samples were obtained at 1.5 hrs after drug treatment, except after debrisoquin, where sampling was at 4 hrs. Plasma norepinephrine (NE) was unaffected by all the treatments. Plasma dihydroxyphenylglycol (DHPG) was reduced significantly by clorgyline (28.1% of saline control), nialamide (32.4%), and debrisoquin (33.4%) but not by deprenyl (114.6%). Decreases in plasma DHPG were greater than decreases in HVA. In striatum, clorgyline and nialamide increased levels of dopamine (DA, 174.8 and 193.8% of control) and decreased levels of dihydroxyphenylacetic acid (DOPAC, 34.6 and 29.5%) and HVA (25.1 and 36.6%), whereas deprenyl and debrisoquin had no effects. The results indicate that plasma DHPG mainly reflects MAO-A activity and that striatal DA is also mainly metabolized by MAO-A. Thus rat plasma HVA and DHPG can be influenced in parallel by inhibition of MAO-A and not MAO-B.

133.15 Genetic Regulation of Catecholamine Enzyme Synthesis in Fischer-344 and Buffalo Rat Adrenals. A. C. Towle, H. Paivarinta, M. J. Evinger and T. H. Joh. Dept. of Neurol., Lab of Mol. Neurobio., Cornell Univ. Med. Coll., 1300 York Ave., N.Y., NY 10021.

Previous investigators have shown that Fischer 344 strain rats have much higher levels of blood epinephrine and adrenal phenylethanolamine n-methyl transferase (PNMT) than Buffalo strain rats. Recently, Evinger et al. (Mol. Brain Res. 1, 63-73, 1986) demonstrated that Fischer adrenals contain 5-fold more PNMT protein and 2-4 fold more PNMT mRNA than Buffalo rat adrenals.

In this study we sought to determine if the genetic differences between these two rat strains was due to a difference in (1) the amount of mRNA per cell, (2) the number of chromaffin cells, or (3) an interaction between mRNA content and cell number. To perform this investigation, rats were perfused with 4% paraformaldehyde and one adrenal was used for immunocytochemistry and the other was used for in situ hybridization. For immunocytochemistry, 25 micron sections through the entire adrenal were first incubated with PNMT antiserum and the immunostaining was shown using DAB. The sections were further incubated with TH antiserum and stained with aminoethylcarbazole. Thus, in the same section, the adrenergic cells were brown and the noradrenergic cells were red. Significantly, the total number of cells in the adrenal medulla appears to be the same for both rat strains. However, the number of cells containing PNMT immunoreactivity was greater in the Fischer than the Buffalo rat adrenals. Approximately 84% of the chromaffin cells contain PNMT in the Fischer rat compared to 60% in the Buffalo rat.

To investigate the possibility that the amount of PNMT mRNA per cell was lower in the Buffalo rat we performed PNMT and TH in situ hybridization. Densitometric quantification of the X-ray film and emulsion coated slides revealed that the number of cells containing PNMT mRNA was lower in the Buffalo than in the Fischer rat adrenal. Furthermore the amount of PNMT mRNA per cell was lower in the Buffalo adrenal. TH mRNA did not appear to be different in the adrenals from the two rat strains.

Thus, the decreased PNMT enzyme activity in the Buffalo rat adrenal is due to a decrease in the number of cell expressing PNMT as well as a decrease in the amount of PNMT mRNA in those cells.

133.16 The Distribution and Dynamics of Epinephrine-Containing Neurons in the Rat Brain: Influence of Adrenocortical State. K.L. Johnson*, C.M. Anderson*, J.K. Nishita, J.C. Ritchie, C.D. Kilts (SPON: R.D. Weiner) Duke Univ. Med. Ctr., Durham, NC 27710.

Epinephrine, a quantitatively minor catecholamine in the CNS, is localized in a variety of hypothalamic, telencephalic, and brainstem nuclei. The topographic distribution of epinephrine(EPI)-containing cells suggests the involvement of these neurons in the regulation of physiological processes such as blood pressure and respiration, neuroendocrine secretions, food and water intake, and thermoregulation. A focal innervation of the parvocellular division of the paraventricular nucleus (PVN) of the hypothalamus by EPI-containing neurons suggests a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Immunocytochemical studies indicate that EPI-containing and corticotropin releasing factor(CRF)-containing neurons make discrete synaptic connections in the PVN (Liposits et al., Histochem. 84:201, 1986) Pharmacological studies using inhibitors of phenylethanolamine-N-methyltransferase (PNMT) have demonstrated an increase in CRF immunoreactivity in the PVN (Mezey et al., Nature 310:140, 1984) and dose-related increases in circulating corticosterone (Roth et al., Life Sci. 28:2389, 1981), suggesting an inhibitory role of central EPI upon the HPA axis. The goals of the present study were to further investigate the distribution of EPI at the level of discrete nuclei and to biochemically estimate the rate of activity of these populations under normal conditions and under defined alterations of the activity of the HPA axis. The EPI content of micropunch dissected brain nuclei was determined by on-line trace enrichment HPLC with electrochemical detection and the activity of EPI neurons was estimated from the rate of depletion with time following administration of a PNMT inhibitor (LY134046). Of the nuclei examined, the following were found to have detectable and significant levels of EPI: PVN> tuberal periventricular nucleus> dorsomedial nucleus> pretuberal periventricular nucleus> C1> lateral hypothalamic nucleus> ventral bed nucleus> arcuate nucleus> C2. The arcuate followed by PVN demonstrated the greatest rates of estimated neuronal activity while cell body rich brainstem nuclei exhibited a significantly slower turnover rate. The collective findings of this study indicate that populations of EPI neurons innervating discrete brain nuclei differ in the estimated density of innervation and the estimated rate of tonic activity. The effects of the administration of glucocorticoids, bilateral surgical adrenalectomy and adrenalectomy plus glucocorticoid supplementation on the biochemically estimated activity of populations of EPI neurons innervating the PVN and other nuclei will be discussed.

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133.17 **PRODUCTION OF POLYCLONAL ANTISERA THAT RECOGNIZE NON-GLYCOSYLATED DOPAMINE β-HYDROXYLASE.** *O. Hwang, A. Towle, and T. H. Joh.* Laboratory of Molecular Neurobiology, Cornell Univ. Med. College, New York, N.Y. 10021.

Dopamine β-Hydroxylase (DBH) is the only enzyme in the catecholamine biosynthetic pathway that has not been characterized by molecular biological cloning. This is partially due to the fact that polyclonal antibodies to native glycosylated DBH have not been effective in recognizing the non-glycosylated enzyme produced in bacterial expression systems. We sought to develop efficient tools for cloning DBH, specifically, to prepare antisera that recognize the non-glycosylated enzyme.

We have previously reported purification of DBH to homogeneity and the sequence of 15 amino acids from the N-terminus (Hwang et al. *Soc. Neurosci. Abst. 12*:602, 1986) To obtain internal peptide sequences purified DBH was digested with trypsin, several peptides were purified by HPLC, and their sequence determined. Based on these sequences, peptides were synthesized and conjugated to Limulus hemocyanin with glutaraldehyde. Antisera were raised in rabbits by immunization at monthly intervals with 1mg of conjugate in complete Freunds adjuvant. Specificity of each antisera was tested by immunobloting, Western blot analysis, and immunocytochemistry. To obtain deglycosylated DBH, 40μg of enzyme was incubated with 0.4 units of glycopeptidase F in the presence of 20 mM potassium phosphate, pH 7 and 10 mM EDTA in a final volume of 50μl; reactions proceeded for 1 - 24 hours. For immunocytochemistry, rats were perfused with 4% paraformaldehyde and 20 micron vibratome sections were incubated with antisera diluted 1:500.

As determined by immunoblot analysis, each of the peptide antisera recognized their respective peptide conjugate both before and after preabsorption with carrier protein, hemocyanin. Surprisingly, highly purified glycosylated DBH was not detected by any of the antisera tested. Thus antisera recognized the synthetic peptides but not the glycosylated enzyme. In order to determine whether these antisera recognize the deglycosylated enzyme, DBH was treated with glycopeptidase F. This treatment resulted in complete deglycosylation as determined by periodic acid Schiff reaction and a decrease in MW from 75, 72, and 69 kD to 66 kD as determined by SDS-PAGE with silver stain detection. Similiar results were reported by Wong and Ciaranello (Soc. Neurosci. Abst. 12:1152, 1986) using a different endoglycosidase. Western blot analysis demonstrates that the anti-peptide antisera recognize the intact, deglycosylated enzyme but not un-treated DBH. Furthermore, none of the peptide antisera stained cell bodies in the locus coeruleous although some nerve terminal were stained indicating that *in vivo* almost all the enzyme is glycosylated. Native DBH antisera, in contrast,stained the cells and nerve teriminals very strongly.

Thus we have produced antisera that are efficient recognizing the non- glycosylated form of DBH, the form that would be present in bacterial expression systems. We are presently using these antisera to identify potential DBH clones from a cDNA expression library.

133.18 ANALYSIS AND ISOLATION OF DOPAMINERGIC NERVE TERMINALS BY FLUORESCENCE-ACTIVATED CELL SORTING. M.E. Wolf and G. Kapatos. Laboratory of Neurochemistry, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

The availability of pure preparations of dopamine (DA) nerve terminals would enable many basic questions about dopaminergic function to be answered. We have developed methods for the analysis and isolation of striatal nerve terminals (synaptosomes) using fluorescence-activated cell sorting techniques (FACS). Synaptosomes were distinguished from free mitochondria and other types of particles based on the following criteria: 1) lysis eliminated the signal attributed to synaptosomes, indicating an osmotically-sensitive compartment 2) synaptosomes, but not free mitochondria, exhibited increased fluorescence after incubation with a voltage-sensitive fluorescent dye, indicating that they possess a membrane potential; furthermore, the fluorescence signal decreased in response to incubation with the depolarizing stimuli 75uM veratridine or 55mM potassium chloride 3) synaptosomes were sorted onto membrane filters and subsequently shown by Western blot techniques to contain TH, a protein previously shown to be localized to DA terminals. Subpopulations of intact synaptosomes were separated by the cell sorter based on differential recognition by fluorescein-conjugated plant lectins and found to contain different amounts of TH. However, no surface marker which selectively identifies DA nerve terminals is presently available. Thus, sorting of dopaminergic synaptosomes was based on immunofluorescent labeling of an intracellular antigen specific to DA terminals, TH, after fixation of synaptosomes to allow penetration by antibodies. A highly fluorescent subpopulation of synaptosomes (9-12%) was detected after sequential incubation with a monoclonal antibody to TH and a fluorescein-conjugated second antibody. Specific labelling was eliminated if synaptosomes were prepared from 6-OHDA lesioned rats. TH-positive nerve terminals were isolated by FACS and proteins separated by SDS-PAGE. Sorted terminals were found to contain 8 times as much TH as an equal number of control terminals. These studies verify the dopaminergic identity of the sorted synaptosomes. This approach will enable DA terminals to be analyzed for the presence of co-transmitters, components of signal transduction systems, and unique protein constituents.

CATECHOLAMINES II

134.1 AUGMENTATION OF AMPHETAMINE-STIMULATED [^{3}H]-DOPAMINE RELEASE AFTER ONE INJECTION OF COCAINE: DO OTHER DOPAMINE UPTAKE BLOCKERS, DOPAMINE RECEPTOR AGONISTS AND LOCAL ANESTHETICS MIMIC THIS EFFECT? J. Peris and N. R. Zahniser. Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

We have reported that a single injection of cocaine augments amphetamine-induced release of tritium from rat striatal slices preloaded with [^{3}H]-dopamine (DA) without affecting electrically-stimulated release (Peris, J. et al. Pharmacol. Biochem. Behav. in press, 1987). This augmentation appears within 24 hrs and persists for 2 weeks even though cocaine is eliminated from brain 2 hrs after injection. It has been suggested that a change in dopaminergic synaptic transmission coincides with this augmentation and could explain sensitization to the behavioral effects of cocaine which appear over the same time course (Lin-Chu, G. et al. Pharmacol. Biochem. Behav. 22:901, 1985). Cocaine has a number of effects on the CNS, including blockade of DA uptake, indirect DA receptor activation and local anesthesia. Therefore it was of interest to determine whether drugs sharing one or more of these actions with cocaine would also augment amphetamine-stimulated [^{3}H]-DA release. Mazindol, a DA uptake blocker and indirect DA receptor agonist (10 mg/kg, i.p.), or lidocaine, a local anesthetic (20 mg/kg, i.p.), was administered to rats 24 hrs before sacrifice. [^{3}H]-DA release from striatal slices of these animals was compared with that from animals which had received either an injection of cocaine (10 mg/kg, i.p.) or an equivolume injection of saline. Release was defined as tritium overflow which is the net result of both DA release and reuptake. While cocaine treatment increased tritium release evoked by 2, 6 and 20 μM D-amphetamine compared to the saline control, mazindol treatment resulted in lower levels of amphetamine-induced release at all doses of amphetamine tested. In addition, whereas cocaine did not affect electrically-stimulated [^{3}H]-DA release, mazindol significantly increased tritium evoked in this manner. These data indicate that mazindol, unlike cocaine, is still present in brain 24 hrs after i.p. injection in sufficient concentrations to block DA uptake. Therefore in subsequent studies, mazindol will be injected 1 week before sacrifice to eliminate drug from brain. If mazindol produces the same long-term augmentation as cocaine, it should be apparent 1 week after injection, once mazindol has been eliminated from brain. Lidocaine treatment 24 hrs previous to sacrifice also caused augmentation of tritium release but, unlike cocaine, only in response to 20 μM amphetamine. It is also possible that lidocaine is present in brain 24 hrs after injection, and the effects of this drug will also be studied 1 week after injection. Similar experiments will be carried out using a selective DA uptake blocker (GBR 13069), another local anesthetic (procaine), and two direct DA receptor agonists (levodopa and apomorphine). From these studies, the mechanism for the augmentation in release and possibly the acute sensitization to the behavioral effects of cocaine may be identified. (Supported by USPHS grants MH 09387, DA 04216 and NS 09199)

134.2 SYSTEMIC OR CEREBRAL ADMINISTRATION OF p-HYDROXYAMPHETAMINE REDUCES DOPAMINE AND SEROTONIN CONCENTRATIONS IN THE RAT CNS. L.A. Matsuda, G.R. Hanson and J.W. Gibb. (SPON: J.A. Madsen) Dept. Pharmacol. & Toxicol., University of Utah, Salt Lake City, UT 84112

In rats treated with amphetamine, the major metabolites formed are p-hydroxyamphetamine (pOHA) and p-hydroxynorephedrine (pOHNor). The ability of these metabolites to deplete norepinephrine stores in both the brain and the periphery has been well characterized. In comparison, however, little is known about the actions of these compounds on central dopaminergic or serotonergic systems. Local administration of pOHA or pOHNor decreased striatal dopamine and its major metabolites, DOPAC and HVA, in a dose-dependent manner. We previously reported that local administration of methamphetamine (100 μg), into the striatum, had no effect on dopaminergic or serotonergic systems (Matsuda et al., Fed. Proc.: 399, 1987). However, as little as 1.0 μg (6.6 nmoles) of pOHA produced significant changes in striatal dopamine (68% of control) and its metabolites 3 hrs after treatment. This effect was reversible; maximal dopamine depletion (43% of control) occurred between 3 and 6 hrs after an intrastriatal injection of pOHA (5 μg) and returned to control levels within 48 hrs after treatment. In addition to their effects on dopamine, local injections of these para-hydroxylated compounds also reduced serotonin concentrations in the striatum; although, compared to dopamine, serotonin stores were less sensitive to the depleting actions of both pOHA and pOHNor. The pOHA-induced effects on striatal dopamine and its metabolites were also observed in animals treated with pOHA systemically. Three hrs after pOHA (15 or 30 mg/kg, s.c.) striatal dopamine decreased to 66% and 53% of control, respectively. This decrease in striatal dopamine was attenuated significantly in rats pretreated with the dopaminergic uptake inhibitor, amfonelic acid (1 mg/kg). Following systemic administration, however, serotonin concentrations were not altered in the striatum or cerebral cortex but were reduced in the hypothalamus and hippocampus. It appears, therefore, that in various regions of the brain, serotonergic neurons display a differential sensitivity to the actions of pOHA. Although the catecholaminergic effects of these para-hydroxylated metabolites of amphetamine have been the focus of numerous studies, a pOHA-induced behavioral response in mice, has recently been demonstrated to be a serotonin-mediated effect (Tadano et al, 1986). Thus, this observation, along with the present results, indicate that additional studies are necessary to appreciate fully the CNS effects of these metabolites of amphetamine. (Supported by USPHA grants DA 00869, DA 04222 and American Foundation for Pharmaceutical Education).

134.3 DOPAMINE (DA) AGONIST ACTIONS ON IDENTIFIED A9 DA NEURONS - ANESTHETIC ACTIONS AND D1/D2 INTERACTIONS. M.D. Kelland, A.S. Freeman and L.A. Chiodo, Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

We have utilized electrophysiological techniques in order to address two issues pertaining to DA agonist actions on the activity of rat A9 DA-containing cells identified by antidromic activation as nigrostriatal DA neurons. First, does the anesthetic condition of the subject alter the actions of DA agonists, as has been reported for DA antagonists? Second, might the selective D1 agonist SKF 38393 exert subtle actions on the activity of A9 DA neurons, either directly or on D2-mediated inhibition of these cells, that have previously gone undetected.

Standard extracellular single-unit recording techniques were used to monitor the activity of A9 DA neurons in vivo. Dose-response curves for the following DA agonists were compared in both the anesthetized and the locally anesthetized, paralyzed preparations: LY171555 (D2 selective), apomorphine (mixed D1/D2) and d-amphetamine (indirect agonist). For each compound, a significantly higher dose was required to achieve inhibition of DA cell discharge in the paralyzed animals (overall basal firing rates of the cells in each condition were not significantly different). Mean ED50 comparisons (anesthetized; paralyzed) were as follows: LY171555, 6.4 $\pm$ 2.4; 24.0 $\pm$ 8.3 ug/kg; apomorphine, 6.5 $\pm$ 4.8; 28.0 $\pm$ 6.3 ug/kg; d-amphetamine, 1.0 $\pm$ 2.8; 1.8 $\pm$ 1.7 mg/kg.

The ability of LY171555 to inhibit A9 DA neuronal firing rate was dependent upon the baseline firing rate of the neuron studied (i.e. faster cells required higher doses in order to achieve either 50% or complete inhibition). Pretreatment with SKF38393 (1.0 mg/kg i.v., 4 minutes) eliminated the rate-dependency of LY171555-induced inhibition of A9 DA neurons. Hemitransections of the forebrain prevented the SKF38393-induced effects, without eliminating the presence of slow or fast firing DA neurons. The selective D1 antagonist SCH23390 also blocked the SKF38393 effects.

The mechanisms underlying the results concerning anesthesia vs. paralysis are unclear at this time. It does appear, however, that anesthetic conditions are an important issue in DA agonist as well as DA antagonist research. The observations in the second set of experiments suggest that the direct-acting D1 agonist SKF38393 exerts a subtle modulatory effect on the responsiveness of A9 DA cells to D2 stimulation via actions it has in the forebrain. Further studies are required to more fully characterize this action of SKF 38393. Moreover, rate-dependent sensitivity of DA neurons to DA agonists is not dependent upon intact long-loop feedback pathways from the striatum.

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134.4 EFFECTS OF MIXED AND SELECTIVE DOPAMINE D-1 AND D-2 AGONISTS ON CAUDATE NEURON ACTIVITY. C.L. Christoffersen* and L.T. Meltzer (Spon: R.J. Anderson) Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105.

Dopamine (DA) D-1 receptors are located on neurons intrinsic to the caudate nucleus (CN), while DA D-2 receptors are located on both intrinsic neurons and terminals of DA neurons which project to the CN (DA autoreceptors). Using extracellular single-unit recording techniques, we have evaluated the effects of mixed and selective DA D-1 and D-2 agonists on the firing activity of spontaneously active neurons in the CN of chloral hydrate anesthetized rats. Recordings were made from both type 1 (negative-positive action potentials) and type 2 (positive-negative action potentials) caudate neurons. In general, no differences were noted between the two neuronal types so the data were combined. Baseline firing rates ranged from 0.3-13.6 Hz with a mean +/- SEM of 2.8 +/- 0.2 Hz (n=65). Drugs were administered i.v. via a tail vein.

An initial bolus injection of apomorphine (APO; 100 ug/kg), a mixed D-1/D-2 agonist, induced increases (n=8) and decreases (n=7) in neuronal firing. Cumulative doses (up to 800 ug/kg) did not produce inhibitions in neurons that were excited by APO. Both the excitatory and inhibitory effects were reversed by haloperidol.

An initial bolus injection of LY 171555 (quinpirole hydrochloride; 500 ug/kg), a selective DA D-2 agonist, induced increases (n=6), decreases (n=2), and no change (n=2) in neuronal firing. Similar to APO, increasing cumulative doses, up to 2 mg/kg, only increased the magnitude of the response, and did not change excitations into inhibitions.

SKF 38393, a selective D-1 agonist, had essentially no effect on CN neuron activity (cumulative dose response from 4-32 mg/kg, n=5).

The doses of APO and LY 171555 tested are much higher than the doses required to completely inhibit DA neuron activity; thus the excitations may be due to a direct effect on CN neurons and not due to disinhibition produced by DA autoreceptor stimulation. It appears that DA D-2 receptors are important for modulating CN neuron activity. The role of DA D-1 receptors is at present unclear.

134.5 EFFECTS OF ACUTE THIORIDAZINE, METOCLOPRAMIDE AND SCH 23390 ON THE BASAL ACTIVITY OF A9 AND A10 DOPAMINE NEURONS. Timothy H. Hand, Richard J. Kasser and Rex Y. Wang, Department of Psychiatry and Behavioral Science, SUNY, Stony Brook, NY 11794.

In a recent paper (Hand et al., Brain Res., in press), we reported that the acute administration of clozapine (considered an "atypical" antipsychotic drug because of its low potential for producing extrapyramidal side effects) elevated the basal firing rate of A10 but not A9 dopamine (DA) cells. By contrast, the neuroleptic haloperidol increased the firing rate of both A9 and A10 DA cells. These acute studies were consistent with previous chronic studies (White and Wang, Science, 1983), which had shown that while clozapine inactivates A10 but not A9 DA cells, haloperidol inactivates DA units of both subpopulations. The agreement between the acute and chronic effects of these two DA antagonists on DA cell subpopulations suggested the potential usefulness of the acute effects of these drugs in predicting their chronic effects. It was also proposed that the differing pharmacological and clinical properties of these two drugs might be related to their respective actions on DA cell subpopulations.

We therefore used this same acute procedure to test three other DA antagonists (thioridazine, TDZ; metoclopramide, MET; SCH 23390) with very different pharmacological and clinical properties, to determine whether there is a consistent relation between these properties and acute electrophysiological effects on DA cell subpopulations. TDZ, like clozapine, is considered an "atypical" antipsychotic drug. MET is a DA antagonist whose antipsychotic properties are weak except at very high doses. SCH 23390 is a selective D-1 receptor antagonist.

MET and TDZ both reversed the suppression of A9 and A10 DA cell activity by the DA agonist apomorphine (APO). SCH 23390 produced only a partial reversal of this suppression. When the antagonists were given alone (eg., with no APO pretreatment), TDZ increased the firing rate of 6/7 A10 cells (mean dose 0.6 mg/kg, i.v.) but only 1/9 A9 cells. On the other hand, MET activated 5/6 A9 cells (1.0 mg/kg) but only 3/7 A10 cells (0.8 mg/kg). SCH 23390 elevated the firing rate of 0/5 A9 cells and 1/5 A10 cells (up to 6.4 mg/kg). These data support the hypothesis that the "atypical" antipsychotic drugs act preferentially on the A10 DA system. They also support the position (White and Wang, JPET, 1984; Napier et al., JPET, 1986) that DA cell activity is not directly regulated by D-1 receptors. Taken together with the results from our previous acute (Hand et al., in press) and chronic (White and Wang, 1983) studies, it appears that the acute and chronic effects of a number of DA antagonists coincide very well, and that the chronic effects of these drugs may be predicted from their acute effects.

(Supported by USPHS Grants MH-41440, 41696 and 00378 to RYW and 09504 to THH)

134.6 EFFECTS OF DOPAMINE D1 AND D2 RECEPTOR AGONISTS ON THE SLEEP-AWAKE CYCLE OF RATS. K.A. Serpa and L.T. Meltzer, Warner-Lambert/-Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI 48105.

Apomorphine (APO), a mixed D1-D2 dopamine (DA) receptor agonist, has a biphasic effect on the behavior of rats. Low doses produce sedation, characterized by locomotor inhibition and a synchronized EEG, while high doses produce increased locomotion, stereotypies, and a desynchronized EEG. The sedative effect of APO has been postulated to be due to selective activation of DA D2 autoreceptors, while the hyperactivity and the stereotypies are attributed to postsynaptic DA D1 and D2 effects. To further characterize the pharmacology of these responses, we have examined the effects of various selective DA agonists and antagonists on the sleep-awake cycle of rats.

Rats implanted with cortical screw electrodes and temporalis muscle wire electrodes were connected via a mercury pool commutator to a polygraph for EEG and EMG recording. Testing was done during the rats' light phase and began immediately following transfer to the recording chamber. EEG records were scored visually for minutes of awake, slow-wave sleep (SWS) and REM sleep. Latencies to first occurrence of deep sleep (S2) and to REM were also measured.

APO decreased latency to S2 and increased total SWS at low doses and decreased SWS and produced stereotypies at high doses. (+)-3PPP, a D2 agonist, had a biphasic effect similar to that of APO. (-)-3PPP, a weak autoreceptor agonist with post-synaptic antagonist activity, increased SWS at all doses. LY 171555, a selective D2 agonist, reduced latency to S2 at all doses. Low doses of LY increased SWS in both the 1st and 2nd h post injection, while higher doses decreased SWS in the 2nd h. The selective D1 agonist SKF 38393 had no effect on latency to S2, and produced a slight decrease in SWS. Both the decrease in latency to S2 and the increase in SWS produced by low doses of APO were antagonized by (-)-sulpiride, a selective D2 antagonist. SCH 23390, a selective D1 antagonist, increased sleep and reduced latency to S2 when administered by itself, and blocked the decrease in SWS and the stereotypy induced by a high dose of APO.

These results confirm and extend the evidence that the sedative effects of DA agonists are mediated by the DA D2 autoreceptor, arousal results from postsynaptic D2 stimulation, and that production of stereotypies requires stimulation of both D1 and D2 receptors.

134.7 DOSE-DEPENDENT EFFECTS OF REPEATED APOMORPHINE TREATMENTS ON LOCOMOTOR ACTIVITY IN RATS. B.A. Mattingly, J.E. Gotsick*, and K. Salamanca*. Dept. of Psychology, Morehead State Univ., Morehead, KY 40351.

In two experiments, the effect of repeated injections of apomorphine on locomotor activity of rats was determined. In each experiment, different groups of rats were injected with either apomorphine (0.2, 1.0, or 5.0 mg/kg) or vehicle at either 24 or 72 hr intervals and tested for locomotor activity in photocell arenas. In Experiment 2, following 13 treatment sessions with various doses, all groups were first tested for activity following a 5.0 mg/kg dose of apomorphine and then given vehicle only prior to the final activity test session. Major findings were as follows: a) repeated injections of 1.0 and 5.0 mg/kg apomorphine produced a progressively greater increase in activity with each injection (i.e., sensitization); b) injections of 0.2 mg/kg of apomorphine produced a slight inhibition of activity which did not change with repeated injections; c) prior treatment with 0.2 mg/kg of apomorphine resulted in a significantly greater activity increase following a 5.0 mg/kg dose of apomorphine than did prior vehicle treatments; and d) chronic pretreatment of rats with apomorphine did not affect their activity level following a vehicle injections. These findings suggest that sensitization to apomorphine is a graded, rather than an all or none, phenomenon dependent on the dose of apomorphine repeatedly administered. In addition, these results are inconsistent with autoreceptor tolerance and conditioning explanations of dopamine agonist-induced sensitization effects.

134.8 DOPAMINE (DA) COMPLEXES WITH TYPICAL ANTIPSYCHOTIC DRUGS (AD) AND WEAKLY OR NOT AT ALL WITH THE ATYPICAL ANTISYCHOTIC DRUGS. J. O. Schenk and T. Patterson (SPON: C. Hughes). Dept. of Chemistry, Program in Biochemistry, Washington State Univ., Pullman, WA 99164-4630.

Acute treatment with AD's increases DA cell firing rates, the number of spontaneously active DA cells, DA release, and DA synthesis and metabolism. Repeated administration of AD's results in a cessation of DA cell firing with decreased spontaneous and depolarization induced release while synthesis and metabolism return to near control levels.

During voltammetric (VA) assessments of the acute effects of AD's on release from striatal tissue in vivo and in vitro we observed negative alterations in the VA assessed [DA] in direct proportion to the [AD]. These effects were observed over 0.27-1.1 uM [AD], the tissue [AD] after a single ca. 0.2 umol/kg i.p. dose (Öhman et al. N.-S. Arch. Pharmacol. 299: 105 (1977)).

This phenomina was then examined chemically in either bicarbonate based buffers (pH 7.0 to 8.0) or in commercial ultra pure water. The following observations were made: 1. the half wave potential (E(1/2)) for the oxidation of DA shifted as a function of [AD] 2. E(1/2) shifts were not observed for methoxylated catechol derivatives 3. u.v.-visible absorption features of DA shift as a function of [AD] 4. no spectral shifts for DA after adding AD were observed at acid pH's and 5. the difference spectra of AD's in the presence of DA (less the spectra of DA) have features that are not found in the spectra of AD alone.

In the case of haloperidol (HAL) (where one can test the commercially available butyrophenone and piperidinyl moeities separately) we found that the basic piperidinyl moeity was essential for the interaction to occur. Phenothiazine AD's also appear to undergo complexation with DA. Based on the data above it is hypothesized that the interaction between AD's and DA is based on acid-base association. However, sulpiride, a basic but so called atypical AD did not complex with DA. The dissociation constants of the complexes that do form is in the range 10^{-4} to 10^{-3} mol/l, values smaller than expected for the simple acid base association between an acidic catechol moeity and the nitrogen based basic sites on AD molecules. The order of ability of individual AD's to complex with DA is Fluphenazine > HAL > SULP.

In conclusion, complex formation between AD's and DA should not appreciably alter DA concentrations in vivo (because of the low [AD] needed for physiological effects) but almost all typical AD molecules would be complexed with DA (due to its excess conc.). This phenomina could be important in the pharmacology of typical AD's and in some of the differences between typical and atypical AD's. Supported by MH-42759 and the State of WA.

134.9 ACTION OF COCAINE ON DOPAMINE (DA) AND SEROTONIN (5HT) CONTAINING NERUONS, M.J. Keegan*, J. Stahl*, and M.P. Galloway (SPON: H. Altman) Lafayette Clinic and Wayne State Univ., Detroit MI

The ability of cocaine (COC) or amphetamine (AMPH) to inhibit neuronal reuptake of monoamines is thought to be one mechanism that contributes to their psychotropic effects. In light of the autoregulatory mechanisms associated with monoaminergic neurons (i.e., modulation of transmitter release, synthesis, and impulse flow), inhibition of reuptake takes on added significance in these neurons. Our studies are focused on the effects of COC and AMPH on 5-HT or DA synthesis (measured after inhibition of decarboxylase with NSD-1015). Monoamine synthesis is utilized as a presynaptic function responsive to autoreceptor (AR) stimulation.

We have previously reported that acute COC dose-dependently suppresses DA and 5HT synthesis in vivo in medial prefrontal cortex (PFC), n. accumbens (NAC), and the striatum (STR). We have recently observed that administration of the D2 antagonist sulpiride (60 umol/kg, ip) blocked the inhibition of DA, but not 5HT, synthesis afforded by cocaine (60 umol/kg, ip) suggesting that the inhibitory effect is D2 receptor mediated. Pretreatment with reserpine (2.5 mg/kg,sc, 6 hrs) depletes striatal DA by 96% (5HT by 88%) but does not block the COC (60 umol/kg, ip) induced attenuation of 5HT or DA synthesis in the accumbens (37% and 22% decrease respectively) or other tissues studied. Inhibition of DA release by administration of GBL prevents the inhibitory effect of COC on striatal DA synthesis.

In striatal slices, DA, but not 5HT, synthesis is dose dependently inhibited by COC and GBR 13098 (EC50 = 0.5 & 1.0 uM, respectively). Curiously, COC (1 uM) induced inhibition of DA synthesis was not blocked by sulpiride, haloperidol, or SCH 23390 (1 uM). In addition, the potency of COC to inhibit DA synthesis was increased in slices from reserpine pre-treated animals.

Repeated administration of COC (10 mg/kg, ip, bid, x14 days) does not substantially alter basal levels of parent monoamines or metabolites in several DA projections, except a 23% increase in striatal DOPAC. However, a consistent observation after repeated COC is a modest increase (15-25%) in the basal rate of in vivo DA synthesis. In addition, in striatal slices obtained from animals treated subchronically with COC (10 mg/kg, ip, tid, x3 days), the ability of the DA agonist quinpirole to inhibit DA synthesis is diminished. Similar decrements in the efficacy of quinpirole in vitro were noted after subchronic treatment with AMPH in vivo.

Taken together, the findings support the hypothesis that COC is an indirect DA agonist capable of altering levels of synaptic DA. Moreover, repeated exposure to COC may lead to a decreased responsiveness of synthesis modulating DA ARs. Support (MPG) from DA-04120, MH-41227, and the State of Michigan-DMH.

134.10 STIMULATION OF MIDBRAIN DOPAMINE NEURONAL ACTIVITY BY RITANSERIN, A NOVEL 5-HT_2 ANTAGONIST. L. Ugedo*, J. Grenhoff* and T.H. Svensson. Dept. of Pharmacology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Anatomical, histochemical and biochemical studies have for long indicated the existence of a projection from the 5-hydroxytryptamine-(5-HT)-containing brainstem raphe nuclei to the substantia nigra (SN) and ventral tegmental area (VTA). However, functional evidence regarding this suggested serotonergic influence on the dopamine (DA) cell groups in the SN and the VTA is still sparse. In the last few years, several new drugs with a purported selectivity for the different 5-HT receptor subtypes have been synthesized. We have used one of these, ritanserin (R 55667, Janssen Pharmaceutica), a selective 5-HT_2 antagonist (see Mol. Pharmacol. 27:600, 1985), to study the serotonergic influence on the DA neurons in the SN and the VTA, which give rise to the nigrostriatal and mesolimbic DA systems, respectively.

Electrophysiological single cell recordings were made from identified DA neurons in SN and VTA in male rats anesthetized with chloral hydrate. Neuronal activity was characterized with respect not only to firing rate, but also to amount of firing in bursts and regularity of firing, respectively, by means of inter-spike interval analysis. One cell per rat (n=24) was recorded before and after intravenous administration of ritanserin (0.5-2.0 mg kg^{-1}).

In SN-DA neurons, 0.5 mg kg^{-1} of ritanserin produced increased burst firing and deregularization of firing pattern without altering the firing rate of the neurons, i.e. a modulatory effect. However, larger doses (1.0-2.0 mg kg^{-1}) increased the firing rate without changing the regularity of firing, i.e. an excitatory effect. In VTA-DA neurons, all doses (0.5-2.0 mg kg^{-1}) increased firing rate and burst firing without altering the regularity of firing. The inhibition (30-100 %) of firing produced by the DA agonist apomorphine after ritanserin indicates that the effects of ritanserin are not due to DA receptor antagonism.

Thus, the following interpretations of our results seem warranted:

1) Midbrain DA neuronal activity may be under tonic, inhibitory 5-HT-mediated control, executed by means of 5-HT_2 receptors.
2) In the SN, the 5-HT influence seems to include a modulatory component, controlling the regularity of firing, as judged by the effects of differential doses of the selective 5-HT_2 antagonist ritanserin.

134.11 CHANGES IN BRAINSTEM NORADRENALINE AND ADRENALINE TURNOVER DURING CLONIDINE TREATMENT AND ITS WITHDRAWAL. L. Lambás-Señas*, J. Atkinson*, J.P. Fluckiger*, M. Sonnay* and B. Renaud. Lab. Neuropharmacologie-UA CNRS 1196, Faculté de Pharmacie, Université Claude Bernard, Lyon (France), Lab. Pharmacodynamie, Faculté de Pharmacie, Université de Nancy I, Nancy (France) and Institut de Pharmacologie de l'Université-CHUV, Lausanne (Switzerland).

This study was undertaken to determine, in the spontaneously hypertensive adult rat, the brain regions involved and the changes occurring in noradrenaline (NA) and adrenaline (A) metabolism i) during a chronic clonidine treatment and ii) at the beginning of the withdrawal syndrome which appears after cessation of a prolonged clonidine treatment. NA and A turnovers (estimated by measuring NA and A depletions after FLA 63 administration) were determined in the A1-C1 and A2-C2 brainstem regions after seven days of treatment with clonidine (0.1 mg/kg/day, i.v., via osmotic minipumps) and at a early (16h), intermediate (40h) and late (64h) delay after withdrawal from a 10 days' treatment. The doses of clonidine and the protocol used have previously been reported to lower blood pressure and produce cardiovascular hyperactivity upon withdrawal (Atkinson et al., Eur. J. Pharmacol., 1986, 121, 97-107).

After a seven days' treatment with clonidine, the NA and A turnovers were unchanged both in the A1-C1 and A2-C2 regions. During withdrawal, the NA turnover was also unchanged in these regions. However, the A turnover was significantly increased 16h ($p < 0.01$) and 40h ($p < 0.05$) after withdrawal in the A1-C1 region and 16h after withdrawal ($p < 0.01$) in the A2-C2 region.

These results show that the NA metabolism is unchanged both during clonidine treatment and during its withdrawal in the brainstem catecholaminergic regions analyzed. In contrast, the increase in A turnover found in the A1-C1 and A2-C2 regions suggests that the adrenergic neurons of the brainstem could be activated during clonidine withdrawal. The adrenergic C1 neurons have been shown by various approaches to be a key element of the sympathetic vasopressor system. Thus, the increase in the A turnover we observed during withdrawal could be at the origin of the sympathetic hyperactivity found after cessation of a prolonged treatment with clonidine.

This work was supported by the "Fondation pour la Recherche Médicale" and by the "INSERM" (Contrat de Recherche externe 85-6017).

134.12 FG 7142, AN ANXIOGENIC BETA CARBOLINE, SELECTIVELY INCREASES TYROSINE HYDROXYLATION IN THE MEDIAL PREFRONTAL CORTEX. A.M. Knorr, A.Y. Deutch, and R.H. Roth. Yale Univ. Sch. Med., New Haven CT 06510.

We have previously demonstrated that footshock stress leads to a selective activation of dopamine (DA) metabolism in the prefrontal cortex of rat. A similar effect is elicited by administration of the anxiogenic beta carboline FG 7142. The activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, increases in the prefrontal cortex following stress exposure (Iuvone and Dunn, 1986), suggesting that an increase in DA synthesis may occur concomitantly with the increase in metabolism.

The current experiments examine FG 7142-induced alterations in DA synthesis as reflected by DOPA accumulation following L-aromatic amino acid decarboxylase inhibition with m-hydroxybenzylhydrazine (NSD 1015). NSD 1015 was administered to rats 30 minutes prior to sacrifice; FG 7142 or vehicle was administered 5 minutes after NSD 1015. Catecholamine levels were measured by HPLC with electrochemical detection. DOPA accumulation was significantly increased in the medial prefrontal cortex but was not altered in the nuc. accumbens or striatum. DA levels in the prefrontal cortex of FG 7142-treated animals were significantly lower than in control subjects, thus confirming an enhancement of DA turnover and metabolism induced by FG 7142. Neither the striatum nor the nuc. accumbens exhibited lowered DA levels. The observed increase in DOPA synthesis in the prefrontal cortex may therefore be secondary to a decrease in feedback inhibition. Alternatively, FG 7142 may influence the kinetic properties of TH by increasing the functional activity of the mesoprefrontal DA neurons. This possibility is consistent with our previous data demonstrating an FG 7142-induced increase in DA metabolite levels in the ventral tegmental area, but not substantia nigra. These data therefore suggest that FG 7142 may be acting directly on the cell body region of mesoprefrontal cortical DA neurons. However, in vitro studies in which prefrontal cortical slices from untreated rats were preincubated with FG 7142 (1-10 um) demonstrated a concentration-dependent increase in tyrosine hydroxylation similar to that observed in vivo. In striatal slices, in contrast to the lack of effect in vivo, DOPA accumulation was significantly decreased. This is consistent with the possibility that FG 7142 may also exert a presynaptic effect on DOPA synthesis which is dependent on the intrinsic organization of the terminal regions. These studies confirm and extend previous data on the effects of the anxiogenic beta carboline FG 7142 by demonstrating that synthesis and metabolism in the mesoprefrontal DA system is preferentially altered by this inverse benzodiazepine agonist.

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134.13 YOHIMBINE INCREASES PLASMA NOREPINEPHRINE APPEARANCE RATE WITHOUT ALTERING CLEARANCE. M.M. Murburg*, E. Villacres*, D.M. Dorsa, G.N. Ko, R.C. Veith* (SPON: D. Bowden). GRECC, Seattle VAMC and University of Washington, Seattle, WA 98108.

The α_2-adrenergic antagonist yohimbine hydrochloride has been reported to increase plasma norepinephrine (NE) in humans. We investigated whether yohimbine increases plasma NE solely by causing a dose-dependent increase in the rate of NE appearance into plasma, or whether it also decreases the rate of NE clearance. Seventeen fasting supine, healthy, nonobese, drug-free men, aged 20 to 34, participated. Plasma catecholamines and basal NE kinetics were measured in arterialized plasma 30, 40 and 50 minutes after a 15μCi/m^2 bolus, followed by a 0.35μCi/m^2/min infusion, of tritiated l-norepinephrine ([^{3}H]-NE). Subjects then randomly received (double-blind) placebo, or yohimbine 20 or 40 mg po. Ninety minutes after the drug, a second bolus and infusion of [^{3}H]-NE were given, with sampling again at 30, 40 and 50 minutes (120, 130, 140 minutes after drug). Plasma NE appearance and clearance rates were calculated using a [^{3}H]-NE isotope dilution technique. Average basal and post-drug steady state catecholamine levels, NE appearance and clearance rates, and vital sign measurements were calculated. One way ANOVA was used to compare mean percent of baseline achieved after each drug treatment for each of these measures.

Basal NE and epinephrine (EPI) levels, NE kinetics and mean arterial pressure (MAP) did not differ among the three drug groups. Placebo, yohimbine 20 mg, and yohimbine 40 mg, respectively, raised plasma NE to 99±25, 157±33 and 213±58 ($\bar{x}$±SD) percent of baseline ($F=11.31$; $p<.005$). The yohimbine-induced increases were accompanied by dose-dependent increases in NE appearance rate, which was 104±27, 175±53 and 230±72% of baseline after placebo, yohimbine 20 mg and yohimbine 40 mg, respectively, ($F=8.12$; $p<.01$). NE clearance was not altered by yohimbine (all 99-108% of basal). EPI levels were 99±17% after placebo, 138±50% after yohimbine 20 mg, and 187±50% after yohimbine 40 mg ($F=6.89$; $p<.02$). Yohimbine increased MAP to (101±4.3; 102±4.7; 108.9±3.6 percent of baseline; ($F=6.01$; $p<.05$) after placebo, yohimbine 20 mg and yohimbine 40 mg, respectively. Increases in human plasma NE levels following yohimbine reflect dose-dependent increases in NE appearance rate, and not alterations in NE clearance, compatible with increased sympathetic outflow in response to blockade of α_2 adrenergic receptors.

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134.14 THYROID HORMONE AND NOREPINEPHRINE: EFFECTS ON RECEPTOR REGULATION AND Na,K-ATPase. Alan C. Swann and Jeffrey S. Steketee. Dept. of Psychiatry, University of Texas Medical School, Houston TX 77225, USA.

Thyroid hormone produces metabolic effects similar to those of stimulation of noradrenergic receptors. It has been reported, however, that norepinephrine turnover is reduced during thyrotoxicosis and that beta-receptor number is increased. Metabolic effects of thyroid hormone may therefore reduce noradrenergic activity by a feedback mechanism. We examined effects of thyroid hormone administration or of production of hypothyroidism with methimazole on receptors associated with regulation of noradrenergic function. Since both norepinephrine and thyroid hormone increase Na,K-ATPase activity, we also examined interactions between effects of thyroid hormone and noradrenergic activity on this enzyme. Treatment with thyroid hormone increased beta-receptor binding, increased alpha-2 receptor binding, and decreased desipramine binding, opposite to effects of hypothyroidism produced by methimazole. Heart was more sensitive than brain to these effects. Treatment with the beta-adrenergic antagonist propranolol or with 6-hydroxydopamine did not prevent the increase in heart Na,K-ATPase associated with thyrotoxicosis. Thyroid hormone also did not prevent the decrease in Na,K-ATPase associated with propranolol or 6-hydroxydopamine. Administration of methimazole did not prevent the stimulation of heart or cerebral cortex Na,K-ATPase by yohimbine; consistent with the increase in alpha-2 receptor number, thyroid hormone increased sensitivity to yohimbine slightly. These data are consistent with reduced noradrenergic activity during hyperthyroidism, possibly mediated by increased alpha-2 receptor function. Thyroid hormone and norepinephrine appear to affect Na,K-ATPase activity by independent mechanisms.

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134.15 EFFECTS OF CHLORAL HYDRATE ANESTHESIA ON LHRH NEURONAL RESPONSIVENESS TO NOREPINEPHRINE. R.D. Hartman* and C.A. Barraclough. (SPON: A. M. Mans) Dept. of Physiology, Sch. of Med., Univ. of Maryland, Baltimore, MD 21201.

Electrical stimulation (ES) of locus coeruleus (LC) or the A1 noradrenergic cell groups, in chloral hydrate (CH) anesthetized rats, amplifies LH release. This effect occurs only after LHRH neurons are partially depolarized by initial electrochemical stimulation (ECS) of the medial preoptic nucleus (MPN). In contrast, plasma LH levels remain basal if only LC or A1 are stimulated. These observations suggest that: (a) inadequate norepinephrine (NE) is released by these stimuli to activate dormant LHRH neurons, or (b) CH elevates thresholds of excitability of LHRH neurons to NE. Accordingly, we evaluated the effects of CH on LH release after infusion of NE into the third ventricle (3V) of control and MPN-ECS rats.

Seven day ovariectomized (OVX) rats (day 0) received Silastic capsules containing estradiol (150 ug/ml in oil, s.c.). On day 2 control rats were anesthetized with ether, whereas experimental rats received CH (400 mg/kg, i.p.). Infusion cannulae were inserted into the 3V and thereafter, NE was infused in 2 ul artificial CSF containing 0.2% ascorbic acid over 1 min. In controls, NE elicited peak LH responses of 250 $\pm$ 30 (5 ug) or 600 $\pm$ 80 ng/ml (15 ug) within 10 min, whereas ACSF alone did not affect basal LH levels. In contrast, in CH-treated rats, peak LH responses reached only 233 $\pm$ 50 (30 ug NE) or 304 $\pm$ 46 ng/ml (45 ug NE). Consequently, one reason why neither LC or A1 ES evoke LH release may be due to the marked suppression of LHRH neuronal responsiveness to NE by CH.

A similar protocol was used to ascertain whether 3V NE infusions would mimic the amplifying effects of LC or A1 ES on LH release. Estrogen-primed OVX rats were anesthetized with CH and the MPN was ECS (100 uA/60 sec, d.c.). Thirty min later ACSF was infused into the 3V of control rats, whereas other groups received either 150 ng, 15 or 45 ug NE in ACSF. Following MPN-ECS, plasma LH rapidly increased from 103 $\pm$ 9 to 597 $\pm$ 42 ng/ml, and this response was not affected by ACSF. Neither 150 ng or 15 ug of NE altered this LH response. However, 45 ug NE markedly amplified MPN-ECS-induced LH release. Within 30 min after NE, plasma LH rose to 1380 $\pm$ 236 vs. control values of 668 $\pm$ 143 ng/ml. By 45 min LH reached levels of 2545 $\pm$ 860 vs. 636 $\pm$ 101, and by 60 min LH was still elevated to 2625 $\pm$ 732 vs. 582 $\pm$ 91 ng/ml for controls. Seemingly, LHRH neurons become more responsive to NE after MPN-ECS compared to unstimulated controls. It is possible that even after MPN-ECS, LHRH neuronal responsiveness to NE is markedly reduced by CH. We currently are searching for other anesthetics which only minimally affect LHRH neuronal responsiveness to NE. Supported by Research Grant HD-02138.

134.16 **NEUROTENSIN EFFECTS ON DOPAMINE RELEASE IN THE STRIATUM AND NUCLEUS ACCUMBENS: SITE SPECIFICITY AND MECHANISMS OF ACTION.** **C.D. Blaha, A.G. Phillips, H.C. Fibiger and R.F. Lane, Depts. of Psychology, Psychiatry and Chemistry, Univ. of British Columbia, Vancouver, B.C. Canada V6T 1Y7.**

The tridecapeptide neurotensin (NT) and dopamine (DA) have been shown to be co-localized in mesolimbic neurons. The observation that intracerebroventricular (i.c.v.) injections of NT produces behavioral effects similar to those of atypical antipsychotics (e.g. blockade of locomotion but not stereotypy induced by amphetamine) has prompted hypothesis about the role of NT in the etiology of schizophrenia. Using in vivo electrochemical techniques (Lane & Blaha, Ann.N.Y.Acad.Sci. 473:50, 1986), we have previously shown that typical antipsychotics (e.g. haloperidol, HAL) increase DA release in both mesolimbic (accumbens) and nigrostriatal (striatum) DA terminal regions, whereas atypical antipsychotics (e.g. clozapine, CLOZ) selectively increase DA release in the accumbens. The present study compares the effects of NT with those of HAL and CLOZ, on DA release in the striatum and accumbens on anesthetized rats. In an effort to understand the mechanisms of action of NT on DA release, the effects of NT and these antipsychotics in combination with apomorphine (APO) and amphetamine (AMP) were also examined.

Administration of various doses of NT (0.1-10 ug/10ul i.c.v.) induced immediate dose-dependent increases in accumbens DA release, whereas striatal DA release was unaffected at these doses. Similar to NT, CLOZ (20 mg/kg s.c.) selectively increased accumbens DA release while having no effect on striatal DA release. In contrast, HAL (0.5 mg/kg s.c.) produced increases in DA release in both brain regions. γ-butyrolactone (GBL) is known to block DA neuronal impulse flow and induce concomitant reductions in forebrain DA release. Dependent upon the time of injection, GBL (200 mg/kg i.p.) either prevented or reversed NT-induced increases in accumbens DA release. Both CLOZ- and HAL-induced increases in accumbens DA release were similarly affected by GBL. APO (50 ug/kg i.v.) was also effective in antagonizing both NT- and antipsychotic-induced changes in DA release. Additionaly, AMP (1 mg/kg i.v.), administered during peak DA stimulatory effects induced by NT or the antipsychotics, produced further increases in release that was comparable in magnitude to that produced after AMP administration alone.

These in vivo results provide evidence that (1) at the doses tested, NT selectively stimulates mesolimbic DA release (2) NT selective effects and interactions with GBL and DA agonists parallel CLOZ effects suggesting common mechanisms of action with atypical antipsychotics and (3) the inability of NT to alter AMP-induced increases in DA release indicates that NT blockade of AMP elicited behaviors is mediated by mechanisms postsynaptic to the DA nerve terminal. **Support: MRC Program Grant-23 and USPHS NS 13556.**

134.17 (-)-3PPP HAS TWO EFFECTS ON DOPAMINERGIC (DA) NEURONS IN THE SUBSTANTIA NIGRA PARS COMPACTA (SNC) OF THE RAT. C.B. Davis* and P.S. Blum. Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477.

(-)3-(3-Hydroxyphenyl)- N-n-propylpiperidine ((-)-3PPP) has been proposed to be an agonist at DA autoreceptors. Part of the evidence for this conclusion includes the observation that a dose-dependent decrease is seen in the activity of DA neurons following administration of (-)-3PPP (ED_{50} 300 µg/kg i.v.). Recently, we investigated the effects of (-)-3PPP on DA neurons in the SNC of chloral hydrate anesthetized Wistar rats. A simple, dose dependent decrease in firing rate was rarely seen. Rather (-)-3PPP produced either no change or an erratic change in neuronal activity. To characterize the effects of (-)-3PPP, therefore, we examined the relationship between neuronal activity and extracellular spike amplitude following (-)-3PPP for 10 DA neurons in the SNC. DA neurons were identified by standard criteria including firing frequency, and duration and shape of the action potential. All recording sites were in the SNC. The total analysis period for each neuron (7-33 min) was divided into 20 sec segments in which the number of spikes was counted and the average spike amplitude and variability in amplitude was determined. Two neurons showed a simple dose-dependent decrease in activity. Twenty µg/kg of (-)-3PPP produced an observable decrease, and inhibition reached 40% with cumulative doses of 75 and 40 µg/kg. There was a reciprocal relationship between frequency and spike amplitude, and there was little second-to-second variability in amplitude. These results are consistent with an action at DA autoreceptors. In three neurons, administration of (-)-3PPP resulted in an abrupt decrease in both frequency and spike amplitude. In two neurons this occurred after one dose of 10 µg/kg and in the other after a cumulative dose of 170 µg/kg. These data suggest that (-)-3PPP has a direct toxic effect on DA neurons that is not receptor mediated. Four neurons showed an intermediate effect; initially there was a dose dependent decrease in activity with an increase in amplitude. This was followed by an abrupt decrease in amplitude and frequency. The threshold for the initial decrease was 10 µg/kg and the threshold for abrupt change was 40-135 µg/kg. One neuron showed no effect of (-)-3PPP (30 µg/kg). These data suggest that (-)-3PPP has two different effects on DA neurons when administered systemically. The first is a decrease in activity mediated by action at DA autoreceptors. The second is a direct effect on the neuronal membrane. Depending upon the sensitivity of the individual neuron, one effect or the other may predominate.

135.1 INTRANIGRAL ANTIEPILEPTIC DRUGS PREVENT SEIZURES INDUCED BY PILOCARPINE. L. Turski, J.S. Andrews*, P.A. Löschmann*, D.N. Stephens*, Z.A. Bortolotto* and E.A. Cavalheiro*. Dept. of Neuropsychopharmacology, Schering, 1000 Berlin 65, West Germany; Dept. of Neurology and Neurosurgery, Escola Paulista de Medicina, CEP-04023 Sao Paulo, Brazil.

Seizures produced in rodents by systemically administered pilocarpine provide a model for studying the generation and spread of convulsive activity in the forebrain. Pilocarpine hydrochloride, 380 mg/kg, induces a sequence of behavioral and electroencephalographic alterations indicative of motor limbic seizures and status epilepticus which is followed by widespread damage to the limbic forebrain resembling that occurring subsequent to prolonged intractable seizures in humans. The efficacy of anticonvulsant drugs in preventing seizures induced by pilocarpine in rats correlates well with their ability to depress the firing rate of neurons in the substantia nigra pars reticulata (Turski et al., Soc. Neurosci. Abstr., 12: 898, 1986; Waszczak et al., J. Pharmacol. Exp. Ther., 239: 606, 1986). Systemic administration of benzodiazepines, barbiturates, trimethadione and valproate prevents seizures produced by pilocarpine, 380 mg/kg, and depresses the firing rate of nigral neurons. In contrast, ethosuximide increases susceptibility of rats to seizures elicited by pilocarpine, while diphenylhydantoin and carbamazepine remain inactive. These effects also correlate with the action of ethosuximide, diphenylhydantoin and carbamazepine on substantia nigra unit activity.

The present study was undertaken to determine whether antiepileptic drugs share an ability to modulate seizures induced by pilocarpine following microinjections into the substantia nigra. Pretreatment of rats with midazolam, ED_{50} 0.038 µmol (0.029-0.052), and phenobarbital, 0.016 µmol (0.007-0.039), prevented the evolution of limbic seizures induced by pilocarpine, 380 mg/kg, while diphenylhydantoin (up to 0.1 µmol) remined inactive. Ethosuximide, 0.038 µmol (0.022-0.065), lowered the threshold for seizures induced by pilocarpine, and converted a non-convulsant dose of pilocarpine, 200 mg/kg, into a convulsant one.

These results show that microinjections of antiepileptic drugs into the substantia nigra are sufficient for an anticonvulsant action.

135.2 EFFECTS OF M1 AND M2 MUSCARINIC AGONISTS ON SEPTO-HIPPOCAMPAL AND OTHER CENTRAL NEURONS IN THE RAT. O. Rascol* and Y. Lamour, INSERM, U. 161, 2 rue d'Alésia, 75014 Paris, France.

The functional role of M1 and M2 muscarinic binding sites in the central nervous system is still poorly understood. It has been suggested that the M1 binding sites could be post-synaptic whereas the M2 sites could be presynaptic. In the present series of experiments the effects of muscarinic M1 (pilocarpine, McN A 343) and M2 (oxotremorine - M, carbachol) agonists and antagonists (pirenzepine, gallamine) applied by iontophoresis were studied on several neuronal populations in the central nervous system of rats anesthetized with urethane. Septo-hippocampal neurons and neurons from hippocampus, subiculum and somatic sensory cortex were studied. Septo-hippocampal neurons (SHNs), taken as example of "presynaptic neurons", were identified by their antidromic response to the electrical stimulation of the fimbria-fornix. Only cholinoceptive neurons (i.e. neurons excited by the iontophoretic application of acetylcholine) were studied. The qualitative (percentage of excitations and inhibitions) and quantitative (current-responses curves) aspects of the effects of the various drugs in the various structures were investigated. Conventional amplification methods were used to record extracellular neuronal activity, using micropipettes filled with 1M NaCl and 2 % pontamine skye blue that were rigidly attached to multibarreled micropipettes (tip diameter 8-10 um) filled with solutions for testing by iontophoresis.

The proportion of SHNs excited by carbachol and oxotremorine-M was close to 90 % whereas only 7 to 9 % of the SHNs were inhibited by these drugs. In contrast pilocarpine and McN A 343 inhibited 25 and 52 % of the SHNs, whereas excitations were observed in only 41 % and 13 % of the SHNs respectively. In the hippocampus and subiculum, the picture was essentially the same with some minor variations. In the cerebral cortex, carbachol and oxotremorine-M were also potent excitants whereas inhibitions were frequent with McN A 343 (41 %) but less so with pilocarpine (11 %). Pirenzepine blocked more easily the M1 than the M2 effects.

These results suggest i) that the "presynaptic" septo-hippocampal neurons as well as the "post-synaptic" hippocampal or cortical neurons both bear M1 as well as M2 receptors which are able to modify neuronal discharge rate. ii) and that M1 and M2 agonists induce changes in neuronal discharge rate in opposite directions. They might therefore act through different mechanisms.

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135.3 DISINHIBITORY ACTION OF ACETYLCHOLINE MEDIATED BY M_1 MUSCARINIC RECEPTOR IN THE LATERAL SEPTAL NUCLEUS OF THE RAT *IN VITRO*. H. Hasuo, J.P. Gallagher, P. Shinnick-Gallagher. Dept. of Pharmacol. & Toxicol., Univ. Texas Med. Br., Galveston, TX. 77550.

Intracellular recording were made from neurons of the dorsolateral septal nuclei (DLSN) contained within a transverse slice of a rat and superfused *in vitro* with artificial cerebrospinal fluid (ACSF). Orthodromic focal stimulation applied to the fimbrial afferents in the slice could generate either a fast inhibitory postsynaptic potential (IPSP) or a fast IPSP followed by a late hyperpolarizing potential(LHP). Bath application of muscarine(0.5-30 µM) either hyperpolarized (70 % neurons tested) or depolarized(20 %) the resting membrane potential of the DLSN neurons; each effect accompanied with decrease or increase in input membrane resistance,respectively. In either case the amplitudes of IPSP and LHP were markedly depressed by muscarine in a dose dependent manner. When we measured the amplitudes of IPSP and LHP, depolarizing or hyperpolarizing dc current was applied to nullify the membrane potential change by muscarine. The amplitudes of IPSP and LHP were depressed by muscarine (10 µM) by 45.2 ± 3.9 % (n=16) and 53.8 ± 2.2 %(n=22), respectively, although the input membrane resistance change was about 10 %. These results indicate that the inhibition of both IPSP and LHP is not simply due to the shunting action caused by a conductance change. Oxotremorine (0.1-10 µM) and bethanechol (10-100 µM) also showed effects similar to muscarine. The membrane potential change and inhibition of IPSP and LHP by muscarine(10 µM) were blocked completely by atropine(1 µM), but were unaffected by mecamylamine (10 µM). In the rat DLSN neuron, IPSP and LHP are considered to be mediated ,at least in part, by GABA receptors (Stevens et al. Synapse, 1:184-190 ,1987). GABA induced potentials produced by pressure application of exogenous GABA were little affected by muscarine(10 µM). Pirenzepine caused a parallel shift in the muscarine dose-response curve. The analysis by the Gaddum-Schild method indicated that pirenzepine blocked competitively the effect of muscarine on IPSP, LHP and resting membrane potential with a K_D value of 30 nM, 40 nM,and 6 nM,respectively. These results suggest that M_1 muscarinic receptors, highly pirenzepine sensitive, may be involved in the presynaptic regulation of GABA release as well as in the muscarine induced resting membrane potential changes of dorsolateral septal neurons. The physiological significance of muscarinic action in the dorsolateral septal neurons is not clear, however since theta rhythm has been shown to be both atropine sensitive and resistant, one type of theta rhythm could be modulated by muscarinic receptors in the dorsolateral septum. (Supported by DAMD-17-86-C-6032 to JPG)

135.4 DIFFERENTIAL EFFECTS OF CHOLINERGIC AGONISTS UPON THE COMPONENTS OF THE COMPLEX EVOKED DISCHARGE OF AUDITORY CORTICAL NEURONS. T.M. McKenna, J.H. Ashe, G.K. Hui, and N.M. Weinberger. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

This experiment studied the effects of cholinergic agents upon responses to controlled pure tone stimulation in the primary auditory cortex of the unanesthetized cat. Because previous reports of physiological plasticity in auditory cortex have noted that learning may differentially affect spontaneous vs. evoked discharges, and various components of evoked discharges, (cf. Weinberger & Diamond, Prog. Neurobio.,1987), and since acetylcholine has been implicated as a factor in neural plasticity, special attention was devoted to these parameters of cellular activity.

Multibarrel micropipettes were used to record activity from 115 acoustically-responsive single neurons and to apply cholinergic agonists acetylcholine (ACh, 2M) or methacholine (MCh, 20 mM), or antagonist atropine (.2M) by micropressure. Most of these neurons had more than one response component to tones (i.e. on, through, or off component).

The most prominent effect of the agonists was a facilitation of "on" discharges, ACh and MCh being equally effective. They also generally produced an increase in "off" responses, but they had opposite effects on "through" responses; ACh was more likely to depress "through" responses than was MCh. Agonist effects on spontaneous activity often paralleled those on "through" responses, but were different from the effects for "on" or "off" components.

Furthermore, agonists had different effects on various response components; in many cases opposite effects (increases and decreases). Moreover, "on" and "off" components were usually changed together. In contrast, changes in "on" responses were weakly related to changes in "through" discharges. Effects of agonists could be blocked by atropine, and atropine effects for "on" and "off" responses also directly covaried.

These findings indicate that ACh and MCh modify evoked activity in a manner more subtle than simple increase or decrease in discharge rate during an acoustic stimulus. Comparison of the effects of cholinergic agents on the different response components revealed that the "on" and "off" responses were modified in concert.

We are currently extending this investigation to neurons tested with a range of tone frequencies.

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135.5 MICROINJECTION OF ATROPINE COMPETITIVELY ANTAGONIZES THE CARBACHOL INDUCED ENHANCEMENT OF DESYNCHRONIZED SLEEP SIGNS. H.A. Baghdoyan, R. Lydic, C.W. Callaway* and J.A. Hobson. Laboratory of Neurophysiology, Harvard Medical School, 74 Fenwood Road , Boston, MA 02115.

Microinjection of cholinergic agonists or an acetylcholinesterase inhibitor into the pontine reticular formation of conscious cats produces a behavioral and electrographic state similar to physiologically occurring desynchronized (D) sleep. This cholinoceptively evoked D sleep-like state is dose dependent and is blocked by centrally administered atropine, indicating that this phenomenon is mediated by muscarinic cholinergic receptors. The purpose of the present study was to determine quantitatively whether the effects of carbachol on D sleep signs can be competitively antagonized by microinjection of the muscarinic receptor blocker atropine.

Three dosages of carbachol (0.4, 1.0, 4.0 micrograms in 250 nl saline) were used. For each injection trial, 15 min prior to carbachol administration cats were pretreated with either atropine (5 micrograms in 250 nl) or saline (250 nl). There were 2 trials per dosage of carbachol, 2 trials of atropine alone, and 2 saline controls, for a total of 16 injections. The percentage of time spent in the carbachol induced D sleep like state (D-carb) was measured for 4 hrs following carbachol administration.

A double reciprocal plot of D-carb percentage (y) vs. carbachol dosage (x) showed the equation for the line to be $y=(0.08)x+0.07$ following microinjection of carbachol, with an x intercept of -0.85; and $y=(0.83)x+0.11$ following the atropine pretreatment trials, with an x intercept of -0.13. Thus, the maximal effect of carbachol was not decreased by atropine, however this pretreatment did reduce the affinity of carbachol for the acetylcholine receptor.

This is the first time that centrally administered atropine has been shown to competitively antagonize the D sleep sign enhancement induced by microinjection of carbachol into the pontine reticular formation. Therefore, these quantitative data provide a new demonstration that D-carb is mediated by muscarinic cholinergic receptors, and support the hypothesis that physiologically occurring D sleep is generated by cholinergic/cholinoceptive mechanisms of the pontine reticular formation.

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135.6 LOCALIZATION OF MUSCARINIC AND GABA RECEPTORS TO APICAL DENDRITIC TUFTS IN CINGULATE CORTEX WITH LAMINAR NEUROTOXIN LESIONS. Brent A. Vogt. Depts. Anatomy and Physiology, Boston University School of Medicine, Boston, MA 02118

Dissociation of adult neurons in combination with radiolabeled ligand binding techniques provide a powerful assay for the localization of receptors to specific classes of cortical neurons and parts of the dendritic tree. This approach, however,includes wet emulsion autoradiography and so requires the use of a limited number of irreversible ligands. In addition, the apical tufts of pyramidal neurons and dendritic spines are pulled off during dissociation.One solution to these problems is to make neurotoxin lesions which are restricted to one or two layers followed by cryostat sectioning, radioligand binding, coverslip autoradiography and quantification of alterations in binding above and below the lesion. Alterations in layer I binding are of particular interest because this is where the apical dendrites of almost all cortical pyramids arborize.

Lesions of either layers II-IV or Vb and VI were made with ibotenic acid in adult, male rats. Two weeks later the animals were perfused with Krebs-Henseleit buffer, the brains removed and frozen and sectioned with a cryomicrotome. The sections were incubated in either pirenzepine (PZ, 15 nM) for localization of M_1 sites or muscimol (20 nM) for localization of GABAa receptors. Nonspecific binding was determined with 1 μM atropine or muscimol,respectively. The sections were then washed, dryed and exposed to emulsion-coated coverslips. After the autoradiograms were developed, single grains were counted in area 29c of posterior cingulate cortex in normal, i.e. unablated, and experimental cases.

In three cases in which layers II-IV were destroyed, there was a reduction in specific PZ binding in layers Ia, b and c of almost 80% in comparison to three normal cases, while reductions in specific muscimol binding were between 32 and 40%. Losses in what remained of layers II-IV were 40-55% for PZ binding and 15-30% for muscimol binding. No significant alterations occurred in layers V and VI for either ligand. Lesions in layers Vb and VI produced 15-20% reductions in PZ binding in layers I-IV.

One of the principal structural heterogeneities in area 29c is the dense dendritic arborizations of pyramidal cell apical tufts in layer I. Pyramids in layers V and VI have apical dendrites which arborize evenly in layer I, while many cells in layers II-IV have a selective termination of their dendrites in layer Ia. The present findings indicate that both M_1 and GABAa sites are on these apical dendritic tufts.

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135.7 CHARACTERIZATION OF MUSCARINIC AUTORECEPTORS IN THE RAT HIPPOCAMPUS AND FRONTAL CORTEX. P. Supavilai*, I. Bobirnac* and M. Karobath (SPON: G. Engel). Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland

Presynaptic muscarinic autoreceptors can regulate acetylcholine release from cholinergic nerve terminals in the central and peripheral nervous system. Studies on electrically evoked release of acetylcholine from brain slices (e.g. Szerb et al, Brain Research 1977; 128:285-291) have shown that inhibitors of acetylcholinesterase (AChE) decrease ^{3}H-acetylcholine release, which is restored by the addition of muscarinic antagonists. The latter drugs added alone have no effect or even slightly enhance ^{3}H-acetylcholine release. Further studies using K^+-evoked release from brain slices and synaptosomes also suggested that acetylcholine release can be inhibited by the stimulation of presynaptic muscarinic autoreceptors. Recently it has been proposed that muscarinic receptors can be divided into 2 major pharmacologically distinct subtypes ie. M1 and M2 receptors with further subdivisions also suggested. Currently it is not clear whether presynaptic muscarinic autoreceptors in a given brain area are of the M1 or M2 type. We therefore investigated the effects of muscarinic agonists, partial agonists, antagonists and AChE inhibitors on electrically-evoked acetylcholine release from rat hippocampus and frontal cortex slices. In some experiments we also studied muscarinic drugs on electrically-evoked acetylcholine release from monkey hippocampus slices. Our results show that the activation of muscarinic receptors can lead to a complete suppression of acetylcholine release, and that the efficacy of muscarinic agonists is similar in rat hippocampus and rat frontal cortex slices. In agreement with observations from other laboratories using currently available receptor subtype selective drugs we suggest that presynaptic muscarinic autoreceptors in rat hippocampus and frontal cortex are of the M2 subtype. Results with brain slices obtained from the hippocampus of squirrel monkeys indicate that no marked species differences in the functional importance and properties of presynaptic muscarinic autoreceptors exist.

135.8 REGULATION OF BRAIN ACETYLCHOLINE (Ach) CONCENTRATION BY MUSCARINIC RECEPTORS. V.H.Sethy and J.W.Francis*, CNS Research, The Upjohn Company, Kalamazoo, Michigan 49001 USA

There is substantial evidence indicating that the function of cholinergic neurons in the central nervous system is subject to a number of regulatory mechanisms. These regulatory mechanisms could involve various neurotransmitter systems making synaptic contact with cholinergic neurons and/or muscarinic receptors located at cholinergic synapses. While the effect of cholinergic drugs on brain Ach levels and the release of this transmitter has already been extensively investigated, very little is known about the relationship between the occupation of muscarinic cholinergic receptors and changes in brain Ach levels. Therefore, it was decided to investigate this relationship after intravenous (i.v.) administration of muscarinic agonists to mice. The peak effect of arecoline (10 umol/kg i.v.) on inhibition of (3H)-oxotremorine-M (3H-OXT) binding and on the increase in brain Ach levels was observed at 1 and 3 minutes, respectively. The maximum effect of i.v. oxotremorine (3 umol/kg) on inhibition of (3H)-OXT binding and on the increase in brain Ach was observed at 30 and 60 minutes, respectively. There was a significant (p 0.001) and dose-dependent inhibition of (3H)-OXT binding and an increase in brain Ach levels after i.v. administration of arecoline (0.3, 1, 3, 10, and 30 umol/kg), oxotremorine (0.1, 0.3, 1, and 3 umol/kg), oxotremorine-1 (0.1, 0.3, 1, 3, and 10 umol/kg), and oxotremorine-3 (0.3, 1, 3, 10, and 30 umol/kg). The correlation coefficient between the ED50 for inhibition of (3H)-OXT binding and the increase in brain Ach levels was 0.94. These results suggest that the muscarinic cholinergic receptor system involved in the regulation of brain Ach levels may lack spare receptors.

135.9 MUSCARINE INCREASES THE DIACYLGLYCEROL CONTENT OF PC12 CELLS. J. Horwitz (SPON: R.L. Perlman) Kennedy Mental Retardation Research Center and Dept. of Pediatrics, Univ. of Chicago, Chicago,IL 60637

Stimulation of muscarinic receptors on PC12 pheochromocytoma cells increases the hydrolysis of inositol containing phospholipids by phospholipase C. The hydrolysis of these phospholipids is thought to lead to the formation of inositol phosphates and diacylglycerol. Previous experiments from this laboratory and others have shown that muscarine increases inositol phosphates in these cells. Diacylglycerol has been shown to activate protein kinase C in vitro. The effect of receptor stimulation on diacylglycerol in cells is usually assessed by measuring the accumulation of [^{3}H]diacylglycerol in cells whose phospholipids have been prelabeled with [^{3}H]arachidonic acid. Muscarine increases [^{3}H]diacylglycerol formation in PC12 cells differentiated with nerve growth factor. After a 5 min incubation at 27°C, muscarine at 100 μM caused approximately a 2-fold increase (cpm/10^6 cells, control: 605±76; muscarine: 1073±49). This, method, however, may not give an accurate indication of diacylglycerol content because [^{3}H]arachidonic acid is incorporated unevenly into all phospholipids. Preiss et al. (J. Biol. Chem. 261, 2597, 1986) have developed an assay that measures the actual amount of diacylglycerol. This assay involves phosphorylation with diacylglycerol kinase and [γ-^{32}P]ATP and measurement of [^{32}P]phosphatidic acid formed. Using this assay, I have measured the effect of muscarinic receptor stimulation on diacylglycerol levels in differentiated PC12 cells. The basal level of diacylglycerol in several experiments ranged from 75 to 200 pmol/10^6 cells. A 5 min incubation with muscarine at 37°C caused a 35% increase in diacylglycerol over basal levels (pmol/10^6 cell, control: 76±4; muscarine: 103±2). Lower temperature (27°C) seemed to enhance the effect of muscarine (control: 115±6; muscarine: 180±6). Thus, muscarine stimulates diacylglycerol formation in PC12 cells and would, therefore, be expected to activate protein kinase C under these conditions. (Supported by NIH grants NS22694 to J.H., HL29025 to R.L.P. and HD04583 to KMRRC)

135.10 CHARACTERISTICS OF MUSCARINIC RECEPTORS AND SECOND MESSENGER RESPONSES OF STRIATAL NEURONS IN PRIMARY CULTURE. J. Ellis, D.E. Kemp*, M. Seidenberg*, R.H. Lenox, and S. Weiss. Neuroscience Research Unit, Department of Psychiatry, University of Vermont, Burlington, VT 05405.

Our preparations of striatum, generated from the fetal mouse brain, are predominantly (>93%) neuronal and are maintained in serum-free monolayer culture. Some advantages of such cultures are: (1) diffusional barriers and contributions from non-neuronal cell types are minimal; (2) the characteristics of these cells are not exclusive to a single cell type, as may be true of cloned, transformed, cell lines; (3) differential distributions of cell types and receptor-mediated responses may be obtained by culturing selected regions of the brain.

Quinuclidinyl benzilate (QNB) and N-methylscopolamine (NMS) bind to these intact neurons in a saturable manner and with high affinity. The values for Bmax and K_D for [^{3}H]QNB are 450 fmol/mg protein and 210 pM; as in many other studies, [^{3}H]NMS has been found to label significantly fewer sites (150 fmol/mg protein, K_D=70 pM). The difference in Bmax between [^{3}H]QNB and [^{3}H]NMS is not likely to be due to uptake or sequestration, because similar values have been obtained from binding assays performed on membranes derived from these cells. In the presence of lithium, carbachol produces a dose-dependent (EC_{50}, 80μM) increase in the accumulation of [^{3}H]inositol monophosphate (IP) in neurons that have been prelabeled with [^{3}H]inositol. At 0.3mM lithium, 1mM carbachol yields greater than 10-fold elevation of IP levels; the presence of 1 μM atropine abolishes this elevation. In identical preparations, carbachol inhibits the accumulation of cyclic AMP (EC_{50}, 0.5 μM). At a concentration of 10 μM, carbachol inhibits about 70% of the stimulation of cyclic AMP accumulation that is produced by the D-1 dopamine agonist SKF 38393 (1 μM). This effect of 10 μM carbachol is reversed by atropine (IC_{50}, 70 nM). Carbachol also reduces basal levels of cyclic AMP by 20%, but has not been found to reduce the elevation of cyclic AMP levels that is induced by forskolin or isoproterenol.

We have also been able to identify cell types immunocytochemically, according to neurotransmitter, and to autoradiographically label muscarinic receptors on the same neurons. It is expected that future studies of this model system will contribute to identifying which subtypes of muscarinic receptors are associated with given cell types, second messengers, and cellular responses such as the regulation of neurotransmitter release.

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135.11 Facilitatory and disfacilitatory actions of acetylcholine in the rat hippocampus may be mediated by M 1 muscarinic receptors. Yanguo Hong* and Kresimir Krnjević, (SPON: D. Watt) Departments of Anaesthesia Research and Physiology McGill University, 3655 Drummond St., Montreal, Que., H3G 1Y6 Canada

In rats under urethane anaesthesia, several cholinergic agonists and antagonists were iontophoretically released in the pyramidal layer and in the apical dendrites of area CA1 (in fewer cases in CA3). The somatic population spike (P.S.) and the dendritic negative field (field EPSP) were evoked by fimbrial and/or commissural stimulation (3.5-6 V, 0.2 ms, 0.7 Hz). Cholinomimetics applied included acetylcholine (ACh), acetyl-B-methylcholine (MeCh), oxotremorine, diphenylpiperazinium iodide (DMPP) and tetramethylammonium hydroxide (TMA).

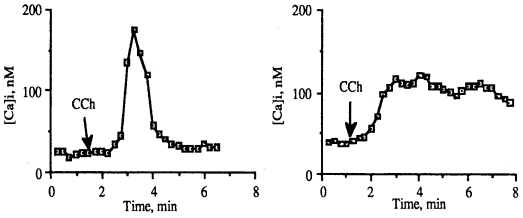
Local ejection of pirenzepine (PRZ) strongly depressed or fully blocked the effects of muscarinic agents on responses to fimbrial stimulation, but had little or no effect on the action of DMPP when they were applied at the same site. Conversely, local applications of curare antagonized the effect of DMPP and TMA without significantly affecting the action of MeCh. At the somatic level, PRZ depressed the facilitatory action of MeCh, ACh and oxotremorine by 67.6 + 18.6% (Mean + SD n=14), 76.5 + 25.6% (n=47), 85.4 + 18.0% (n=14) respectively. At the apical dendritic level, PRZ reduced the effects of Ach by 70.0 + 19.3% in 14 tests. These results were compared with those of atropine. There was no statistically significant difference between PRZ and atropine in respect of their depressant efficancy (76.5 + 25.6%, n=47; 81 + 24%, n=31, respectively), suggesting that the muscarinic receptors involved in chrolinergic facilitation of population spike in the pyramidal layer as well as those responsible for disfacilitation at the dendritic area belong to the M 1 subtype.

Supported by the MRC of Canada.

135.12 CARBACHOL INCREASES CYTOPLASMIC FREE CALCIUM IN RAT BRAIN CORTICAL CELLS VIA MUSCARINIC RECEPTORS. LJ. Reynolds, G.S. Shangold*, E. Lee* and R.J.Miller. Dept. Pharmacol. and Physiol. Sci., Univ. Chicago, Chicago IL 60637.

Many studies have demonstrated that muscarinic agonists can increase the metabolism of polyphosphoinositides. Generation of inositol triphosphate and inositol tetrakisphosphate is believed to be coupled to the increase of intracellular free calcium ([Ca^{2+}]i) by release from intracellular stores or by opening of a membrane channel. However this process has not been demonstrated directly in neurons. We have measured [Ca^{2+}]i in single cells in monolayer cultures of fetal rat cortex using the calcium sensitive dye fura-2.

Cortical cells were taken from embryonic day 18 rat fetuses and grown in vitro for 14 days. At this age the cells have muscarinic receptors demonstrated by [^{3}H] QNB binding (kD 50pM, Bmax 338fmoles/mg protein). Addition of 100uM carbachol to cortical cells produced a rise in[Ca^{2+}]i in approximately 50% of the cells examined. The average peak response seen was an increase of 77nM above baseline. Broadly, the responses could be equally divided into two types; a fast transient response and a slower more sustained response. Typical responses from single cells are shown below.



In the presence of atropine (1mM) the frequency of response to carbachol diminished to about 10% , while the response was not altered in the presence of hexamethonium (100mM). This suggests that the response was mediated by muscarinic acetylcholine receptors. This conclusion is supported by the observation that oxotremorine produced similar responses. Similar results have also been obtained in cultures of striatal cells.

We have demonstrated that muscarinic receptors in cultures of rat brain cortex can produce a rise in [Ca^{2+}]i. The source of the Ca^{2+}that produces the rise is not clear at this time and may originate from release of intracellular stores or from entry following cell depolarization. Possible mechanisms are currently under investigation.

135.13 THE AFFINITY RATIO FOR ANTAGONIST AND AGONIST LABELED BINDING SITES PREDICTS MUSCARINIC RECEPTOR AGONIST/ANTAGONIST ACTION IN CENTRAL AND PERIPHERAL TISSUES. L.L. Coughenour*, W.G. Berghoff*, S.L. Myers*, R.D. Schwarz, W.H. Moos, and R.E. Davis (SPON: R.F. Bruns). Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105.

Numerous studies have shown that muscarinic agonists bind to multiple affinity states of the muscarinic receptor. In contrast classical muscarinic antagonists usually detect only a single affinity state of the receptor. Using a rapid filtration assay (Vickroy et al. JPET 229, 1984), we have selectively labeled the high affinity state of the muscarinic receptor with [^{3}H]cismethyldioxolane (CMD) and the antagonist state of the receptor with [^{3}H]quinuclidinyl benzilate (QNB) and determined the antagonist/agonist affinity ratio (QNB/CMD ratio) in these two assays for a number of cholinergic agents.

The QNB/CMD ratio was about 1000 or greater for efficacious agonists such as carbachol, muscarine, oxa-22, and cismethyldioxolane. It was markedly lower for partial agonists, such as pilocarpine, BM-5, RS-86 and AF-30 and approached one for antagonists such as atropine, scopolamine, pirenzepine and AF-DX-116. The QNB/CMD ratio was correlated with agonist efficacy in decreasing the release of [^{3}H]acetylcholine and in stimulating phosphatidylinositol turnover in vitro and the ability to reverse scopolamine induced swimming activity in vivo (R.D. Schwarz et al., this meeting).

The M1 selective agonists, McN-A-343 and pilocarpine, could be distinguished by their higher affinity for [^{3}H]CMD binding in the cortex (M1) compared to [^{3}H]CMD binding in the heart (M2) but not by [^{3}H]QNB binding in the cortex and heart. On the other hand, the M1 selective antagonists, pirenzepine and trihexyphenidyl, could be distinguished using [^{3}H]QNB binding in the cortex and heart but not by [^{3}H]CMD binding.

Various classes of compounds including acetylcholinesterase inhibitors (AChEI) have been reported to perturb binding to the muscarinic receptor. There were differences among the binding interactions of AChEIs. Physostigmine and miotine exhibited high affinity for CMD binding and QNB/CMD ratios like those of muscarinic agonists. Ambenonium exhibited high affinity for both CMD and QNB binding and a QNB/CMD ratio like that of a muscarinic antagonist. In contrast tacrine did not exhibit high affinity for either CMD or QNB binding. Since all of these are about equipotent as AChEIs (R. Davis et al., this meeting), these findings suggest that interactions of these agents with the muscarinic receptor may not be related to their acetylcholinesterase inhibition. In conclusion the QNB/CMD ratio in these two assays appears to predict the agonist efficacy of the agent.

135.14 EFFICACY AT CENTRAL MUSCARINIC RECEPTORS: CORRELATION OF RECEPTOR BINDING WITH PHOSPHATIDYLINOSITOL TURNOVER, ^{3}H-ACETYLCHOLINE RELEASE, AND IN VIVO BEHAVIORAL ACTIVITY. R.D. Schwarz, L.L. Coughenour, R.E. Davis, W.H. Moos, and C.J. Spencer*. Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105.

Muscarinic agonists (e.g. arecoline, oxotremorine) have been shown in both humans and rodents to produce significant positive effects on cerebral activity while muscarinic antagonists (e.g. scopolamine, atropine) decrease cognitive function. These findings gain importance with the recent observations that marked cholinergic dysfunction occurs in age-related memory disorders such as senile dementia of the Alzheimer type (SDAT). Greater understanding of molecular events at central muscarinic receptors and their relationship to in vivo behavioral activity will aid in the development of more efficacious cholinergic agents useful in the treatment of age-related memory disorders.

In a series of binding experiments it was found that muscarinic agonists showed higher affinity for sites labeled by the agonist ^{3}H-cis-methyldioxolane (CMD) than those labeled by the antagonist ^{3}H-quinuclidinyl benzilate (QNB), while antagonists displayed equal affinity for both sites. Calculation of the QNB/CMD ratio for agonists extended from 5500 (carbachol) to 100 (RS-86), whereas antagonists produced ratios of 1.0. Compounds having a mixed profile, such as BM-5, had ratios between 1-100 (L.L. Coughenour et al., this meeting). As a measure of muscarinic postsynaptic activity, the stimulation of phosphatidylinositol (PI) turnover was determined in rat cortical slices. Full agonists such as carbachol, muscarine, and CMD produced maximal stimulation while all other agonists displayed partial activity. Muscarinic presynaptic activity was assessed by measuring K^+-stimulated ^{3}H-ACh release from rat cortical slices. While a maximal inhibition of 45-50% was observed for any agonist tested, in general as the QNB/CMD ratio decreased, the amount of inhibition was also reduced. The ability to reverse scopolamine-induced swimming (SIS) activity was used as an index of in vivo cholinomimetic activity. Of those compounds which are centrally available upon systemic injection, SIS activity was also predicted by the QNB/CMD ratio. The greater the ratio, the greater the ability to reverse scopolamine.

In conclusion, the ratio of QNB/CMD binding may be used as a predictor of efficacy at central muscarinic receptors as defined both in vitro and in vivo.

135.15 EVALUATION OF THE CENTRAL PHARMACOLOGY OF TACRINE (TETRAHYDROAMINOACRIDINE: THA). R. E. Davis, L. L. Coughenour*, E. Gamzu, W. H. Moos and R. D. Schwarz, Pharmaceutical Research, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48105.

Within restricted dose ranges cholinomimetics can improve learning and memory in animals. In addition, senile dementia of the Alzheimer's type (SDAT) in man is associated with forebrain cholinergic hypofunction and the cholinesterase inhibitors, physostigmine and tacrine, may ameliorate the cognitive deficits accompanying senile dementia. Because of the recent claims of dramatic improvement in cognitive performance of SDAT patients after tacrine, we reevaluated the 'in vitro' and 'in vivo' pharmacology of tacrine in comparison to other known cholinesterase inhibitors.

As a cholinesterase inhibitor tacrine was equipotent to physostigmine and miotine (3-(1-dimethylaminoethyl)-phenyl-N-methylcarbamate). All three also decreased K+-stimulated release of acetylcholine from cortical slices. However, unlike physostigmine and miotine, tacrine did not bind to muscarinic sites labelled by [3H]-cis-methyldioxolane (L. L. Coughenour, this meeting). These compounds reverse scopolamine-induced swimming activity in rats, demonstrating 'in vivo' cholinomimetic activity. The onset of this effect was rapid for physostigmine and miotine (< 15 min) but was delayed for tacrine (2 hr). Both tacrine and physostigmine improved the performance of hippocampally-deficient, C57/B10jsnc mice in a water maze but with extremely steep dose effect curves. In the water maze, effective doses were only slightly below toxic doses. Neither tacrine nor physostigmine improved delayed response performance of aged-rhesus monkeys. These cholinesterase inhibitors also increased local cortical blood flow in rats through a central cholinergic mechanism.

These data demonstrate that tacrine is a potent cholinomimetic 'in vitro' and 'in vivo' working through inhibition of acetylcholinesterase. It has a much longer duration of action than physostigmine and miotine in rats.

135.16 NEUROTOXIN (AF64A) MODEL OF CHOLINERGIC HYPOFUNCTION: AN IMPROVED SYNTHETIC PROCEDURE FOR AF64A. C.J. Spencer*, W.H. Moos, R.E. Davis, R.D. Schwarz, J. Kinsora*, M.E. Smith* (SPON: J. Symons). Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis, Pharmaceutical Research, 2800 Plymouth Road, Ann Arbor, MI 48105.

The cholinergic deficit that accompanies aging and dementia has prompted a search for appropriate preclinical models of cholinergic hypofunction. One model that has received particular attention involves the use of aziridinium salts such as AF64A (1-ethyl-1-(2-hydroxyethyl)aziridinium). In an effort to both simplify and improve the reproducibility of the AF64A model, we have developed a new and straightforward synthetic procedure using stoichiometric base.

Within 45 min of its preparation, AF64A was injected bilaterally into the lateral cerebral ventricles (ICV) of male Long-Evans rats (3 nmoles/side, 1 nmole/ul over 15 min). An additional group received ICV injections of 0.9% saline vehicle. Five weeks later, these rats were tested in a hidden platform water maze task. An additional group of rats previously trained on a discrete trial delayed alternation task received similar AF64A or vehicle ICV injections. One week later, these animals were retested and if necessary retrained on the delayed alternation task. To biochemically assess the lesions, cholineacetyltransferase (ChAT) activity then was determined by the method of Fonnum (J. Neurochem 24: 407, 1975) in the corpus striatum, hippocampus and cerebral cortex from all rats, some at 6 weeks, others at 6 months after injection.

AF64A reduced hippocampal but not cortical or striatal ChAT activity. Hippocampal ChAT activity was inversely related to the latency in the water maze task with latencies increasing with decreasing ChAT activity. AF64A lesioned rats also were impaired on the delayed alternation task.

The behavioral and biochemical observations thus confirm the effectiveness of the lesions produced by the AF64A synthesized by this improved procedure.

135.17 **CENTRAL MUSCARINIC PHARMACOLOGY OF A SERIES OF ARECAIDINE ESTERS.** W.H. Moos, L.L. Coughenour, R.E. Davis, C.C. Humblet*, and R.D. Schwarz. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

It is now well established that the muscarinic receptor agonist arecoline (arecaidine methyl ester) can improve learning and memory in animals within restricted dose ranges. These findings gain additional importance from evidence that senile dementia is associated with forebrain cholinergic hypofunction. Despite this association, clinical trials with muscarinic agonists have yet to produce unequivocal, clinically relevant improvement in the symptoms accompanying senile cognitive decline. Limitations of arecoline in treating human cognitive dysfunction arise largely from pharmacokinetic liabilities such as a short duration of action. As a preliminary step toward developing more suitable agents for treating senile dementia, we have studied the central cholinergic pharmacology of a series of arecaidine esters whose cholinomimetic activity in cardiac and smooth muscle preparations is known.

Using the finding that known muscarinic receptor agonists exhibit higher affinities for sites labeled by the quaternary muscarinic receptor agonist [^{3}H]-cis-methyldioxolane (CMD) than for sites labelled by the quaternary muscarinic receptor antagonist [^{3}H]-quinuclidinyl benzilate (QNB) (Schwarz, et al., 1987), relative affinities (ratio of binding affinities (QNB/CMD)) of compounds from this series were determined for rat cerebral cortical muscarinic sites. Muscarinic receptor agonists decrease potassium-stimulated release of acetylcholine from cortical slices (NTRA), stimulate phosphatidylinositol turnover (PIT), and reverse scopolamine-induced increases in swimming activity (SIS). Agonist activity in NTRA, PIT, and SIS was reduced as the QNB/CMD ratio decreased.

Arecoline and arecaidine esters with electron-donor moieties (e.g., propargyl and butynyl esters) displayed potent agonist-like activity in NTRA, PIT, and SIS, as predicted by the QNB/CMD ratio. Increasing the size of the ester group, however, generally decreased agonist-like activity in this series. Meaningful correlations amongst biological data and physicochemical properties were observed.

These data demonstrate the QNB/CMD ratio in this series of arecaidine esters to be predictive of both in vitro and in vivo central muscarinic cholinergic activity.

DOPAMINE RECEPTOR FUNCTIONS

136.1 OPPOSING ACTIONS OF D-1 AND D-2 DOPAMINE RECEPTORS ON cAMP FORMATION DURING NEURONAL DOPAMINE RELEASE: THE PREDOMINANCE OF D-1 RECEPTOR ACTIVATION. A.J. Azzaro, J. Liccione and J. Lucci* Departments of Neurology, Pharmacology/Toxicology and Psychiatry, West Virginia University Medical Center, Morgantown, WV 26506.

The results of many studies have provided strong evidence for a functional interaction between D-1 and D-2 dopamine (DA) receptors in brain tissue. At the molecular level, activation of D-1 or D-2 DA receptors (with selective DA receptor agonists) leads to opposing actions on the formation of cAMP. Evidence that such interactions between D-1 and D-2 receptors occur under conditions of neurotransmitter release are presented here in studies with d-amphetamine (AMPH) in rat striatal brain slices. AMPH (0.1uM to 20uM), an agent which releases newly-synthesized DA from DA nerve terminals, stimulated the formation of cAMP in a concentration-dependent manner. This action of AMPH was related to the release of the endogenous DA since experiments performed in the presence of 3-iodotyrosine (an inhibitor of DA synthesis) + reserpine (an inhibitor of DA storage) failed to demonstrate a change in cAMP formation at all concentrations of AMPH.

To examine the nature of this DA mediated response, a variety of DA, norepinephrine and serotonin receptor antagonists were examined. Only the D-1 (Sch 23390) or D-2 (sulpiride) DA receptor antagonist were able to alter the AMPH-induced change of cAMP formation. Sch 23390 (10uM) completely abolished while (-)sulpiride (10uM) potentiated (2 fold) the actions of 20uM AMPH on cAMP formation.

The relationship between the amount of DA released and the change in cAMP formation seen after AMPH was examined by simultaneous assay of DA and cAMP in superfusates of striatal brain slices. A strong correlation was observed between the increase in DA release, by each concentration of AMPH, and the stimulation of cAMP formation. Moreover, when synaptic catabolism of DA was inhibited with 0.1uM clorgyline (a selective type A MAO inhibitor), the larger concentration of synaptic DA was reflected in an enhanced stimulation of cAMP formation by AMPH.

Superfusion experiments were performed in the presence and absence of 50uM (-)sulpiride in order to determine the role of each DA receptor subtype in the formaton of cAMP. At each concentration of AMPH, (-)sulpiride significantly potentiated cAMP formation but was without effect on DA release. With increasing concentrations of AMPH, the inhibitory role of D-2 receptors on cAMP formations rose from 10% at 5uM AMPH to 30% at 20uM AMPH.

These experiments demonstrate that opposing actions of D-1 and D-2 receptors are seen under conditions where DA is released from DA nerve terminals. However, D-1 induced activation always greatly exceeded D-2 induced inhibition of cAMP formation, suggesting a modulator role for D-2 DA receptors in this striatal second messenger system. (Supported by WVU. Corp. & F.O.E.)

136.2 EFFECTS OF CHRONIC DOPAMINE RECEPTOR ANTAGONIST TREATMENT ON THE BEHAVIORAL AND BIOCHEMICAL PROPERTIES OF D_1 AND D_2 DOPAMINE RECEPTORS. Ellen J. Hess*, Andrew B. Norman*, Diane Swick* and Ian Creese, Dept. of Neuroscience, UCSD School of Medicine, La Jolla, CA 92093.

We have investigated the effects of chronic dopamine receptor antagonist treatment on the behavioral and pharmacologic properties of D_1 and D_2 dopamine receptors. Rats were treated with 0.5 mg/kg/day SCH23390, 0.2 mg/kg/day spiperone, 1 mg/kg/day cis-flupentixol, or 0.5 mg/kg/day SCH23390 plus 0.2 mg/kg/day spiperone for 21 days. During the treatment, rats were tested for the cataleptic response to their administered drug. Chronic treatment with SCH23390 resulted in a significant increase (16%) in D_1 receptors, labeled by [^{3}H]SCH-23390, but no change in D_2 receptors, labeled by [^{3}H]spiperone, was observed. Conversely, treatment with spiperone resulted in no significant change in D_1 receptors while D_2 receptors were significantly increased (24%). Paradoxically, chronic treatment with the mixed D_1/D_2 receptor antagonist cis-flupentixol resulted in a significant increase in only D_2 receptors (24%) with no change observed in D_1 receptors. Treatment with SCH23390 plus spiperone resulted in a significant increase in both D_1 (19%) and D_2 (32%) receptors. Thus, under these conditions, simultaneous D_1 and D_2 receptor antagonism did not appear to modulate D_1 receptor regulation, as one might hypothesize from the results of chronic cis-flupentixol treatment. In fact, our studies utilizing in vivo cis-flupentixol administration followed by in vivo EEDQ treatment demonstrate that cis-flupentixol preferentially interacts with D_2 receptors in vivo.

Catalepsy scores for rats chronically treated with spiperone decreased over the course of treatment with a significant reduction in catalepsy scores occurring by day 5. Rats receiving chronic administration of SCH23390 for 21 days demonstrated no tolerance to its potent cataleptogenic action. Rats receiving chronic cis-flupentixol demonstrated no tolerance to its cataleptogenic action two hours post-injection. However, the pattern of catalepsy observed over the two hour catalepsy scoring session changed over the duration of treatment; in the first 80 min of the scoring session, the animals appeared to become progressively tolerant to the cataleptogenic effects of cis-flupentixol. Those rats receiving SCH23390 plus spiperone demonstrated no tolerance to the cataleptogenic effects of these drugs. Rats treated with SCH23390 for 21 days and then administered an acute injection of spiperone on day 22 demonstrated tolerance to the cataleptic effects of spiperone. Conversely, rats challenged with an acute dose of SCH23390 after 21 day spiperone treatment were profoundly cataleptic. Thus, although there is a reciprocal interaction between D_1 and D_2 receptors, apparently behavioral tolerance may only occur through D_2 receptors. Additionally, it appears that D_2 receptor upregulation is not a prerequisite for the development of tolerance to neuroleptic-induced catalepsy. Supported by MH32990,MH09535 & Scottish Rite Schizophrenia Res. Prog

136.3 BEHAVIORAL EFFECTS OF CHRONIC EXPOSURE TO SELECTIVE D-1 AND D-2 DOPAMINE RECEPTOR ANTAGONISTS AND AGONISTS ADMININSTERED ALONE AND IN COMBINATION A.R. Braun*, M.M. Mouradian* and T.N. Chase (SPON: H. Weinstein) Experimental Therapeutics Branch, NINCDS, NIH, Bethesda MD 20892

Dopamine receptor antagonists alter spontaneous motor activity, catalepsy and behavioral responses to apomorphine when they are administered chronically. In order to identify the roles played by D-1 and D-2 dopamine receptors in the generation of such effects, experiments were performed utilizing the selective D-1 receptor antagonist SCH-23390 and agonist SKF-38393 and the selective D-2 receptor antagonist metoclopramide and agonist LY-171555.

Six groups of ten male Sprague Dawley rats were injected i.p. twice daily for 28 days with one of the following regimens: SCH-23390 1 mg/kg; metoclopramide 20 mg/kg; SCH-23390 plus metoclopramide; SCH-23390 plus LY-171555 1.5 mg/kg; metoclopramide plus SKF-38393 8 mg/kg; or saline. Spontaneous motor activity was rated blindly on a scale of 0-3, 18 hours after the first and last drug treatment. Catalepsy was assessed by measuring the time animals remained suspended by all four paws on a wire grid, 45 minutes following the first and last treatment. Stereotypic behavior elicited by 675 mg/kg apomorphine s.c. was rated using a modified Ernst scale prior to chronic treatment and after a 72 hour drug-free interval which followed the last injection.

Spontaneous motor activity did not differ from that observed in saline treated controls 18 hours following the initial dose of any drug combination. Following chronic treatment, however, activity was significantly reduced in animals that had received either the D-2 antagonist metoclopramide alone or metoclopramide plus SKF-38393 and was significantly increased in animals that had received the D-1 antagonist SCH-23390.

Acute treatment with all drug combinations produced elevated catalepsy scores which did not significantly differ from one another except for the group which received both SCH-23390 and metoclopramide, in which scores were on the average 350% higher. All animals showed habituation of the cataleptic response following chronic treatment except those receiving SCH-23390 plus metoclopramide or metoclopramide plus SKF-38393.

Chronic treatment with either metoclopramide or SCH-23390 alone resulted in a supersensitive behavioral response to subsequent apomorphine challenge (Ernst scores were 213% and 198% of saline controls, respectively). Chronic treatment with both selective antagonists produced a significantly greater response (325% of control animals). The induction of behavioral supersensitivity by either selective antagonist, however, was blocked by concurrent treatment with the complementary receptor agonist: scores in animals treated with SCH-23390 plus LY-171555 or metoclopramide plus SKF-38393 did not significantly differ from those of control animals.

Evaluation of spontaneous motor activity suggests distinct and opposing effects of chronic D-1 and D-2 receptor blockade. Results of all three behavioral experiments illustrate a complex synergistic interaction between D-1 and D-2 receptor subtypes in the generation of chronic behavioral effects in the intact rat.

136.4 **DOPAMINERGIC RECEPTORS IN YOUNG CHICKENS.**

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Apomorphine (0,05 -0,5 mg.kg-1 I.M) induced in young chickens a compulsive type of behavior pecking which could be easily quantified. Pecking was dose dependant. Other dopaminergic agonists have been studied. D1 agonist bromocriptine (1-4mg.kg-1) induces pecking. D2 agonist Lylli 171555 (1-10 mg.kg-1) is ineffective. Amphétamine (1-10 mg.kg-1) and amantidine (1-10 mg.kg-1 I.M), which release dopamine , are ineffective . Pecking response to apomorphine (0,25 mg.kg-1 I.M) was antagonised by the neuroleptic agents pimozide (1 mg .kg-1) and spipérone (1 mg.kg-1), by the sympathomimetic agents clonidine (0,3 mg.kg-1) and clenbuterol (2 mg.kg-1), by the alpha-adrenoceptor blocking agent yohimbine (2 mg.kg-1), by the ß adrenoceptor blocking agent propranolol (5 mg.kg-1) and by the antiserotoninergic agent methysergide (1mg.kg-1).

Methylphenidate (12 mg.kg-1), imipramine (12 mg.kg-1) but not amitriptyline (1-12 mg.kg-1) induce a very strong pecking .

This model specific for D1 agonists allows to study dopamine receptors D1 involved in the release of other neurotransmitters

136.5 DO D-1/D-2 RECEPTOR INTERACTIONS OCCUR DIRECTLY IN THE SUBSTANTIA NIGRA PARS RETICULATA? B.G.Weick and J.R.Walters. Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20892.

Recent behavioral and neurophysiological studies have suggested that concurrent stimulation of D-1 and D-2 receptors is necessary for the expression of apomorphine-like postsynaptic effects in the rat; the two dopamine (DA) receptor subtypes appear to interact in a synergistic way to affect basal ganglia output[1-4]. To gain insight into where the interacting receptors may be located, we have explored the possibility that D-1 and D-2 receptors within the substantia nigra pars reticulata (SNpr) might be involved in mediating the synergistic effects induced by coadministration of D-1 and D-2 receptor selective DA agonists on SNpr neurons.

D-1 and D-2 agonists were co-iontophoresed onto cells in the SNpr and their ability to affect the firing rates of these neurons was determined in conjunction with their ability to attenuate the inhibitory effects of GABA. Previous studies have shown that iontophoresis of DA in both the globus pallidus and the SNpr results in a modest increase in the activity of pallidal and SNpr neurons, and, more consistently, effectively attenuates the inhibitory effects of GABA in these brain regions[5-6]. The D-2 selective agonist quinpirole produced modest increases in SNpr firing rate in chloral hydrate anesthetized rats and attenuated the inhibitory effects of GABA iontophoresis. These effects did not appear dependent on the presence of endogenous DA providing simultaneous D-1 receptor stimulation since they were not attenuated in animals pretreated with alpha-methyl-para-tyrosine; thus they did not appear to involve D-1/D-2 receptor interactions. Both the selective D-1 agonist R-SKF 38393 and the selective D-1 agonist R-fenoldopam (R-SKF 82526) produced increases in firing rate in approximately 85% of neurons tested. With increasing currents, both R- and RS-SKF 38393 induced complete diminution of spike amplitude in 85% of reticulata neurons examined (n=13). Both R-fenoldopam and R-SKF 38393 also attenuated the effects of GABA in 50% of the cells recorded. However, none of these effects were potentiated by co-iontophoresis of quinpirole. Moreover, the mechanism underlying these effects is unclear: in 66% of the cells recorded (n=15), the D-1 inactive enantiomer, S-SKF 38393, also produced increases in firing rate that preceded complete diminution of spike amplitude and produced significant attenuation of the effects of GABA in 41% of neurons (n=17). The effects of the R-and S-enantiomers of SKF 38393 on spike amplitude and GABA mediated inhibition occurred at similar currents for both enantiomers in all neurons in which both enantiomers were tested (n=5).

These results do not support the idea that local interactions of D-1 and D-2 receptors in the SNpr are involved in mediating the potentiated responses in SNpr activity observed with systemic coadministration of D-1 and D-2 dopamine agonists.

[1]Braun and Chase, Soc. Neurosci. Abstr. 11:671, 1985. [2]Carlson et al., Brain Res. 400: 205, 1987. [3]Weick and Walters, Brain Res. 405: 234 1987. [4]Walters et al, Science, in press. [5]Waszczak and Walters, Science 220: 218, 1983. [6]Bergstrom and Walters, Brain Res. 310: 23, 1984.

136.6 APOMORPHINE'S EFFECTS ON THE ACTIVITY OF GLOBUS PALLIDUS NEURONS ARE RELATED TO ROTATIONAL BEHAVIOR IN RATS WITH UNILATERAL QUINOLINIC ACID LESION OF THE STRIATUM. H.S. Pan, D.A. Bergstrom, J.H. Carlson, T.M. Engber, T.N. Chase and J.R. Walters. NINCDS, Bethesda, MD 20892.

Doses of postsynaptically active apomorphine (APO) which cause behavioral activation in rats also induce marked increases in firing rates of neurons in the globus pallidus (GP). GP receives a sparse but widespread direct dopamine (DA) input from substantia nigra pars compacta as well as an extensive indirectly mediated DAergic influence via the striatopallidal pathway. The relative importance of the direct versus the indirect DAergic influence on GP is not known. It is also unclear to what extent the electrophysiological changes in GP and any of the behavioral phenomena induced by APO are related. To examine these issues, we studied APO's effects on GP neuronal firing rate and rotational response to APO after unilateral quinolinic acid lesion of the striatum.

Male Sprague-Dawley rats (300 g) received an injection of quinolinic acid (300 nmol/0.5 ul/min) in the left striatum. After 1 week of recovery, rotational behavior induced by APO (0.5 mg/kg, s.c.) was measured in a rotometer for 1 hour. One week after the behavioral testing, extracellular single unit recording techniques were used to study the effects of APO (0.3 mg/kg, i.v.) on GP cell firing rates in locally anesthetized, gallamine treated and artificially respired rats. Only rats with histologically confirmed recording sites and lesions affecting at least the rostral half of the striatum with minimal involvement of the cortex and GP were used in the data analyses.

Lesioned animals were divided into two groups on the basis of turning response to APO administration; nonturners were those which rotated less than 60 turns/hr (mean ± SEM: 13.3 ± 3.8 turns/hr, n=14), and turners were those rotating more than 60 turns/hr (mean ± SEM: 113.6 ± 14.8 turns/hr, n=12). In control nonlesioned rats, APO induced firing rate increases of at least 20% baseline in 27 of 30 (90%) cells, yielding an average increase of 93 ± 9%. In nonturners, APO induced increases in firing in 11 of 14 (79%) GP cells, yielding an average increase of 63 ± 17%. In contrast, in turners, only 4 of 12 (33%) GP cells showed firing rate increases of more than 20% baseline and 3 of 12 (25%) cells showed decreases of more than 20% baseline with APO administration. The overall rate change induced by APO in turners was an increase of 10 ± 19% over baseline, an effect which was significantly different from that of both controls and nonturners.

These results indicate that unilateral lesions involving the rostral half of the striatum can cause changes in both behavioral and GP electrophysiological responses to APO. The appearance of rotational behavior seems related to an altered pharmacological response of GP cells to APO in turners. The absence of rotational response in nonturners appears related to increased GP cell firing, as seen in normal rats. The data suggest that the influence of DA via the striatum plays a more important role than the direct nigropallidal DA input in mediating DA and DA agonists' effects on GP neuron activity.

136.7 EVIDENCE FOR DOPAMINE D-1 RECEPTOR RESERVE IN RAT STRIATUM AND SUBSTANTIA NIGRA. M.S. Ackerman*, L.D. Artman*, R.G. MacKenzie and J.W. Kebabian. Abbott Laboratories, Abbott Park, IL 60064.

The presence of receptor reserve or "spare" receptors in a system means that a maximal response can be evoked by a full agonist at submaximal receptor occupancy. The maximal response of a partial agonist, however, is only achieved at full receptor occupancy and is thereby limited by receptor number (Ruffolo, R.R. J. Auton. Pharmacol. 2:277, 1982). Therefore, in systems with receptor reserve, reducing receptor number should have a greater impact on the maximal response evoked by a partial rather than a full agonist. Results from a recent study based on the above approach suggest the existence of a dopamine D-1 receptor reserve in rat striatum (Hess, E.J. et al. Mol. Pharmacol. 31:50, 1987). In the present study, we compare the effects of reducing D-1 receptors on the maximal stimulation of adenylate cyclase by a partial and a full agonist in rat striatum and substantia nigra.

Rats were injected i.p. with 3 mg/kg of the irreversible receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihyroquinoline (EEDQ), sacrificed the following day, and the striata and nigra were removed and frozen. Adenylate cyclase activity and D-1 binding parameters were measured in the same tissue homogenates. In the striatum, EEDQ reduced B_{max} by 51% whereas maximally stimulated adenylate cylase by dopamine (a full agonist) was reduced by 19% and the maximal effect of SKF-38393 (a partial agonist) was reduced by 31%. In the substantia nigra, B_{max} was reduced by 36% and the maximal effects of dopamine and SKF-38393 on cyclase were reduced by 35% and 64%, respectively. In both tissues, EEDQ treatment had no effect on basal or forskolin-stimulated cyclase or D-1 receptor affinity, indicating that the effects of the treatment were confined to reductions in receptor number. We suggest that these results are consistent with the existence of a D-1 receptor reserve in both striatum and substantia nigra and that this reserve might be greater in the striatum.

136.8 DOPAMINE AUTORECEPTOR RESERVE ON SUBSTANTIA NIGRA DOPAMINE NEURONS CAN MASK PARTIAL AGONIST PROPERTIES OF PUTATIVE AUTORECEPTOR AGONISTS. D.A. Bergstrom, J.H. Carlson, H.S. Pan and J.R. Walters. NIH, NINCDS, Bethesda, MD 20892 and Univ. of Calif. San Diego, La Jolla, CA 92093.

Studies have suggested that pharmacologically exploitable differences exist between dopamine (DA) autoreceptors and postsynaptic DA receptors in the CNS. Indeed, certain drugs appear selective for DA autoreceptors. A more substantial DA receptor reserve at the presynaptic site would tend to increase relative autoreceptor agonist potency and efficacy and might explain these findings.

In the present neurophysiological study, the effects of 2 putatively selective DA autoreceptor agonists, BHT 920 and EMD 38362, on DA autoreceptors and postsynaptic DA receptors were compared with those of the nonselective DA agonist apomorphine (APO) in normal rats and in rats pretreated with the irreversible receptor inactivating agent EEDQ to decrease DA receptor number. Extracellular single unit activities of substantia nigra DA neurons and globus pallidus (GP) neurons provided indices of pre- and postsynaptically mediated DA receptor effects, respectively. Drugs were administered intravenously. Like APO, BHT 920 and EMD 38362 were effective agonists at DA autoreceptors, producing haloperidol-reversible inhibitions of DA cell firing. Maximal inhibitions (99-100%) of DA cell activity were observed with doses of 160 ug/kg APO and 40 ug/kg BHT 920 or less; ED_{50}s for APO and BHT 920 were 6.3±1.6 (n=13) and 2.3±0.5 (n=16) ug/kg, respectively. EMD 38362 was slightly less effective with an ED_{50} of 13.8± 4.6 ug/kg (n=12) and typically inhibited activity 95-100% with doses of 80 ug/kg or less. In the GP, BHT 920 and EMD 38362 were not as effective as APO in increasing neuronal firing rates. Maximal firing rate increases of 93±9% (n=30), 54±10% (n=22) and 25±14% (n=17) were induced by 0.3 mg/kg APO, 2.8 mg/kg BHT 920 and 0.4 mg/kg EMD 38362, respectively. EMD 38362, but not BHT 920, antagonized the effects of APO on GP cell activity.

In rats treated with EEDQ (6 mg/kg, i.p.) 24 hr. prior to recording, the dose-response curve for APO on DA cells was not significantly shifted (ED_{50}=11.6±2.9 ug/kg, n=8) and maximal inhibition of DA cell firing was still observed. In contrast, the dose-response curves for BHT 920 and EMD 38362 were shifted to the right by EEDQ pretreatment. Moreover, the activities of fewer neurons were completely inhibited by these drugs and thus, the maximal inhibitions induced by BHT 920 (92%, n=10) and EMD 38362 (77%, n=6) were significantly less than those in control rats. In the GP, pretreatment with this dose of EEDQ markedly attenuated the rate-increasing effects of APO; an average increase in pallidal neuron activity of 35±14% (n=10) was induced by 0.3 mg/kg APO in the EEDQ-treated rats.

These results lend neurophysiological support to the idea (Meller et al., Eur. J. Pharmacol. 123: 311, 1986) that a greater DA receptor reserve on DA neurons, as compared to postsynaptic cells, may explain the apparent autoreceptor selectivity of drugs like BHT 920 and EMD 38362 which appear, relative to APO, to be partial DA agonists.

136.9 COMPARISON OF IN VITRO AND IN VIVO MODELS OF STRIATAL DOPAMINE (DA) AUTORECEPTOR FUNCTION. K. Bohmaker,* M. Goldstein and E. Meller. Department of Psychiatry, New York University Medical Center, New York, NY 10016.

We have recently determined the relative potencies and relative efficacies of several DA agonists, including N-propylnorapomorphine (NPA) and the DA autoreceptor selective agents (+) and (-)3-PPP, utilizing the in vivo γ-butyrolactone (GBL) method (Meller et al., Eur. J. Pharmacol. 123:311-314, 1986; ibid., Mol. Pharmacol. in press, 1987). A large receptor reserve was also found at the autoreceptor for full agonists such as NPA. We have now examined these drugs using an in vitro model in order to compare their potency ratios and intrinsic activities in vivo and in vitro.

Strait and Kuczenski (JPET 29:561-569, 1986) recently studied DA agonist inhibition of synaptosomal TH after its activation by forskolin. Their results, unlike those of several recent studies (Fowler et al., Naunyn-Schmied. Arch. Pharmacol. 331:12-19, 1985; Brautigam et al., Mol. Pharmacol. 8:515-520, 1985) were consistent with an autoreceptor model, presumably because they measured DA agonist inhibition of an "activated" form of the enzyme similar to that obtained after treatment with GBL in vivo.

As previously reported (Strait and Kuczenski, op. cit.), incubation of striatal synaptosomes with forskolin (0.15-100 uM) elicited a dose-dependent increase in the activity of TH subsequently solubilized and assayed; activation of the enzyme was associated with an apparent change in forms of the enzyme with multiple Km's for tetrahydrobiopterin (BH_4) (curvilinear Lineweaver-Burk plots) to a single form with a low Km (30 uM) for the cofactor (linear Lineweaver-Burk plots). Forskolin (1.5 uM) stimulated TH activity about 70-80 percent at low BH_4 concentrations (33 uM). NPA dose-dependently inhibited forskolin (1.5 uM) stimulated TH activity with an IC_{50} of 5 nM; maximal reversal was about 80 percent. (+)3-PPP had an IC_{50} of 2 uM, and maximal reversal (appx.75%) was not significantly different from that elicited by NPA, whereas the IC_{50} for (-)3-PPP was about 1 uM and its maximal effect was only about 35% reversal. The relative potencies of NPA, (+)3-PPP and (-)3-PPP in vitro (1, 0.0025 and 0.005, respectively) were very similar to those obtained in vivo (1, 0.0007 and 0.001). Likewise, the relative intrinsic activities were also nearly identical (1, 0.9 and 0.4 in vitro and 1, 1 and 0.5 in vivo). These results support the idea that unlike inhibition of basal TH activity, which yields IC_{50} values for NPA and (+) and (-)3-PPP at least 100 times larger (Fowler et al., op. cit.), inhibition of forskolin-stimulated TH in vitro may be an accurate reflection of autoreceptor-mediated regulation of DA synthesis in rat striatum in vivo. Supported in part by grants NS-23618, MH-02717 and MH-35976.

136.10 K+-INDUCED DEPOLARIZATION OF RAT STRIATAL NEURONS IN VITRO ALTERS FUNCTIONAL EFFECTS OF DOPAMINE (DA) AGONISTS AT DA SYNTHESIS-MODULATING AUTORECEPTORS: DEPENDENCE ON THE RELATIVE EFFICACY OF DOPAMINE AGONISTS? D. Clark*1, R.S. Salah2 and M.P. Galloway2. (SPON: R.B. Holman) 1Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235, and Neuropsychopharm. Lab., Dept. of Psychology, University of Reading, England, 2Neurochemical Pharmacology Research Unit, Lafayette Clinic, Detroit, MI 48207.

Previous in vivo biochemical and electrophysiological evidence indicates that the enantiomers of 3-hydroxyphenyl-N-n-propyl-piperidine (3-PPP) act as agonists at dopamine (DA) autoreceptors, with (-)-3-PPP exhibiting lower relative efficacy (Clark et al., J. Neural Transm. 62: 171, 1985; Meller et al., Soc. Neurosci. Abstr. 12, Part 1: 194, 1986). In the present study, we have investigated the effects of these compounds on DA synthesis in rat striatal slices. Comparisons were made with the selective D2 DA agonist quinpirole, and the putative partial DA receptor agonist transdihydrolisuride (TDHL; Kehr, Eur. J. Pharmacol. 97: 111, 1984). The enantiomers of 3-PPP concentration-dependently reduced basal DA synthesis, measured as DOPA accumulation after decarboxylase inhibition by NSD 1015, although they were considerably less potent (IC50: 1.8-3.2 uM) than quinpirole (IC50: 0.1 uM) and TDHL (IC50: <0.1 uM) in this regard. Similar inhibitory effects of the 3-PPP enantiomers were observed in slices depleted of DA by a prior in vivo pretreatment with reserpine. Although both (+)-3-PPP and quinpirole were able to completely reverse the increase in DA synthesis in normal slices produced by addition of excess K+ (30uM) to the incubation medium, (-)-3-PPP and TDHL were ineffective in this regard. Instead, the latter compounds reversed the inhibitory effects of quinpirole under these conditions. This switch in pharmacological profile of (-)-3-PPP and TDHL may be related to their previously demonstrated low relative efficacy, and to the presence of varying synaptic DA concentrations under our different conditions. This hypothesis is currently being further investigated. The present findings suggest caution in interpreting the profile of novel DA receptor agonists in certain DA autoreceptor models. Thus, the previously reported antagonist profile of (-)-3-PPP at DA release-modulating autoreceptors may be related to a partial agonist action of the drug at these sites and significant receptor concentrations of competing endogenous transmitter. (The present research was supported by grants MH-41227 and DA-04120 to MPG).

136.11 COMPARISON OF STRIATAL D1 DOPAMINE RECEPTORS AND ENDOGENOUS DOPAMINE RELEASE IN AGING MICE. K.A. Young [1], J.A.Severson [2], J.J.Woodward [1], S.W.Leslie [1], P.K. Randall [2] and R.E.Wilcox [1]. [1] Institute for Neurological Sciences Research, College of Pharmacy, University of Texas, Austin, TX, 78712. [2] University of Southern California, Los Angeles, CA.

While D2 DA receptors decrease in the aged rodent, there is controversy concerning age effects on neuronal [and especially synaptic] markers of DA neuronal function in striatum. We have previously demonstrated in C57BL/6 mice that high-affinity binding of DA to D2 dopamine receptors in striatum is reduced with age. It was of interest to compare effects of age on the D1 DA receptor and on the release of endogenous DA in the same strain.

Male, C57BL/6 mice were used. For D1 binding studies the ages were 3,8, and 24 mo., for up uptake/release studies the ages were 3,12, and 24 mo. Striata from 3 mice were pooled for each receptor binding or calcium uptake/DA release assay. In both D1 receptor and uptake/release assays sets of three experiments were conducted at one time, with tissue from each of the three ages represented in each assay. For D1 binding, 8 experiments in duplicate were run for each age. The procedure of Hess et al. [Eur. J. Pharmacol., 121:31, 1986] was used for the D1 binding assay. Saturation isotherms using 6 concentrations of 3H-SCH23390 [250-3000pM] and displacement of 750pM 3H-SCH23390 by 15 DA concentrations [0.1mM to 0.1nM] were run in duplicate with 300nM cis-flupentixol as a blank to define nonspecific binding. Six 45Ca++ uptake/DA release experiments were conducted in duplicate. The procedure of Leslie, S., et al., Brain Res., 325: 99, 1985] was used in the present experiment. Synaptosomes were prepared and separate portions were incubated in microcentrifuge tubes for 1, 3, 5, 15, or 30 sec. Uptake/release under resting conditions [5mM K+] was subtracted from uptake/release under depolarizing conditions [30mM K+] to provide net uptake/release through voltage-sensitive channels.

The Bmax for D1 DA receptors was unchanged from 3 to 8 mo., but was reduced by 24 mo. [1606 ± 207, 1637 ± 207, and 1119 ± 105 fmole/mg protein, respectively]. Kd's were unchanged from 0.74 nM. The percentage of D1 receptors exhibiting high-affinity binding for DA was unchanged from 54.6 ± 5% under our assay conditions. However, the capacity of the high affinity agonist state of the D1 receptor were significantly reduced between 8 and 24 mo. [by 22%]. Thus, when 3H-SCH23390 and cis-flupentixol are used for D1 DA receptor assays, clear cut reductions in both high-affinity binding of DA and in total D1 sites occur with aging.

Neither 45Ca++ uptake through voltage-sensitive channels nor DA release was altered by aging. The expected increases in 45Ca++ uptake and in DA release from 1 through 30 sec. occurred, with most of each over by the end of the 1-3 sec. fast-phase period as we have previously described. Also, values for 45Ca++ uptake and DA release which we obtained in 3 mo. old C57BL/6 mice were found to be comparable to those which we had previously obtained using 3 mo. CD-1 mice. Thus, values obtained in all 3 age groups were similar to those observed in the 3 mo. group: (1) 45Ca++ uptake [nmoles/mg protein were 1 sec. 89 ± 16, 3 sec. 260 ± 23, 5 sec. 413 ± 23, 15 sec. 515 ± 35, and 30 sec. 527 ± 24; [2] DA release [pmoles/mg protein] were 1 sec. 12 ± 2, 3 sec. 21 ± 2, 5 sec. 25 ± 2, 15 sec. 34 ± 3, and 30 sec. 34 ± 3. The coupling ratios for calcium/DA were also found to be the same in the 3 age groups. Total DA levels in striatum were also found to be constant across the 3 ages in the present study. Thus, when the fast-phase endogenous release of the transmitter coupled to 45Ca++ entry is used an index, aging to 24 mo. does not appear to reduce either process.

Overall, our previous results on age effects on the striatal D2 DA receptor in conjunction with the present results suggest that, in mice of this strain assayed under the described conditions, age produces reductions in the high-affinity binding of DA to the D1 and D2 sites without altering the stimulus-secretion coupling process for the transmitter. [Supported by NS20827 (REW & WW Spirduso), MH09269 (JJW), AA05809 (SWL) and a Texas Technology Grant (SWL & REW)].

136.12 EFFECTS OF CHRONIC APOMORPHINE TREATMENT ON MEASURES OF STRIATAL DOPAMINE FUNCTION. R.E.Wilcox [1], W.H.Riffee [1], J.A.Severson [2], J.J. Woodward [1], S.W.Leslie [1], and D.M.Vaughn [3]. [1] Dept. of Pharmacology & Toxicology, College of Pharmacy, and Neuropharmacology Research Program, Institute for Neurological Sciences Research, University of Texas, Austin, TX 78712. [2] Dept. of Psychiatry, School of Medicine, University of Southern California, Los Angeles, CA 90033. [3] Scott-Ritchey Research Program, School of Veternary Medicine, Auburn University, Wire Road, Auburn, AL 36849.

Subchronic [14 once daily injections] treatments of mice with apomorphine [APO] induce a behavioral sensitization to the stereotypic effects of challenge doses of APO. In the present report we attempted to establish a biochemical basis for the behavioral effect by evaluating the effects of subchronic vs. single APO treatments on striatal dopamine (DA) metabolites, DA synthesis, in vitro DA release, and on the high-affinity agonist binding of DA to the D2 DA receptor. Mice received 14 daily injections of APO (30 mg/kg, ip; a subchronic injection routine) or saline. Three days after the last subchronic dosing (which is the time of peak behavioral sensitization) mice were challenged with saline or APO (2 mg/kg, ip). In parallel experiments, the effects of a single APO treatment 24 hr prior to euthanasia on striatal DA metaboism, synthesis, release and on D2 DA receptors were evaluated to suggest rates of adaptation to the agonist for these markers. Subchronic APO administration (1) increased the stereotypic response to APO in a dose-dependent manner for the 1 and 2 mg/kg challenge doses of APO, but not the 4 mg/kg dose; (2) increased basal striatal levels of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and the ratios of DOPAC/DA and HVA/DA; (3) attenuated the ability of APO challenge to lower DOPAC/DA and HVA/DA ratios; (4) increased striatal l-dopa accumulation after decarboxylase inhibition; (5) left unaltered the ability of APO challenge to lower l-dopa accumulation; (6) left unaltered fast-phase (3 sec. depolarization) endogenous DA release; and (7) left unaltered the percentage of D2 DA receptors binding DA with high-affinity. Single APO pretreatments (24 hr prior to euthanasia) (1) did not affect striatal DA metabolites or DA synthesis but (2) reduced fast- (but not the slow-) phase release of DA and (3) increased the percentage of D2 DA receptors binding DA with high-afinity. These results are consistent with an ability of subchronic APO treatment to induce altered synaptic DA content in striatum independent of receptor mechanisms. Our findings suggest that alterations in dopamine synthesis or metabolism in response to subchronic DA agonist treatment may occur at different rates than changes in DA release and binding and that such neuronal alterations may play a role in the behavioral changes which are associated with multiple dopamine agonist pretreatments. These results are also consistent with a rapid development of tolerance to the nigrostriatal neuron and D2 receptor effects of APO in which a reciprocal pattern of changes occur in DA release and in the percentage of D2 DA receptors in the high-affinity agonist state. [Supported by MH33442 (WHR & REW), NS20827 (REW & WW Spirduso), MH09269 (JJW), AA05809 (SWL) and a Texas Technology Grant (SWL & REW)].

136.13 AMPHETAMINE-INDUCED INHIBITION OF VENTRAL TEGMENTAL AREA DOPAMINERGIC NEURONAL IMPULSE FLOW: IN VITRO STUDIES IN THE MOUSE. E. Viscardi, G. Bernardini and D. C. German. Depts. of Psychiat. and Physiol., Univ. of Texas Health Sci Cntr., Dallas, TX 75235.

The effect of d-amphetamine (d-AMP) on ventral tegmental area (VTA) dopamine (DA)-containing neurons is thought to be critical for the enhanced motoric effects of this drug. d-AMP is a potent releaser of DA and inhibits VTA DA neuronal impulse flow in vivo. The inhibition of cell firing is thought to be due to a local effect of d-AMP in releasing dendritic DA onto cell body autoreceptors (Groves et al.,Science 190:522, 1975; Wang, Brain Res. Rev.,3: 153,1981). The purpose of the present study was to investigate whether d-AMP inhibits mouse VTA DA neuronal impulse flow in vitro. We have previously demonstrated that mouse midbrain DA neurons exhibit spontaneous activity in vitro and that DA inhibits neuronal impulse flow (Sanghera et al. Neurosci. 12:793, 1984). Using the in vitro slice preparation, the effects of d-AMP can be studied directly without the influence of feedback and afferent inputs to the VTA neurons. Brains from BALB/c and CBA mice were rapidly removed from the skull and sectioned in the coronal plane at a thickness of 400 um. Recordings were obtained from neurons in the VTA whose firing characteristics resembled those previously described for DA neurons. The tissue was superfused with d-AMP in increasing doses (12.5, 25, 50, 100 uM). In 12 cells examined, doses as low as 25 uM induced over 90% inhibition of cell firing. This inhibition was reversed by superfusion with the DA type-2 receptor antagonist, (-) sulpiride (0.1 uM). Pretreatment of mice with the DA-synthesis inhibitor, alpha-methylparatyrosine (250 mg/kg, i.p.), 90 min. prior to sacrifice, blocked the inhibitory effects of d-AMP, yet superfusion with DA still inhibited cell firing. These data are consistent with the hypothesis that d-AMP releases newly synthesized dendritic DA to inhibit VTA DA neuronal impulse flow. Research supported by grants from the NIMH (MH-30546) and the Dallas Area Parkinsonism Society.

136.14 DOPAMINE RECEPTOR BLOCKADE ANTAGONIZES AMPHETAMINE-INDUCED ASCORBATE RELEASE IN THE NEOSTRIATUM. George V. Rebec, Choonjeong Oh*, and Thomas W. Gardiner. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Ascorbate (AA), which exists in high concentration in the extracellular fluid of the neostriatum, appears to modulate both the firing rate of neostriatal neurons (Gardiner et al., Brain Res. 344:181, 1985) and behavioral responses mediated by neostriatal dopamine (Rebec et al., Science 227:438, 1985). Amphetamine, a well-known dopamine agonist, nearly doubles the extracellular concentration of neostriatal AA. Although this effect is not abolished by destruction of dopaminergic terminals in the neostriatum, several studies suggest that dopamine receptors may play a role in AA release. The present study was designed to examine this hypothesis.

Electrochemically-modified microvoltammetric electrodes were positioned in the neostriatum of urethane-anesthetized rats. These electrodes yield a voltammetric wave for AA that is resolved easily from that for catechols and all other electroactive species in the mammalian brain. Voltammetric scans, displayed in a differential form, were obtained at 2-min intervals. The animals received 2.5 mg/kg d-amphetamine and were challenged 30 min later with one of several dopamine antagonists or with vehicle. Both haloperidol and clozapine reversed the amphetamine-induced rise in AA. This was not the case following administration of either SCH-23390, which selectively blocks D-1 dopamine receptors, or sulpiride, a selective D-2 dopamine antagonist. When administered together, however, these drugs blocked AA release. It appears, therefore, that a blockade of both D-1 and D-2 dopamine receptors is required to reverse the increase in neostriatal AA produced by amphetamine.

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137.1 EXTERNAL ENVELOPE PROTEIN (gp120) of HIV PRODUCES NEURONAL DEATH IN HIPPOCAMPAL CULTURES. D.E. Brenneman, C. Pert and G.Westbrook Lab. of Develop. Neurobiol., NICHD and Section on Brain Biochem., Clin. Neurosci. Branch, NIMH, Bethesda, MD 20892.

Cognitive impairment and neuronal deficits are clinical features of Acquired Immune Deficiency Syndrome. Previous studies have indicated that several of the immunological defects associated with Human Immunodeficiency Virus (HIV) infection are mediated by nonviable viral proteins (Pahwa,S. et al.,PNAS,82: 8198,1985). We have tested if the 120-kDa external HIV envelope glycoprotein (gp120) produced neuronal death when incubated with dissociated cultures from the hippocampus.

Hippocampal neurons obtained from 16-17 day old fetal mice were plated on confluent hippocampal feeder cultures prepared from newborn mice. The neuronal cultures were maintained in defined medium supplemented with 5% horse serum/MEM and the feeder cultures were grown in 10% fetal bovine serum/MEM until seeded with neurons. Gp120 and peptide treatments were added to 7 day old cultures and treatment was continued for 5 days. To assay, the neurons were immunocytochemically identified with antibodies to neuron specific enolase and counted. Gp120 was purified from HIV isolate $HTLV\text{-}III_B$ by immunoaffinity chromatography and preparative PAGE as described (Robey,W. et al., PNAS,83:7023,1986).

Addition of gp120 to hippocampal cultures produced a concentration-dependent decrease in neuronal survival as compared to control cultures. Decrements were evident at 10^{-13} M, with maximal neuronal deficits (67-75% of control values) being observed with 10^{-12} M. However, neuronal losses associated with gp120 treatment were attenuated at concentrations of gp120 greater than 10^{-11} M.

Vasoactive intestinal peptide (VIP) has been shown to increase neuronal survival during development in culture (Brenneman,D. et al.,PNAS,83:1159,1986). A five amino acid sequence of VIP (TDNYT) shares homology with sequences of the gp120 molecules. Because of this similarity, we have examined the effect of VIP and an analog of an octapeptide sequence from the envelope, ($D\text{-}Ala^1$) Peptide T amide, on gp120-induced neuronal death. Addition of 0.1 nM VIP to hippocampal cultures produced a significant increase (25%) in the number of neurons and also prevented losses produced with gp120. Treatment with the Peptide T derivative alone had no effect on hippocampal neuron survival; but this substance, like VIP, also attenuated gp120 associated neuronal death. These data indicate that in cell culture, the nonviable external envelope protein of HIV can produce neuronal death. The attenuation of gp120-induced death by VIP and the Peptide T derivative suggests an important link between the action of these substances and protection against HIV-associated neurotoxicity.

137.2 NEUROCHEMICAL CHANGES IN THE CORTEX OF HUNTINGTON'S DISEASE PATIENTS: POSSIBLE BASIS FOR AFFECTIVE DISORDER. J.C. Hedreen and G.L. Wenk. Departments of Neuropathology and Psychology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Huntington's Disease (HD) has four principal clinical manifestations: (1) Chorea and other involuntary movements; (2) Disturbances in voluntary motor control; (3) Dementia; and (4) Emotional (affective) disorders including depression, irritability, aggression hallucinations and suicide. The principal histopathologic change in HD is a loss of the small/medium projection neurons in the caudate and putamen. The neurochemical changes in the striatum include decreased levels of gamma-aminobutyric acid, acetylcholine, substance P, and dynorphin, and increased levels of somatostatin and neuropeptide Y. Few investigators have examined changes in neocortical neurochemistry. In the present study, we have examined the levels of various biochemical markers for the cholinergic, serotonergic, dopaminergic, and noradrenergic neurotransmitter systems in neocortex of patients with pathologically confirmed HD and in age-matched controls. These measures include choline acetyltransferase activity, endogenous levels of dopamine, serotonin, norepinephrine, 5-hydroxyindoleacetic acid, homovanillic acid, and the number of serotonergic type-2 receptors (as determined by [^{3}H]-ketanserin), as well as the density of high-affinity norepinephrine uptake sites (as determined by [^{3}H]mazindol). There was no correlation between levels of the biochemical markers and post-mortem delay or drug therapy. The ratio of 5-hydroxyindoleacetic acid to serotonin, and the number of serotonergic type-2 receptors were decreased, suggesting decreased activity in raphe neurons. Levels of dopamine metabolites were increased, and levels of dopamine were decreased, suggesting increased activity in mesocortical dopaminergic systems. The density of norepinephrine uptake sites was decreased, consistent with decreased release of norepinephrine. The data support the hypothesis that alterations in cortical monoamine neurotransmitter systems may underlie some of the affective disorder symptomatology associated with HD.

137.3 HUNTINGTON'S DISEASE: STUDIES ON BRAIN FREE AMINO ACIDS. E. Bonilla, A.L.N. Prasad* and A. Arrieta*. Instituto de Investigaciones Clínicas and INBIOMED-FUNDACITE. Apartado 376. Maracaibo, Venezuela.

Huntington's disease (HD) is a chronic neurodegenerative disorder characterized by involuntary choreiform movements, psychological symptoms and dementia. It is inherited in an autosomal dominant fashion with complete penetrance. Although a genetic marker linked to the Huntington's disease locus located in chromosome 4, has been identified (Gusella, J.F. et al, Nature 306: 234, 1983), the affected gene and the resultant biochemical deficit is unknown. In this report, we investigated the changes in free amino acid levels in putamen and Brodmann's area 10 of patients who died with Huntington's disease.

Brains were obtained from 12 patients with HD (7 male, 5 female; mean age, 60.5 years; range 36-80 years) and 13 non-neurologic controls (9 male, 4 female; mean age, 67.7 years; range 34-84 years). The diagnosis of HD was confirmed by neuropathologic examination. Free amino acids were detected using a high performance liquid chromatographic procedure (Jones, B.N. et al Liquid. Chromat. 4: 565, 1981).

Gamma-amino-butyric acid (GABA), glutamate and alpha-amino-n-butyric acid levels were found to be significantly reduced in the putamen of HD patients. In Brodmann's area 10, the concentrations of glutamate, histidine and lysine were highly decreased, but the levels of aspartate, GABA, glycine, serine and taurine were increased.

We have confirmed previous reports stating that the concentration of GABA decreases in the putamen of HD patients. A reduction in GABA neurons has been considered to be responsible for the disinhibition of the dopamine nigrostriatal system and would explain the improvement of choreic movements by dopaminergic antagonists. However, the fact that GABA levels are normal in other brain regions or increased, as we have found in Brodmann's area 10, makes it unlikely that GABA is solely responsible for the pathogenesis of HD. The reductions in the levels of glutamate do not necessarily preclude the possible role of glutamate in the pathogenesis of HD since its buildup at the synaptic cleft of excitatory synapses is all it takes to produce the neurotoxic effect of this amino acid on postsynaptic neurons. The increase in glycine content observed in Brodmann's area 10 may be a direct consequence of the elevation in serine levels. Further work is needed to define the significance of the changes in these amino acid for the development of the neurologic and psychiatric symptoms in HD.

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137.4 PHARMACOKINETICS AND METABOLIC DISPOSITION OF THE COGNITION ACTIVATOR ^{14}C-CI-933 IN DOGS. A. Black and T. Chang*, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

CI-933, 1-(4-methoxybenzoyl)-5-oxo-pyrrolidinepropanoic acid, is a cognition activator currently undergoing Phase 1 clinical evaluation. A two-way crossover study in beagle dogs was performed by giving single 10 mg/kg IV and PO doses of ^{14}C-CI-933. Heparinized blood, urine and feces were collected at specified time periods. Total radioactivity was determined in plasma, urine and feces by liquid scintillation spectometry. Plasma CI-933 concentrations were assayed by a validated HPLC method. Plasma protein binding was determined by ultracentrifugation. HPLC with radioactivity detection (RAM) was used to detect metabolites in urine and plasma.

Following single IV doses, plasma ^{14}C activity declined in a polyexponential manner with a mean $t_{\frac{1}{2}}$ of 4 hours. By six hr postdose ^{14}C concentrations were 0.1 ug/ml or less. After single PO doses, mean peak ^{14}C activity (20.9) ug/ml) occurred 0.6 hr postdose. Similar concentration-time profiles were obtained and mean elimination $t_{\frac{1}{2}}$ was 3.5 hrs. RBC ^{14}C activity was approximately one-third of plasma ^{14}C activity after either route of administration. Plasma CI-933 concentrations declined in a monoexponential manner after single IV doses and were undetectable by 6 hr. Following single PO doses, peak CI-933 plasma concentrations (19.9 ug/ml) were observed less than one hr post dose. Elimination $t_{\frac{1}{2}}$ was approximately 0.5 hours and independent of route of administration. Plasma protein binding of CI-933 over a 100-fold concentration range averaged 25%. Based on plasma AUC comparisons, absolute oral bioavailability of CI-933 was 82%.

Excretion patterns of ^{14}C activity were similar after either route of administration indicating complete oral absorption. Urinary excretion was the major route of elimination accounting for 88% of the radioactivity by 24 hr post dose. Fecal recovery ranged from 1-7% of the dose. HPLC-RAM analysis of urine after IV and PO doses indicated that intact CI-933 accounted for approximately 76% of the dose. The remainder of radioactivity (11.7%) was in the form of 5-oxo-2-pyrrolidinepropanoic acid metabolite. This metabolite was also detected in plasma at later time intervals.

137.5 Early Leukocyte Migration into a Bacterial Cerebritis. W. Lo and D. McNeely Department of Pediatrics, Columbus Children's Hospital, Columbus, Ohio 43205.

The initial steps by which bacteria impair blood-brain barrier integrity and eventually cause edema are not well understood. We hypothesized that bacteria stimulate neutrophils to migrate from the blood to the brain, the neutrophils phagocytose the bacteria, and the chemical products of phagocytosis damage microvessel integrity, thereby the blood-brain barrier is damaged. We examined the first step of this hypothesis in a rat model of a bacterial brain abscess (Winn, H.R. et al., J. Neurosurg 51:685-690, 1979). Sprague-Dawley rats, (250-300 gms) were anesthetized with pentobarbital (40mgs/kg), and intracerebrally inoculated with a 10^4 colonies/ml suspension of log phase Staphylococcus aureus (PG-114-1, a non-delta-, and non-alpha-toxin producing strain). The animals were perfused and sacrificed at 3, 6, 12, 24, and 48 hours after inoculation (n=3-4), and the brains were prepared for hematoxylin and eosin staining. The experimental animals showed little cellular change at 3 hours except for a scant amount of neutrophil margination in microvessels near the inoculation site. By six hours after inoculation, neutrophils accumulated into a measurable volume, 0.136 ± 0.090 mm^3 (n=3), and the volume of leukocyte infiltration steadily increased in size, to 0.385 ± 0.142 mm^3 (n=3) by 48 hours. In contrast, control animals injected with sterile medium showed only a slight collection of red blood cells along the needle path at the early time points. A few neutrophils appeared only 24 to 48 hours after inoculation, and never achieved measurable volume.

Our results indicate that Staphyloccous aureus produces chemotactic factor(s) that stimulate neutrophil chemotaxis across the microvascular wall. Neutrophil migration begins as early as three hours after inoculation. The migration occurs when there is no histologic evidence of tissue destruction or necrosis.

Our results suggest that neutrophil chemotaxis across the microvessel wall is an early step in the development of a cerebritis. Our findings suggest that neutrophil migration may precede alteration of the blood-brain barrier, and that the barrier may be affected by the products of neutrophils phagocytosing bacteria.

Supported by grants from the Children's Hospital Research Foundation and Central Ohio Heart Association.

137.6 INDUCTION AND SUPPRESSION OF AUTOIMMUNE NEUROPATHIES F. C. Westall and R. S. Root-Bernstein. Institute for Disease Research, 6171 Kinlock Ave., Rancho Cucamunga, CA 91730.

Post-infectious and post-vaccinal neuropathies (PINs and PVNs) occur very rarely (1/10,000 to 1/500,000 per infection or injection). Like other autoimmune diseases, they are usually regarded as resulting from "mistakes" in the self-recognition system. However, under experimental conditions, autoimmunity can be induced in many species with almost 100% success. This observation suggests that the induction of autoimmunity is not a mistake, but due to factors not normally encountered. Experimental autoimmune diseases, including experimental autoimmune encephalomyelitis, thyroiditis, and castration, all require both a protein "antigen" and one or more bacterial "adjuvants" for their induction. It is not possible to induce any of these autoimmune diseases with the protein or peptide alone, or with the "adjuvant" alone. Furthermore, the "adjuvant" induces a separate immune response from the protein or peptide "antigen". As with "antigen", the pretreatment of animals with large doses of "adjuvant" can prevent induction of autoimmunity, and large doses of either "antigen" or "adjuvant" can suppress the disease once induced. In short, the "adjuvant" has all of the properties of a second "antigen", and both antigens must be present concurrently to induce an autoimmune response. A dual antigen mechanism for the induction of autoimmunity would explain many currently puzzling facts concerning PINs and PVNs. Viruses, including measles and Epstein-Barr, have coat-protein sequences similar to myelin basic protein. Injection of animals with these viruses alone does not, however, lead to autoimmune neuropathies. As with other autoimmune diseases, we suggest that an appropriate second antigen, bacterial in origin (e.g., a Mycoplasma or B. pertussis), is necessary to induce PINs and PVNs. The necessity for concommitant viral and bacterial infections would explain the very low incidence of PINs and PVNs; is supported by hospital records of patients admitted with PINs and PVNs; and suggests the possibility of treatment of such patients with suppressive doses of bacterial "adjuvants" or myelin basic protein. (Westall and Root-Bernstein, Mol Immunol 20 (1983) 169-77; Lancet (2 Aug 1986) 251-2).

137.7 INHIBITORY EFFECTS OF PHENIDONE ON COLD-INDUCED VASOGENIC EDEMA AND LEUKOTRIENE PRODUCTION IN RODENT BRAIN. L. J. Robichaud* and F. W. Marcoux. Department of Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Extracts from sections of guinea pig and rat brains cryogenically injured 15 or 60 minutes before sacrifice were assayed for leukotriene (LT)C_4 by radioimmunoassay. LTC4 levels in injured brain extracts were elevated as compared to controls. The lipoxygenase inhibitor, phenidone (30 mg/kg, IP), was tested in guinea pigs and completely blocked the cold-induced increase in LTC_4. LTC_4, which was not detected in normal rodent brains, increases cerebral vascular permeability in rats (Black & Hoff, 1985), and has been implicated in post-ischemic reperfusion injury in gerbil brain (Kiwak et al, 1985). We hypothesized that LT might also be an important mediator in cold-induced vasogenic brain edema.

Transcranial cold injury induced extravasation of albumin (A) labeled with either ^{125}I or Evans-blue (EB) dye. Assessments of ^{125}I-A and EB-A in tissue after injury were interpreted as measures of the intensity and extent of edema, respectively. Phenidone (30 and 100 mg/kg, IP) inhibited cerebral tissue accumulation of ^{125}I-A and reduced the extent of EB-A accumulation in rats and guinea pigs subjected to cold injury.

Phenidone has various pharmacological actions (e.g. oxyradical scavenger and inhibitor of ornithine decarboxylase activity). In order to further characterize the edema model and the mechanisms involved, additional pharmacologic agents were tested. Several inhibitors of oxy-radicals and/or their effects were inactive in the edema model: allopurinol (100 mg/kg IP 48, 24 and 0.5 hrs before challenge), mannitol (4mg/kg IV), DMSO (1%, 4 ml/kg IP), catalase-PEG (3600 IU/kg, IV) and superoxide dismutase-PEG (3600 IU/kg, IV). Catalase and superoxide dismutase combined potentiated the intensity of the edema (73%). The ornithine decarboxylase inhibitor 3400 MDL (100 mg/kg IP) had no effect on vasogenic edema. These data further support a role for leukotriene as a mediator of cold-induced vasogenic brain edema in rats, and suggest that the inhibitory effects of phenidone on edema may be due to inhibition of lipoxygenase but not to inhibition of oxy-radicals.

137.8 CALCIUM HOMEOSTASIS IS ALTERED IN SYNAPTOSOMES FROM CATS WITH GM_1 GANGLIOSIDOSIS. M.L. Koenig, R.S. Jope, H.J. Baker, and J.A. Monti. Dept. of Pharmacology and Neuropsychiatry Program, Univ. of Alabama at Birmingham, Birmingham, AL 35294 and Dept. of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

GM_1 gangliosidosis is one of several lysosomal storage diseases which are characterized by severe, progressive dysfunction of the central nervous system (CNS). Although much has been learned about the specific defects in lysosomal biochemistry associated with these diseases, the underlying pathogenetic mechanisms responsible for CNS dysfunction remain poorly understood. Our previous studies of cholinergic metabolism in a well characterized feline model of GM_1 gangliosidosis have shown that the depolarization-dependent release of acetylcholine is increased in diseased cats relative to controls (J. Neurochem. 46:1567, 1986). Subsequent studies of calcium fluxes in synaptosomes prepared from cats with GM_1 gangliosidosis and from phenotypically normal controls have shown that both the fast and slow phases of voltage dependent calcium influx as well as Na^+ dependent efflux are reduced significantly in the diseased cats (Brain Res., in press). These results suggest that the intrasynaptosomal calcium concentration might be increased chronically in cats with GM_1 gangliosidosis.

The intra synaptosomal free Ca^{2+} concentration ($[Ca^{2+}]_i$) in diseased and control cats, measured using the fluorescent Ca^{2+} indicator indo-1, was higher under resting conditions, was less affected by a high K^+ (50 mM) induced depolarization of the synaptosomal membrane, and appeared to be less effectively buffered in synaptosomes prepared from GM_1 mutant cats. Subsequent studies of the sequestering capacity of synaptosomal mitochondria and endoplasmic reticulum have indicated that the ATP-dependent uptake of calcium into the endoplasmic reticulum of synaptosomes from GM_1 mutants is 30-40% lower than controls.

Because altered intrasynaptosomal calcium concentrations will affect both the rate and extent of protein phosphorylation and dephosphorylation, we also are investigating the possibility that phosphorylation of synaptosomal proteins is altered in cats with GM_1 gangliosidosis. Differences in the phosphorylation patterns of at least five synaptosomal proteins have been identified. Our present effort is focused on determining the extent to which these changes can be attributed to changes in the activities of the well characterized Ca^{2+} dependent protein kinases, Ca^{2+}-calmodulin dependent protein kinase and Ca^{2+}-phospholipid dependent protein kinase (protein kinase C).

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137.9 IMMUNOPATHOLOGY OF THEILER'S VIRUS DEMYELINATION.
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We are investigating the immunopathology of the demyelinating process associated with Theiler's murine encephalomyelitis virus (TMEV). We have previously shown [J. Immunol. 136:2136, 1986] that some splenocytes are infected with the virus following intracerebral inoculation. We suggested, as have others [Clatch, et al J. Immunol. 136:920, 1986], that the macrophages were the most likely cell type to be infected. Using both a bioassay and immunocytochemical staining, we have identified plastic-adherent, esterase positive cells in the spleen that are infected. After 10-12 weeks, spleens from intracerebrally infected mice showing clinical signs of demyelination were removed. Following separation into plastic-adherent and non-adherent populations, the plastic non-adherent cells were passed over a nylon-wool column and the remaining non-adherent cells recovered. An aliquot of the adherent and non-adherent cells were subjected to repeated freeze-thawing and the supernatant injected intracerebrally into 2 day old suckling mice for a bioassay. The remainder of the two cell populations were fixed, treated with saponin to permeabilize the cell membrane, and immunocytochemically stained for TMEV protein. The primary antibody was a rabbit anti-TMEV with a fluoresceinated goat anti-rabbit-IgG secondary antibody. After identification of fluorescent positive cells, the coverslips were removed and the cells stained for esterase. Infected cells were found in the plastic-adherent, esterase-positive cell population. Only the freeze-thaw supernatant from the plastic-adherent cell population was infective in the bioassay. We have also reported [J. Neuroimmunol., In press] immune responses directed against both the TMEV and myelin basic protein (MBP) in these infected mice. To further investigate the immunopathology of this disease we sought cytotoxic lymphocytes from TMEV-infected mice. Using a modification of the method of McCarthy and deVellis [J. Cell Biol. 85:890, 1980; Knapp et al, Dev. Biol. In press], we prepared a monolayer of cells, rich in oligodendroglia, from a primary culture. The plastic non-adherent, nylon-wool non-adherent cell population is the source of cytotoxic lymphocytes. Expression of MHC antigens on target cells is induced using a concanavalin A conditioned media eluant and verified by immunocytochemical staining. In cytotoxic assays the oligodendroglia, after lymphocyte exposure, are monitored for the integrity of their membrane sheets and characterized by immunocytochemical staining for MBP and galactocerebroside. Controls are identical cultures exposed to ConA eluant only or to nylon-wool non-adherent lymphocytes from CFA immunized donors.
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137.10 PLATELET PROTEIN MAPPING IN NORMAL AND AUTISTIC SUBJECTS USING TWO-DIMENSIONAL "GIANT GEL" ELECTROPHORESIS.
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One successfull approach to determining genetic defects in inherited disorders has been to identify and characterize specific disease-related proteins and to then determine their corresponding genes. Recent work in Alzheimer's Disease has demonstrated the feasibility of this approach in the molecular analysis of neuropsychiatric disorders. In most cases the most difficult step in this process would appear to be the initial identification of altered proteins. Several groups have employed standard two-dimensional gel electrophoresis in attempting to locate altered proteins in CSF, lymphocytes, or fibroblasts obtained from patients with neuropsychiatric disorders.

In order to perform as complete as possible mapping and inventory of cellular protiens in CSF, fibroblasts, platelets and brain we have used the "giant gel" methodology of Young (Clin. Chem. 30:2104) and Klose & Zeindl (Clin. Chem. 30:2014). Two-dimensional "giant gel" were obtained using 32 cm isoelectric focusing tube gels (3 mm I.D., ampholytes 4:1, pH 5-7 : pH 3-10) followed by 35 cm SDS-PAGE seperation in the second dimension (12-17% acrylamide, exponetial gradient) Typically 0.5-1.0 mg of brain, fibroblast, or platelet protein has been electrophoresed and approximately 1200 discrete protein spots have been observed after silver staining. Results of "giant gel" mapping of brain (cortex), fibroblast, and platelet proteins using silver staining will be presented as will mapping studies of fibroblast proteins metabolically-labeled with ^{35}S-methionine.

Our first clinical application of the method has been to the study of platelet proteins in autism. Recent research on the mechanism of the well-replicated increase in platelet serotonin (5-HT) levels seen in autism has stongly indicated that some aspect of the platelet's uptake or storage of 5-HT is altered in autism. This has suggested the platelet as a potentially fertile area for the molecular analysis of autism. We will present results of the "giant gel" inventory of platelet proteins, as well as more specific studies of 5-HT related proteins, in autistic and normal subjects.

Work supported by NIH grants HD03008 and MH30929, The MacArthur Foundation, and Mr. and Mrs. Victor Gettner.

137.11 AKINESIA AND CATALEPSIA INDUCED BY CARBACHOL INJECTED INTO THE PONS AND EEG CORRELATES.
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The basal ganglia are considered as the anatomical substrate of catalepsia induced by systemic administration of neuroleptic drugs. However, it was reported that the mesencephalic reticular formation may also be a site for the cataleptic effect of neuroleptics (Hartgraves and Kelly, Brain Res., 307:47, 1984). Here we report our findings about the role of the pons in catalepsia.

Carbachol (4-10 ug in 1 ul saline) was injected unilaterally into the mesencephalon and pons of freely moving male Wistar rats. The effect obtained in the two brain areas was similar, but stronger in the pons. Several minutes after the injection the animals acquired a characteristic standing immobile posture. The horizontal bar test revealed an intense, long standing cataleptic state. The bracing, clinging and righting tests indicate a state similar with the neuroleptic induced catalepsia (De Rick et al., Brain Res. 201:143, 1980).

Higher amounts of Carbachol induced EEG epileptiform seizures recorded from the injection site. The seizures were associated with strong catalepsia which was the first motor correlate observed. Generalized convulsions started later from a backgroung of catalepsia and were followed by catalepsia.

Intraventricular injections of 6-hydroxydopamine decreased, after two weeks, the noradrenaline content of the pons by 50 percent. No spontaneous catalepsia was seen in these animals but correspondingly smaller amounts of Carbachol were necessary to induce both catalepsia and epileptiform seizures. Atropine injected in the same pontine sites abolished both catalepsia and seizure activity induced by Carbachol.

Our findings show: 1. the pons may be a site for the generation of catalepsia; 2. cholinergic pontine mechanisms may be involved in the generation of catalepsia; 3. the association between catalepsia and epileptiform activity and the parallel facilitation of both phenomena by reduction of the noradrenaline content suggest a relation between the pontine mechanisms of catalepsia and generalized seizures.

137.12 2,5 HEXANEDIONE AFFECTS THE POSITION SENSITIVITY OF DE-EFFERENTED MUSCLE SPINDLES.
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The neurotoxicants acrylamide (ACR) and 2,5 Hexanedione (HD) induce a distal axonopathy (DA) with an accumulation of 10nm neurofilaments distally prior to axonal degeneration. ACR has previously been shown to effect muscle spindle afferents. In this study the effect of HD on the position sensitivity of primary and secondary muscle spindles was studied in cats to determine if a similar functional pathogenesis occurs. The neurotoxicant was administered as a 0.5% solution in distilled water. Cats drank ad libitum, either distilled water or until either 2.5 or 5.0 ml/kg HD was consumed. On the following day the cat was anesthetized and a dorsal laminectomy was performed. The soleus nerve and muscle were isolated in situ, and a hindlimb de-afferentation was performed. The cat was mounted on a spinal unit and the leg was held rigid. L6-S1 dorsal and ventral roots were sectioned proximally. Single soleus muscle spindle afferents were recorded from the teased L7 dorsal root and isolated via an amplitude discriminator coupled to a frequency analyzer. Afferents were classified as primary or secondary muscle spindle endings based on their conduction velocities (CV) and their characteristic response to stretch. Static discharge frequencies were determined by stretching the soleus muscle from 0 to 14 mm in 2 mm increments. Frequency-response curves (stretch vs. frequency) were generated and comparisons made between control and HD groups.

Animals displayed behavioral signs of DA in a dose-dependent manner. The length-frequency relationships of both the primary and secondary muscle spindle afferents were significantly depressed when compared to control at both doses studied. Mean slopes of the frequency-response curves for the primary muscle spindle afferents were 4.4±0.22, 1.3±0.20, 1.4±0.19 Hz/mm and their mean peak frequency response (at 14 mm) were 71±3.4, 24±3.6, 24±3.6 Hz for control, 2.5 and 5.0 ml/kg HD, respectively. Mean slopes of the frequency-response curves for the secondary muscle spindle afferents were 4.1±0.56, 1.3±0.54, 1.8±0.18 Hz/mm and their mean peak frequency response were 75±8.1, 33±7.9, 30±5.8 Hz for control, 2.5 and 5.0 ml/kg HD, respectively. Mean CV of both the primary, and secondary muscle spindle afferents were depressed in a dose-dependent manner.

In summary, these data show that HD affects the position sensitivity of primary and secondary muscle spindle afferents. There was no difference in the frequency-response relationship between the two doses studied. However, the mean CV exhibited a dose-response relationship. Supported by NS 18664.

137.13 COMPARISON OF BRAIN SIZES OF CATS WITH AN INHERITED SPINOCEREBELLAR DISEASE WITH NONAFFECTED CATS-Gerald A. Hegreberg. College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

An inherited progressive neurodegenerative spinocerebellar disorder which is transmitted as an autosomal recessive trait has been recognized in cats. Intention tremors, the earliest clinical manifestation, are observed in kittens as early as 11 days of age. The tremors are apparent in all affected kittens by the age of 4-5 weeks. The tremors progress in severity with age and are throbbing, rhythmical tremors of high amplitude at the clinical apogee, usually reached by 16 weeks of age. Other neurologic and behavioral changes occur as the disease progresses, including hypermetria, posterior ataxia, posterior paresis, generalized seizures, limb rigidity, moderate to severe visual impairment, unprovoked agitation and rage, dementia, catatonia, stereotypy, and apparent hallucinations.

Brain sizes were examined from 5 adult affected female cats and compared to 23 nonaffected adult female cats. Brains were removed immediately after necropsy and were weighed. Visual examination suggested that the brain of the affected cats were smaller than the brains from nonaffected cats and that all regions of the brain appeared involved to some exte Comparisons of the brain weights were made using the students test for grouped data. The results indicated that the mea brain size of the affected cats was smaller than the mean brain size from the nonaffected cats and that this change is highly significant ($P<0.001$). The mean and 1 standard deviation of the brain sizes from the affected cats and nonaffected cats were 12.6 Gm $\pm$ 0.15 Gm and 23.8 Gm $\pm$ 1.5 Gm respectively.

Based on these studies, we could not ascertain whether this change involves a disturbance in growth of the brain or atrophy after maturation. Although the disease is first associated with young developing animals, morphologic changes observed in mature affected animals indicate that there is severe neuronal degeneration. This feline disorder may provide a means of unraveling mechanisms resulting in neurodegeneration and the relationship of this disorder to human spinocerebellar diseases and epilepsy.

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137.14 EFFECT OF INTERLEUKIN ADMINISTRATION ON GROWTH OF TRANSPLANTED C_6 GLIOMA CELLS

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Studies in a number of laboratories underlie the importance of lymphokines in glial function. The present study was conducted to determine if exogenous interleukin 1 (IL-1) or 2 (IL-2) can alter the growth of transplanted C_6 glioma cells in the rat.

Aggregates of C_6 cells were injected into caudates of Sprague-Dawley rats. Elvax pellets containing IL-1, IL-2 or no additives were inserted into cortical tissue uverlying the caudate at time of C_6 cell injection or 9 days later. Animals were sacrificed 16 days after initial cell injection. Brains were fixed in formalin, embedded in parafin, and sections taken every 250 microns. Tumor cross-sectional area was determined in H and E stained sections and volume of tumor tissue ascertained. Results showed that insert ion of low dose IL-2 pellets (50 units/brain) at time of C_6 injection markedly prevented establishment of tumors when compared to rats in which additive-free pellets were employed (three-fold differences observed between groups). In contrast, IL-1 pellet insertion at time of cell injection resulted in tumors with volumes of more than 2 times that of controls. Implantation of high dose IL-2 pellets (200 units) at 9 days after C_6 cell injection significantly decreased tumor growth by approximately 40%, low dose pellets having no effect.

Subsequent studies were conducted to determine if these effects were due to (a) direct inhibitory action on C_6 cells, or (b) alteration of "host" brain tissue. The former involved growing C_6 cells on 35 mm dishes in the presence or absence of IL-1 or IL-2 (200 U/ml) for 24 hours. No significant effect on proliferation was noted with IL-2, whereas IL-1-treated cultures contained approximately 50% less cells than control cultures.

In vivo experiments were conduted to determine the extent to which lymphokine pellets affect host brain tissue. Saline was injected into caudates of rats and IL-1, IL-2 or additive-free pellets implanted. Five days later, serial sections of brain were obtained and assessed for infiltration by inflammatory cells. Increased infiltration was noted in IL-2-exposed brains, IL-1 rats displaying less of this effect than in additive- free pellet groups.

Results obtained indicate that exogenous IL-2 can inhibit establishment and growth of transplanted C_6 cell tumors, whereas IL-1 appears to enhance tumor growth. These studies (a) offer an approach to deliver exogenous interleukins to tumor tissue and (b) describe an experimental model tha can be used to study the mechanisms underlying interleukin-directed changes in tumor growth.

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137.15 CHARACTERIZATION OF THE DIVERSITY OF MURINE BRAIN REACTIVE AUTOANTIBODIES.

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Autoantibodies to brain antigens have been demonstrated in patients with systemic lupus erythematosus (SLE). In addition, sera from murine models of autoimmunity have also been shown to contain such antibodies. If these autoantibodies play a role in the neuropsychiatric manifestations associated with SLE, then it is important to properly characterize them and determine their diversity in order to better understand their relationship to disease activity. Toward these ends, we have studied the antigen binding specificities and isotypes of anti-brain antibodies, reactive with cell surface antigens, from one non-autoimmune and three autoimmune strains of mice.

Integral brain membrane proteins of a non-disease manifesting autoimmune strain of mouse (viz., MRL/mp) was isolated by detergent extraction and phase separation. These proteins were separated on polyacrylamide gels under non-reducing conditions, transferred onto nitrocellulose membranes and incubated with sera from Balb/c, MRL/l, BXSB and NZB/W mice. Positions of reactive antigens were visualized using an indirect immunoenzymatic technique, with enzyme labeled, heavy chain specific, anti-mouse immunoglobulin antisera. The results of these assays, testing large numbers of sera, indicate the following: (1) Not all individuals of these autoimmune strains produced detectable antibodies against brain membrane antigens. (2) Autoantibodies of both IgG and IgM isotypes were produced in all three strains. (3) Considerable heterogeneity in binding specificities was observed in MRL/l and BXSB mice. Interestingly, a high proportion of NZB/W mice produced IgM antibodies against two high molecular weight proteins and IgG antibodies to a peptide with an apparent molecular weight around 75KD. Further information on the biochemical properties of these NZB/W autoantibody reactive brain membrane antigens are presented. We will discuss the implications of these findings.

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137.16 **Cerebral Glucose Metabolism in Cortical and Subcortical Regions of Drug-Free Schizophrenic and Normal Brain using PET/FDG**

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Schizophrenia is a chronic psychotic illness whose mechanism and etiology remain unknown. Studies of its pathogenesis have focused on brain dopamine systems because of the potent antipsychotic effect of neuroleptic drugs. Until recently, no consistent abnormality of CNS dopamine systems had been identified in schizophenic patients. But, now, an increase in striatal DA receptors identified by in vivo 11C-NMSP binding and PET, has been reported in schizophrenia by Wong and his colleagues (Science 234:1558, 1986). This finding has refocused interest in a potential pathophysiologic role of DA in schizophrenia. In the studies reported here, that question has been further explored by measuring glucose metabolism in several DA terminal areas of mesencephalic DA-containing neurons (Table 1). Eleven neuroleptic-free psychotic patients with a DSM-III diagnosis of schizophrenia and eleven normal controls received 18F-2-deoxyglucose and were scanned with a NeuroPET scanner (5-7 mm resolution); data in μmoles glucose/100 gm tissue/min were calculated from the Brooks modification of the Sokoloff equation. Subjects were scanned at rest with eyes closed and ears plugged.

Results indicate no metabolic differences in this group of schizophrenics in caudate or various cortical areas containing DA receptors. However, significant decreases in glucose utilization were apparent in the anterior cingulate cortex, and the parahippocampal gyrus. These results are in agreement with postmortem morphometric studies in schiozphrenia. And they serve to focus our attention and studies on the hippocampus and related subcortical structures in schizophrenia.

Table
Glucose Utilization in Human Brain
μmole glucose/100 gm tissue/min)
(mean $\pm$ SD)

Area	Schizophrenic (N=11)	Normal (N=11)	P
Caudate	10.2 $\pm$ 2.3	11.9 $\pm$ 3.6	NS
Ant. Cingulate C.	9.6 $\pm$ 1.9*	12.9 $\pm$ 3.2	.008
Sup. Frontal C.	9.7 $\pm$ 2.0	11.2 $\pm$ 2.8	NS
Hippocampal C	6.9 $\pm$ 1.6*	9.5 $\pm$ 1.7	.001
Sup. Temporal C.	9.5 $\pm$ 2.2	10.3 $\pm$ 2.1	NS

137.17 THROMBOSPONDIN: QUANTITATION AND DISTRIBUTION ON MUSCLE BASEMENT MEMBRANES IN NEUROMUSCULAR DISEASES. J.S. Rao*, D. Hantai*, B.W. Festoff (SPON: L.T.Giron). Dept. of Neurology, UKMC, Kansas City, KS. and Neurobiology Research Laboratory at VAMC, Kansas City, MO. 64128.

We have studied the distribution of thrombospondin (TSP), fibronectin, laminin, heparan sulfate proteoglycan (HSPG) and collagen types I, III and IV by immunofluorescence in frozen sections of muscle from normal, disease control and amyotrophic lateral sclerosis (ALS) patients. In ALS, normal and disease control muscle, types I and III collagen were localized to the endomysium and the perimysium. Type IV collagen and laminin precisely delineated each muscle fiber (basement membrane) but did not stain the perimysium. We found no marked qualitative difference in the distribution of these ECM proteins, or HSPG, in ALS patients compared to controls. However, TSP showed a marked increase in its distribution in ALS patients' muscle. Quantitative analysis by ELISA also indicated that TSP was increased in ALS patient's muscle. Since TSP is known to be released from platelet α-granules in response to thrombin stimulation its elevation implies involvement of the extravascular thrombolytic system in ALS. Other studies have indicated decreased circulating protease inhibitors and increased plasminogen activators in this disorder.

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137.18 MUSCLE PLASMINOGEN ACTIVATORS (PAS) INCREASE ON NERVE CRUSH AND RECEDE ON REINNERVATION. D. Hantai*, J.S. Rao*, C.B. Kahler* and B.W. Festoff (SPON: A. Dick). Dept. of Neurology, UKMC, Kansas City, KS. and Neurobiology Research Laboratory at VAMC, Kansas City, MO 64128.

Our previous studies (J.Cell Biol. 103:1415-1421, 1986) showed 8-10 fold activation of 1st urokinase (uPA) and then tissue (tPA) PAs in muscle after denervation. In addition, specific components of the muscle fiber basement membrane (BM) are susceptible to locally-generated plasmin produced by PA activation of plasminogen (Exp. Neurol. 95:44-55, 1987). These studies suggest neural regulation of extracellular matrix of muscle in attmepted regeneration after injury. The present studies sought to further explore the neural regulation of PA activities in muscle. The nerve crush paradigm was used and both muscle contraction on nerve stimulation and return of choline acetyltransferase (CAT) activity was used to monitor re-innervation. uPA began to rise 24 hours after sciatic nerve crush in sciatic-innervated muscles. This was noted 1st in cytosol prior to a rise in membrane-bound activity. By 7 days, maximal membrane-bound uPA, approximately 7-8 fold above control levels, was detected using the S-2251 assay. tPA followed, both in soluble and membrane fractions, but uPA was last to return to baseline by 40 days. Coincident with return of nerve-evoked muscle contraction and CAT activity at 21 days, both PA activities showed their greatest drop from previous high levels. These results show tight regulation of PA levels in muscle by some neural influence. The mechanism of this influence is currently under study.

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137.19 NEONATAL HERPES SIMPLEX VIRUS INFECTION OF MICE: AVOIDANCE LEARNING AND VIRUS CHARACTERISTICS. L. S. Crnic and L. I. Pizer* (SPON: J. Nolte) Depts. Pediatr., Psychiat., Microbiol., Univ. Colo. Sch. Med., Denver, CO 80262.

We have previously reported that neonatal infection with a mutant herpes simplex type 1 (HSV-1) virus produces transient CNS infection, interferes with granule cell migration in the cerebellum, and produces hyperactivity and deficits in passive avoidance but not in radial maze learning. We now report further behavioral effects and characterize the mutant virus.

Wild-type HSV-1 (14-120) was mutated and virions selected that grew in primate, but not mouse cells in vitro. BALB/c mice were injected subcutaneously in the shoulder on the second day of life with 2.5×10^6 plaque forming units of the mutant virus. The infection was mild, producing no deficits in body weight. Mice selected for high activity (> 95 squares crossed in a 3 min. open field test) were compared to control mice that had been injected with a killed virus preparation. Mice were trained in a shuttle box avoidance task signalled with 5 seconds of light and buzzer preceding 200 μa shock. Intertrial intervals were 30 sec. and 30 trials were run per day. There was no difference between infected and control groups (mean trials to criterion 18 ± 5 for infected mice, 20 ± 7 for control mice). While we have previously shown neonatally infected mice to be poor at passive avoidance learning, the present results show that they learn active avoidance normally, and thus likely have a deficit in inhibition of behavior rather than any difficulty with shock avoidance.

Several studies were undertaken to characterize the differences between wild-type virus, which kills neonates, and mutant virus, which produces mild infections. DNA was extracted from both wild-type and mutant virus and another strain of HSV-1, KOS. Viral DNA was cut with restriction enzymes (Bgl I, Eco R1, Hind III, Sal I) and run on a polyacrylamide gel. There were no differences between wild-type and mutant on major bands and few minor band differences. In contrast, both wild-type and mutant differed from KOS in several major bands. Thus, the nature of the mutations must not involve major rearrangement of or deletions from the genome.

The ability of the virus to make thymidine kinase (TK) determines neurovirulence in HSV-1. Virus infected cells were exposed to ^{14}C labeled thymidine, washed and then exposed to film. Wild-type but not mutant virus demonstrated TK activity. 3 of 10 lines retrieved after passage of mutant virus through mouse brain showed normal TK activity. Thus, the virus can revert to the wild-type TK phenotype in vivo. We postulate that a minor mutation in the TK gene is responsible for the reduced virulence of the mutant. Supported by NIH HD18378.

137.20 EXPERIMENTAL HERPES SIMPLEX (TYPE 1) ENCEPHALITIS IN RAT: DISTRIBUTION OF INFECTED NEURONS DEMONSTRATED BY IN SITU HYBRIDIZATION. J.R. O'Kusky, D. Walker*, J.B. Hudson* and P.L. McGeer. Division of Medical Microbiology, Department of Pathology and the Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Herpes simplex virus (HSV) is a major cause of human viral encephalitis. Autopsy findings frequently indicate a selective involvement of temporal and orbital neocortex, hippocampus and amygdala. This anatomical specificity has led to speculation that HSV may reach the central nervous system via the olfactory pathway and its connections with the limbic system. The distribution of infected neurons in the brains of adult rats following intraventricular inoculation of HSV (type 1) was examined using in situ hybridization with cloned HSV-1 DNA probes.

Herpes simplex type 1 virus (strain F) was suspended in Eagle's minimal essential medium and inoculated into anesthetized Sprague-Dawley rats. Stereotaxic injections (5 μl of virus suspension containing 50, 500 or 2500 pfu) were made into the dorsal aspect of the left lateral ventricle at the level of the decussation of the anterior commissure. Control animals received similar injections of Eagle's medium. Infected animals were moribund by 7-9 days after inoculation, at which time they were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were embedded in paraffin and serial sections were cut in the frontal plane at 7 μm. These sections were processed for in situ hybridization using a modification of the procedure of Brigati et al. (Virology, 126: 32, 1983). One cloned HSV-1 fragment from HSV-1 strain F (Eco R1 fragment G) was used as probe. The cloned HSV-1 DNA was labeled by random priming with ^{35}S dCTP to a specific activity of approximately 10^9 cpm/μg. Slides were coated in liquid film emulsion and exposed for 1-2 weeks.

Infected neurons were found to be concentrated bilaterally throughout the olfactory tubercle and the adjacent pyriform cortex. Smaller patches of infected neurons were detected in the caudate-putamen and basal forebrain. In the neocortex scattered neurons were seen most frequently in the cerebral hemisphere ipsilateral to the site of injection. The preferential distribution of infected neurons in the olfactory tubercle and pyriform cortex appears to be independent of an olfactory route of entry. Other factors which determine the neurotropism and localization of HSV infection within the brain are likely to include the distribution of HSV receptors. (Supported by the Medical Research Council of Canada)

138.1 A COMPARISON OF TWO N-METHYL-D-ASPARTATE ANTAGONISTS, CPP AND CGS 19755, IN THE GERBIL FOREBRAIN ISCHEMIA MODEL. C.A. Boast, S.C. Gerhardt*, G. Pastor*, J.C. Lehmann, P.E. Etienne and J.M. Liebman. Research Dept, Pharma Division, CIBA-GEIGY Corp., Summit, NJ 07901.

Previous reports have indicated that N-methyl-D-aspartate (NMDA) antagonists, administered intracerebrally, can have protective effects against brain damage following ischemia (Simon et al, 1984) or hypoglycemia (Wieloch, 1985). Systemic administration of 2-amino-7-phosphonoheptanoic acid (AP7) (Boast et al, 1987) or ((+)3-(-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) (Gerhardt & Boast, 1986) to gerbils can also limit ischemic brain damage. We now report a detailed comparison of CPP and a novel NMDA antagonist, cis 4-(phosphonomethyl)-2-piperidine-carboxylic acid (CGS 19755) (Lehmann et al, in preparation) in the gerbil ischemia model.

Bilateral carotid occlusion (20 min) of female Mongolian gerbils (TUM:[Mon], Tumblebrook Farms, West Brookfield, MA, 50 - 70 g) was conducted under methoxyflurane anesthesia. The drugs were administered at various doses intraperitoneally (i.p.) four times 2 hr apart, beginning at various times relative to the initiation of ischemia. Motor activity was measured, using Omnitech Digiscan chambers, 1 and 4 days later. Brains were removed, and sections were evaluated for evidence of hippocampal damage using a 5 point rating scale.

Both drugs showed statistically significant effects on ischemia-induced hypermotility and hippocampal damage. When drug administration began 15 min prior to the carotid occlusion, CPP was effective at 30 mg/kg, while CGS 19755 was effective at both 10 and 30 mg/kg. When 30 mg/kg of either drug was administered beginning some time after the initiation of carotid occlusion, CGS 19755 retained protective effects even with a delay of 4 hr, while CPP was not effective when a 1 hr delay was used. After a 24 hr delay between ischemia and drug treatment, neither drug showed positive effects.

These data support the growing body of evidence that NMDA antagonists may be useful in treating human brain ischemia which results from stroke or cardiac arrest. In particular, CGS 19755 appears to be a potent compound with protective effects that persist despite treatment delays of several hours.

138.2 THE NON-SEDATING BENZODIAZEPINE PARTIAL AGONIST CGS 9896 REDUCES ISCHEMIC HIPPOCAMPAL DAMAGE IN GERBILS. S. C. Gerhardt*, G. Pastor* and C. A. Boast. (SPON: R. Katz) Neuroscience Research, Pharma Division, CIBA-GEIGY Corp., Summit, NJ 07901

Degeneration of hippocampal neurons following ischemia in gerbils may be reduced by drug treatments acting through various mechanisms. Several drug classes (barbiturates, excitatory amino acid antagonists, benzodiazepines) that have been effective against ischemic damage also have sedative properties. It is not known whether the sedative properties of any of these compounds are essential for the observed protective effects. Non-sedating anxiolytics, such as CGS 9896 (Bennett and Petrack, 1984), which acts via benzodiazepine modulation, have now been described. We used the gerbil ischemia model to assess the effects of CGS 9896 and the sedating benzodiazepine, diazepam.

Female Mongolian gerbils (TUM:[Mon], Tumblebrook Farms, West Brookfield, MA) weighing 50-70 g were anesthetized with methoxyflurane and subjected to 20 min of bilateral carotid occlusion. Drug-treated animals received two injections (30 mg/kg i.p.) 4 hr apart, beginning either 15 min prior to carotid occlusion or immediately after occlusion. Ischemic controls received no injections.

Motor activity was recorded for 30 min in Omnitech Digiscan (Columbus, Ohio) chambers on the first and fourth day after the occlusion. Following the final motor activity assessment, brains were removed, sectioned, stained and evaluated for hippocampal damage using a 5-point rating scale.

Ischemia-induced motor activation, which has been shown to be correlated with the degree of hippocampal neuronal damage (Gerhardt and Boast, 1987), was reduced by pretreatment with CGS 9896 or diazepam. Post-treatment with diazepam was also effective. Hippocampal damage was decreased by both drugs, whether given before or after occlusion.

These results might be explained by a number of possible mechanisms, some of which may be related to the anticonvulsant properties of these drugs. Direct mechanisms include inhibition of abnormally high firing rates of hippocampal neurons, GABA facilitation, or alterations in postsynaptic potentials and resultant changes in Cl^- and Ca^{++} ion flux. It is also possible that there is an indirect mechanism by which the drugs would have effects on neurons other than those that are destroyed after ischemic insult. Regardless of the mechanism of action, it is now clear that sedation per se is not required in order for drugs acting via the benzodiazepine receptor complex to protect hippocampal neurons from ischemic damage.

138.3 GERBIL FOREBRAIN ISCHEMIA INCREASES ACETYLCHOLINE TURNOVER IN THE HIPPOCAMPUS BUT NOT IN STRIATUM. L.L. Peoples*, C. Cosi*, S.C. Gerhardt*, P.L. Wood and C.A. Boast. (SPON: R. Infurna) Psychol. Dept., Rutgers Univ., New Brunswick, NJ 08903 and Neuroscience/ Cardiovascular Research, Pharma Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Reversible forebrain ischemia has been shown to produce selective hippocampal damage in rodents. Cognitive impairments associated with hippocampal damage have also been demonstrated in ischemic animals. Alterations in acetylcholine (ACh) function have been implicated in a variety of cognitive impairments in animals and humans. This study investigated the changes in ACh turnover (Tr) in hippocampus and striatum of gerbils which had been subjected to forebrain ischemia.

Under methoxyflurane anesthesia jugular catheters and loose carotid ligatures (Chandler et al, 1985) were externalized in female Mongolian gerbils (TUM: [Mon], Tumblebrook Farms, West Brookfield, MA). The gerbils were allowed to recover for 24 to 48 hr and then the carotid ligatures were tightened in an experimental group, thus occluding blood flow for 5 min. Twenty four hr later motor activity was measured in both experimental and control animals using Omnitech Digiscan apparatus (Columbus, OH). Increased motor activity at that time has been shown to be correlated with the degree of resulting hippocampal damage (Gerhardt & Boast, 1987). Five or six days after carotid occlusion phosphoryl-D9-choline dissolved in saline was infused via the jugular catheter at a rate of 15 umol/kg/min in a volume of 0.1 cc/min for 9 min. Gerbils were killed by focused microwave irradiation, brains were removed and bilateral samples of dissected hippocampus and striatum were frozen separately. The tissue samples were later prepared for gas chromatography - mass spectrometry (GC-MS) analysis of choline and ACh content (Hanin, 1974). The percentage of deuterium incorporated into regional brain choline or acetylcholine was monitored by GC-MS using the positive ionization mode (Wood & Peloquin, 1981). ACh Tr rates were calculated using the single infusion time method (Racagni et al, 1974).

Initial studies on non-ischemic gerbils were conducted to determine the necessary rate constants related to the time course of choline uptake and incorporation into ACh. These rate constants were found to be similar to those previously determined for the rat (Racagni et al, 1974). In ischemic gerbils ACh turnover in the hippocampus was significantly increased (average > 100%), but ACh turnover was not significantly affected in the striatum (average < 25%).

These data indicate that forebrain ischemia in the gerbil can result in selective increases in ACh Tr in the hippocampus. Since selective neuronal damage is also seen in this brain region, it is reasonable to postulate that the neurons that are damaged by ischemia usually participate in a negative feedback loop onto the cells of origin for ACh. Removal of this feedback could then result in increased ACh Tr. The possible implications for cognitive function are not clear at this time.

138.4 A NOVEL N-METHYL-D-ASPARTATE ANTAGONIST, CGS 19755, PREVENTS ISCHEMIA-INDUCED REDUCTIONS IN ADENOSINE A-1 RECEPTORS IN GERBIL BRAIN: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. Michael F. Jarvis, Deborah E. Murphy, Michael Williams, Susan C. Gerhardt and Carl A. Boast. Drug Discovery Division, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

The CA-1 region of the hippocampus contains the greatest density of adenosine A-1 receptors in the mammalian brain and transient brain ischemia results in the destruction of the pyramidal cell layer within this region. Brain damage following an ischemic insult appears to result from the release of toxic quantities of endogenous excitatory amino acids (EAA; Hagberg et al.,1985). This mechanism is further supported by demonstrations that EAA receptor antagonists can block ischemic brain damage (Simon et al., 1984; Boast et al., 1987). In the present study, quantitative autoradiography of adenosine A-1 receptors was used to evaluate the protective effects of a new N-methyl-D-aspartate (NMDA) antagonist, CGS 19755 (Lehmann et al., this meeting). Under methoxyflurane anesthesia, female Mongolian gerbils (TUM:[MON]) had both common carotid arteries occluded for 20 min with microaneurysm clips. Drug-treated gerbils received four injections of CGS 19755 (30 mg/kg i.p.) 1, 3, 5 and 7 hours after the ischemic episode. Ischemic and normal controls received no injections. Four days following the ischemic episode, animals were sacrificed and [^{3}H]CHA (Cyclohexyladenosine) used to label adenosine A-1 receptors in 20 µ coronal brain sections. Tissue sections were incubated in Tris-HCl buffer (pH, 7.4) for 2 h using 4 nM [^{3}H]CHA. Non-specific binding was measured in the presence of 20 µM-2-CADO. Following washing and drying, sections were opposed to tritium-sensitive film for a month. Resulting autoradiograms were analysed using video densitometry.

[^{3}H]CHA binding sites were heterogenously distributed in gerbil brain with the greatest density of A-1 receptors in the stratum radiatum of the hippocampus followed by stratum oriens > cerebral cortex > thalamus. The 20 min ischemic episode caused a 35% reduction in [^{3}H]CHA binding in hippocampus and cortex but not thalamus. Post ischemic treatment with CGS 19755 resulted in a significant protection of the ischemia-induced reduction in A-1 receptor binding in both hippocampus and cortex. These results indicate that A-1 receptors in the CA-1 region of the hippocampus may be associated with pyramidal cells in agreement with other reports (Lee and Kreutzberg, 1987). Additionally, the selective NMDA-antagonist, CGS 19755, is effective post episode in reducing ischemia-induced alterations in adenosine A-1 receptors in the hippocampus and cortex.

138.5 FURTHER PROBING THE BRAIN LIPID CHANGES IN POST-DECAPITATIVE ISCHEMIA. R. Chandrasekhar* and G.Y. Sun (SPON: D. O'Brien). Biochemistry Dept. and Sinclair Comparative Medicine Research Farm, University of Missouri, Columbia, MO 65203.

Previous studies in our laboratory with rats injected ^{32}Pi (i.p.), ^{32}P-ATP and ^{3}H-inositol (intracerebrally) have indicated rapid decline of poly-phosphoinositides (Poly-PI) with time of post-decapitative ischemia. Concomitantly, there is an increase in the levels of diacylglycerols (DG) and free fatty acids (FFA). With poly-PI labeled with ^{3}H-inositol, the decrease in poly-PI is also marked by an increased turnover of inositol-phosphates which can be followed with time. Although the increase in DG can be largely accounted for by the decrease in poly-PI, the 3-5 fold increase in FFA remains unexplained. The present studies are directed towards probing the effects of post-decapitative ischemia on brain phospholipids that are prelabeled with ^{14}C-acetate. Three to four week-old Sprague Dawley rats were injected intracerebrally with 25 uCi of 1-^{14}C-acetic acid two hours prior to decapitative ischemic treatment (5 min). Under this condition, labeling of brain phospholipids due to acetate was attributed mainly to the de novo synthesis of fatty acids and subsequent phospholipid biosynthesis. Approximately 45% of the label was found in phosphatidylcholines although all other phospholipids, neutral glycerides, and cholesterol were also labeled. Ischemic treatment (5 min) resulted in no observable decrease in the major phospholipids except for a decrease in labeled phosphatidic acid and an increase in labeled DG. There was no obvious increase in the labeled FFA. These results suggest that the phospholipid pool represented by de novo biosynthesis did not participate in the FFA release due to post-decapitative ischemic treatment. Studies using another lipid precursor (e.g. ^{14}C-arachidonic acid) are presently in progress to further elucidate the mechanism of FFA release due to the ischemic insult. (Supported in part by AA 06661 from NIAAA).

138.6 ELECTROPHYSIOLOGICAL CORRELATES OF ISCHEMICALLY INDUCED NEURONAL DAMAGE IN THE RAT HIPPOCAMPAL FORMATION. J.J. Miller, J.M. Klancnik and L.A. Mudrick. Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The mechanisms involved in the selective neuronal degeneration of hippocampal CA1 neurons, following cerebral ischemia, are poorly understood. It has been hypothesized that changes in calcium gradients may initiate a cascade of cellular events that cause delayed cell death. Utilizing an in vivo preparation we have followed the effects of ischemia on evoked population responses in the CA1 and dentate gyrus (DG), in order to to elucidate the events occurring during and after ischemia.

Male Wistar rats (350-500g) were anesthetized with urethane, the femoral artery cannulated and baseline mean arterial pressure (MAP) was recorded. Electrodes were implanted in the perforant path (PP) and ipsilateral DG to stimulate and record population responses in the DG, and in area CA3 and contralateral area CA1 to stimulate and record responses in the CA1. Paired pulse stimuli were applied from both PP and CA3 electrodes with 20 msec intervals between the first and second pulse of each pair. Responses were evoked and recorded before, during and for 24 hrs after an ischemic episode induced by haemorrhaging the animal until MAP reached 30 mmHg followed by bilateral carotid occlusion for 20 mins. Following release of the carotids, blood from the reservoir was reinfused. This model of ischemia produces consistent, highly selective damage to 93% of area CA1.

During ischemia all responses in both DG and CA1 rapidly disappeared. Recovery of population responses in the DG began within 1/2 hr following reinfusion. DG population spike amplitude rapidly increased to 200-300% of baseline value, without a corresponding increase in population EPSP slope, and maintained this increased amplitude for the duration of the experiment. The paired pulse responses indicated a loss of recurrent inhibition both at the onset of ischemia and during the early phase of post-ischemia recovery. CA1 population responses did not usually start to recover until about four hours after reinfusion. These responses recovered at most to baseline values, then attenuated and usually disappeared within 24 hrs. No changes in recurrent inhibition were found in the CA1. In addition, after ischemia, population spike latencies in both regions increased by up to 4 msec for the entire duration of the experiment.

These data indicate that functional damage to the CA1 pyramidal cells occurs within 24 hrs of ischemic insult. In contrast, the dentate granule cells demonstrate greatly potentiated responses. It is possible that this potentiation may be contributing to the pathophysiological processes occurring in CA1. (Supported by a Canadian MRC Program Grant to J.J.M.)

138.7 DETECTION OF 60k, A PUTATIVE PROTEIN KINASE C SUBSTRATE, IN THE CSF OF PATIENTS WITH PARANEOPLASTIC CEREBELLAR DEGENERATION. S. Gandy, J. A. Grebb, N. Rosen, K. Albert*, N. Anderson*, J. Cedarbaum, G. Sedvall*, J. B. Posner and P. Greengard. The Karolinska Institute, Stockholm, Sweden and Memorial Sloan-Kettering Cancer Center, The New York Hospital-Cornell Medical Center and The Rockefeller University, New York, NY 10021.

A variety of protein kinase substrate proteins, possibly neuron-specific, have been detected in human cerebrospinal fluid (CSF) by radioactive phosphorylation using 32P-ATP and protein kinases, followed by imaging with PAGE/autoradiography (Gandy et al, Neurology 37(Suppl 1): 193, 1987). Among the proteins which contribute to the CSF, those which participate in phosphorylation systems are likely to be neuron-specific because of the relative abundance of protein kinases and their substrate proteins within neurons. A systematic survey of phosphoprotein patterns in CSF from normal individuals and from patients with various neurologic and psychiatric diseases is in progress. One such illness, termed paraneoplastic cerebellar degeneration (PCD), is associated with rapid selective necrosis of Purkinje cells in the setting of active systemic cancer. Circulating anti-Purkinje-cell-antibodies (APCAs) may also be present. Using the CSF phosphoprotein pattern assay, we have detected the presence of a putative protein kinase C substrate of apparent Mr 60kd in 6 of 12 patients with PCD. This 60kd phosphorylatable protein was not seen in CSF from over 150 other patients, including normals and non-PCD ataxic disease controls such as olivopontocerebellar atrophy. Stratification by tumor type and presence of serum APCA disclosed that 60k was detectable in the CSF of 2/5 lung cancer patients (all APCA negative), 2/2 Hodgkin's disease patients (both APCA negative), 1/2 ovarian cancer patients (both APCA positive) and 1/2 patients with adenocarcinoma, primary unknown (both APCA positive). Neither serum APCA nor CSF 60k was present in a patient with breast cancer and PCD. The detection of this presumed Purkinje-cell-specific 60kd protein in PCD but not in other ataxias may be due to the more rapid course of PCD, leading to the relatively rapid accumulation of previously intracellular proteins in the CSF. The identification of neuron-specific phosphoproteins in human CSF may advance our understanding of the biochemical composition of at-risk neurons in various clinical situations. Further, disease-associated CSF phosphoprotein patterns may aid in antemortem diagnosis, differential diagnosis, nosology of neurologic and psychiatric diseases, and objective assessments of disease "activity" and response to therapy. [Supported by the Dystonia Medical Research Foundation, Huntington's Disease Foundation and The New York Academy of Medicine]

138.8 INCREASED 3-HYDROXYANTHRANILIC ACID OXIDASE AND DECREASED PICOLINIC CARBOXYLASE ACTIVITIES IN C6-GLIOBLASTOMA CELLS, FOLLOWING SERUM DEPRIVATION OR HERPES VIRUS INFECTION. E.G. Shaskan. Dept. of Medicine, Univ. of Connecticut Health Ctr., VA Medical Ctr., Newington, CT 06111.

Intermediary metabolism of tryptophan (kynurenine pathway) has long been associated with infectious diseases in man and recently has been linked to pathogenesis of human neurodegenerative disorders. The branch-point around 3-hydroxyanthranilic acid (3-HA) is theoretically important, since the products formed are potential intracellular chelators of Fe^{3+} (picolinic acid = PA) and Fe^{2+} (quinolinic acid = QUIN) and, thus, may play roles in iron-withholding as host defenses against intracellular microbes. Additionally, QUIN may be an endogenous neurotoxin (excitotoxin).

3-Hydroxyanthranilic acid oxidase (HAO) catalyzes the conversion of 3-HA to α-amino-β-carboxymuconic semialdehyde which then can be a substrate for picolinic carboxylase (PC) or spontaneously rearrange to form QUIN. Since HAO has been localized to astroglial cells in situ by immunohistochemistry, its activity was assessed in rat C6-glioblastoma cells which, in my laboratory, are also studied for their unique ability to naturally establish persistent infections by herpes simplex virus type 1 (HSV1). Although no measurable HAO activity could be demonstrated in C6 cells under standard serum conditions at cellular confluence, following serum deprivation for 1 hour more than three-times boiled-blank values of ^{14}C-QUIN were isolated from supernatants fractionated by standardized Dowex 50W cation exchange columns (method of Foster, et al., J. Neurochem. 47, 23, 1986), corresponding to values of approximately 100 pmol QUIN formed per mg protein per hour. Measured as $^{14}CO_2$ evolved from the same high speed supernatants, PC activity fell from stable baseline values of approximately 35 pmol per mg protein per hour under standard serum conditions to unmeasurable levels following 1 hour serum deprivation. This and related inverse relationships between HAO and PC activity, in direct temporal response to serum alterations, was also observed in VERO cells (transformed monkey kidney) and in primary cultures of human fibroblasts (HFFs). Independent of serum conditions, acute HSV1 infection increases HAO activity and decrease PC activity by an early mechanism associated with internalization of the virus, as determined from temperature studies. Absolute magnitudes for these host-cell enzyme responses to HSV1 infection seem to be more accentuated in permissive cells (VEROs and HFFs) than in restrictive cells (C6), although it remains speculative that modulation of this host cell response bears causal relationship to establishment of persistent HSV1 infection of C6 cells. Although precise mechanisms for alternate activation and inhibition of these branch-point enzymes requires further investigation, preliminary evidence suggests a role for ferrous iron. (Supported by The U.S. Veterans Administration and by the Univ. of Connecticut Research Foundation.)

138.9 SCHWANN CELL REMYELINATION RESTORES SECURE CONDUCTION TO CENTRAL DEMYELINATED NERVE FIBERS. P.A. Felts and K.J. Smith (Spon. G.E. Goode), Department of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501 USA

Demyelinated central nerve fibers can be remyelinated by Schwann cells, but the conduction properties of the remyelinated fibers are not known. These properties are of interest since it is now possible to remyelinate persistently demyelinated central axons by the artificial introduction of Schwann cells into the lesion (Blakemore, Neuropath. Appl. Neurobiol. 8:365-375, 1982), and this finding has been advanced as of potential importance with respect to diseases such as multiple sclerosis.

Five rats were implanted with pairs of stimulating electrodes positioned extradurally over the dorsal columns at T_{12} and L_1. Recording electrodes were also implanted adjacent to each sciatic nerve at the ischium, so that recordings could be made (from anesthetized and paralyzed animals) every 1-2 weeks, for several months. It was therefore possible to obtain serial records of activity conducted through the lesion site, both before the lesion was induced, and during the periods of demyelination and remyelination.

After a control recording period of four weeks, the rats were again anesthetized, and ethidium bromide (1.0 µl of 0.5 mg/ml; Blakemore and Crang, J. Neurol. Sci., 70:207-223, 1985) was injected via a micropipette into the left dorsal column at T_{13}, to produce a unilateral demyelinating lesion in which all the affected fibers later underwent repair by remyelination. Records taken one hour after injection were normal, but during the period of demyelination (examined between 7-21 days) conduction in the affected fibers was blocked. Conduction was progressively restored during the period of remyelination, such that the compound action potential regained 60% of its normal amplitude by 10 weeks. The conducting fibers initially had prolonged refractory periods of transmission, but this parameter returned to within normal limits by 10 weeks. The number of conducting fibers could initially (between three and six weeks) be increased by reducing the body temperature, but this temperature sensitivity eventually disappeared. All records obtained omitting the lesion from the conduction pathway remained normal throughout the recording period.

Histological examination revealed the presence of a large (up to 80% of the ipsilateral dorsal column) lesion of Schwann cell remyelinated fibers, bordered by a thin band of fibers (2-15 wide) remyelinated by oligodendrocytes. The results show that Schwann cell remyelination of rat dorsal column axons is effective in restoring secure conduction.

138.10 **IMPROVEMENT OF THE CNS PATHOLOGY IN THE TWITCHER MOUSE FOLLOWING BONE MARROW TRANSPLANTATION.** **K.Suzuki, P. Hoogerbrugge*,B. Poorthuis* and K. Suzuki. University of North Carolina at Chapel Hill, N.C.; TNO Radiobiological Institute, Rijswijk, University of Leiden, Leiden, The Netherlands.**

The twitcher is an authentic murine model of globoid cell leukodystrophy (GLD), caused by a genetic deficiency of a lysosomal enzyme, galactosylceramidase. Its neuropathological hallmark is severe demyelination of the CNS white matter associated with degeneration of oligodendrocytes, gliosis and infiltration of macrophages. On the ultrastructural level, typical tubular and/or crystalloid inclusions are found in the cytoplasm of oligodendrocytes as well as in these macrophages. Various degrees of demyelination involve the PNS as well. The twitcher develops neurological symptoms around the day 20 postnatal and usually dies around the day 40. Following isogenic bone marrow transplantation (BMT), specific activity of galactosylceramidase increased and reached to the donner level in the bone marrow and spleen at day 14 after BMT. In the brain the enzyme activity increased slowly but steadily and reached 14-16% of control value at day 80-100 after BMT. Light and electronmicroscopic studies were carried out on these twitcher mice at 44, 102, 106 and 109 days after BMT. At 44 days, CNS pathology does not differ significantly from untreated twitcher mouse. Degeneration of myelin was extensive and macrophages containing typical inclusions of GLD were numerous in particular in the white matter of the cerebellum, brainstem and spinal cord. Vesiculation of myelin lamellae and myelin stripping by macrophages were observed. In addition to the typical macrophages of GLD, however, some lipid-laden macrophages were also found in the white matter. On some occasions macrophages contained both typical GLD inclusions and foamy lipid inclusions. In the CNS of the twitcher more than 100 days after BMT, many lipid-laden macrophages were observed but typical macrophages of GLD were either not observed or extremely rare. Degeneration of myelin lamellae was rare, and instead many thinly myelinated nerve fibers suggestive of remyelination were observed. GLD inclusions were, however, found in the cytoplasm of apparently remyelinating oligodendrocytes. Remyelination was evident in the PNS as well, and remyelinating Schwann cells also contained GLD inclusions. These findings suggest that following BMT, donner macrophages infiltrate into the Twitcher CNS and contributed to an improvement of the CNS pathology by supplying deficient enzyme, galactosylceramidase.

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138.11 **CONTRAST ENHANCEMENT (POINT PROCESS) AND EDGE DETECTION TECHNIQUES IN COMPUTER TOMOGRAPHY AND NUCLEAR MAGNETIC RESONANCE BRAIN IMAGING.** T. Goldberg,* M.F. Casanova,* R. Suddath,* D. Daniel*, D. Weinberger. Clinical Brain Disorders Branch, St. Elizabeth's Hospital (NIMH, Washington, D.C. 20032

Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) have revolutionized the practice of neurology by allowing accurate diagnosis of structural abnormalities in a non-invasive manner. While these techniques produce "scans" that are usually interpreted in a qualitative fashion, they generate quantitative information. Unfortunately, the quantitative interpretation of resultant images is limited by artifacts, partial volume effects, the physical properties of the structure visualized and physiological biases in image perception by the human eye. The use of a computer image analysis system (LOATS) makes interpretation easier by enhancing both contrast and edges. Images are digitized from X-ray films placed over a light box. Resolution is maximized by lowering the camera until the image of interest covers the whole field of view. Software routines allow calibration of background elimination. Contrast is enhanced by limiting the gray scale used to the transmission values of interest and selectively stretching its dark, midportion or bright regions. In those cases where the gray and white matter of the brain are studied, transmission values of cerebrospinal fluid and bone are noise clipped. Since the human eye can distinguish different colors better than shades of gray, optical density values are assigned to a fixed color scale. We have found gray scale reversal to be of use for cases where saturation is present at the dark end of the spectrum. Cinematographic sequencing of gray scale reversal, different coloration schemes and either dark or bright region stretching provides detailed information about image contours. Edge enhancement is implemented by Laplace transformation or Nomarski pseudo-optics. Both methods create high spatial frequencies of points which are emphasized by the human eye and give an idea as to the roughness of a section. Smoothing provides a linear weighting of adjacent pixels that deteriorates contrast and edges but is useful for correcting film artifacts. Enhancing contrast and edges of CT scan and NMR films maximizes their signal to noise ratio and emphasizes features which would otherwise be lost by cursory visual examination.

138.12 FURTHER STUDIES OF THE EXCITABLE PROPERTIES OF TE-671 CELLS. E. K. Stauffer and R. J. Ziegler. Depts. Physiol. & Microbiol. Univ. Minnesota-Duluth, Sch. of Medicine, Duluth, MN 55812.

We have reported (Ziegler & Stauffer, 1986. Soc. Neurosci. Absts. 12:87) that the presence of mumps virus changes the distribution of stimulus-evoked action potentials (SEAPs) of persistently-infected (PI) TE-671 cells from one composed primarily of normal, rapidly-rising, all-or-none SEAPS to one dominated by abnormal, slowly-rising, graded responses or by no responses at all. Short-term (48 hrs) treatment with rabbit anti-mumps antibody (RAA) did not reverse the effect.

The present data are from control studies which were designed to more fully describe the electrical characteristics of the TE-671 cell line, and from test studies designed to extend our previous observations by examining for certain membrane-associated phenomena which might be associated with the viral effect. Long-term (240 hrs) exposure to RAA was also investigated.

SEAPs were recorded intracellularly (single electrode current- and/or voltage-clamp) from cell cultures (mock-infected [MI] and PI) in response to anode-break stimulation. Standard ionic channel blockers (tetrodotoxin [TTX], tetraethylammonium ion [TEA], and cobalt ion [Co^{++}]) and/or ion substitutes were used to differentiate and identify specific voltage-gated ionic currents. Membrane electrical properties (resistance [R], time constant [τ], and capacitance [C]) were measured and/or calculated.

There were no significant differences of R, τ, or C between MI and PI cells. Long-term RAA treatment did not reverse the distribution of PI SEAPS to that found for the MI cells. Initial control studies from MI cells have shown rapid, inward currents to be associated with the rising depolarization phase of SEAPs, but there were no outward currents during the falling repolarization phase. TEA had no observable effect on the SEAPs. Instead, τ of the falling phase was similar to τ of electrotonic potentials recorded from the same cell. TTX greatly reduced the magnitude and rate of rise of SEAPs; Co^{++} eliminated the residual SEAP. Conductances calculated for SEAP repolarizations were not different from those of the resting state. These data are consistent with other results of virus-induced alterations of voltage-gated Na^+ channels (Fukuda et al. 1983. Brain Res. 262:79-89).

Supported in part by a grant from the Minnesota Medical Foundation.

138.13 ASTROCYTOSIS IN CAPRINE β-MANNOSIDOSIS. K. L. Lovell. Dept. of Pathology, Michigan State University, E. Lansing, MI 48824

Caprine β-mannosidosis, a genetic dysmyelinating disorder, is associated with a deficiency of β-mannosidase activity and tissue accumulation of oligosaccharides. Cytoplasmic vacuolation occurs in many cell types in the viscera, and, to various extents, in neurons and glia in the central nervous system. Clinical symptoms present at birth in goats include inability to stand and intention tremor. Morphological changes previously reported in fetal and postnatal goats with β -mannosidosis include severe myelin paucity in the brain and very mild deficits in the spinal cord. Consistent regional variation in the extent of myelin deficiency is an important feature of the brain pathology. Gliosis in some regions has been reported, but the astrocyte involvement has not been extensively investigated. In the present study, immunocytochemistry to localize glial fibrillary acidic protein (GFAP), a marker for astrocytes, was performed on paraffin-embedded and Epon-embedded sections from various regions in fetal (124/150 days gestation) and postnatal affected and control goats. The density and distribution of GFAP reactivity was analyzed in these sections. In some regions, changes in the distribution of glial processes were determined by electron microscopy and compared to immunocytochemical results.

In cerebellar white matter, both paraffin-embedded and Epon-embedded sections showed considerably more GFAP reactivity in the affected postnatal animals than in the control animals. However, the patterns of GFAP staining in the internal granular layer and the molecular layer were not substantially different between affected and control animals. In the optic nerve, increased numbers of astrocytic processes were present as early as 124 days gestation; previous studies demonstrated a substantial deficit in the number of myelinated axons at this relatively early stage of myelination. Regions of the cerebral white matter in postnatal affected goats showed more numerous astrocytic processes stained for GFAP than in control goats. These results indicate that early, as well as late, astrocytosis occurs as a prominent component of the pathological changes in β-mannosidosis. Further analysis of the development of the astrocyte and oligodendrocyte changes in this disorder will help define the pathogenesis of the dysmyelinogenesis and the gliosis.

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138.14 DETECTION OF SOMATOSTATIN mRNA BY IN SITU HYBRIDIZATION CYTOCHEMISTRY IN NEURONS OF THE CAUDATE NUCLEUS OF THE HUMAN. W.A. Stoutenger[1,2], and M.-F. Chesselet[2]. Depts. of Neurosurgery[1], Pharmacology and Anatomy[2], Med. Col. of Pa., Philadelphia, PA 19129.

Neurons expressing somatostatin-like immunoreactivity have been described in the caudate nucleus of the rat, cat, monkey, and human. These somatostatin-positive neurons have been described morphologically as medium aspiny neurons and also contain NADPH-diaphorase activity.

In this study we sought to determine whether the mRNA for pre-pro-somatostatin (the precursor of somatostatin) could also be detected in post-mortem human striatum. An ^{35}S UTP labelled RNA probe complimentary to the mRNA for pre-pro-somatostatin was transcribed from a cDNA inserted in the SP65 vector (obtained from R. Goodman-Tufts Univ.). Human post-mortem tissue was obtained at autopsy; caudate nucleus was quickly frozen on dry ice, sectioned at 10 um, and kept at -70 C° until the morning of the experiment. Sections were quickly defrosted, fixed in paraformaldehyde, acetylated, dehydrated in graded ethanol, and hybridized for 3 1/2 hours at 50 C°. Following hybridization stringent rinses were applied to remove excess probe and RNAse treatment was performed to hydrolyse probe remaining as single strand non-hybridized RNA. Autoradiography was performed with Kodak NTB3 emulsion[1]. Some sections were stained for NADPH-diaphorase activity prior to in-situ hybridization.

Heavily labelled neurons of medium size were found scattered throughout the caudate and in tissue stained for NADPH-diaphorase activity these neurons were found to also be NADPH-diaphorase positive.

These results show that the mRNA for pre-pro-somatostatin can be detected by in situ hybridization cytochemistry in somatostatinergic neurons of the human caudate nucleus in autopsy material. Because neurons expressing somatostatin immunoreactivity have been shown to be preferentially preserved in the caudate nucleus of patients who died with Huntington's Disease we are now using the same approach to analyze levels of pre-pro-somatostatin mRNA in single cells in post mortem Huntington's Disease brain.

[1] Chesselet and Affolter, Brain Res., 1987, in press.

We thank Dr. K. Weidenheim, Dept. of Pathology, for providing the autopsy material.

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138.15 LYME BORRELIOSIS PATIENTS' SERA INHIBIT SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES. N. T. Carnevale, J. Monckton, J. J. Halperin Dept. of Neurology, SUNY, Stony Brook NY 11794

We have previously reported that many patients with chronic infection with Borrelia Burgdorferi have significant, reversible intellectual impairment, including memory dysfunction and fatigue. Clinical anatomic (CT and MRI scanning) and physiologic (EEG and evoked potentials) tests have failed to demonstrate a pathophysiologic basis for this disorder. Since there is physiologic and immunocytochemical evidence that serum factors may impair the functioning of CNS neurons in other diseases (eg multiple sclerosis, Sydenham's chorea, lupus erythematosis) we studied the effects of sera from patients with Lyme borreliosis (L.b.) on synpatic transmission in hippocampal slices in vitro.

Sera were obtained from normal volunteers, from L.b. patients with CNS symptoms, and from L.b. patients without CNS dysfunction. Samples were dialyzed (mw cutoff 3500) against hippocampal Ringer solution, and then diluted 1:4 in Ringer's. Rat hippocampal slices were prepared and maintained using standard techniques. Extracellular recordings were obtained from the CA1 region in response to stimulation of the Schaffer collaterals. Measurements were performed before and during exposure to test solutions, and after return to normal Ringer.

Control sera had no effect, but sera from many patients depressed the amplitude of the field EPSP and population spike (on average by approximately 20%). We conclude that sera from L.b. patients contains factors that affect neuronal function. This effect may be related to the CNS symptoms experienced by these patients. Work is in progress to identify these factors and the cellular mechanism(s) responsible for their effects.

This work was supported, in part, by a Veterans' Administration merit review grant (NTC).

138.16 THE DEVELOPMENT OF FUNCTIONAL ISLET CELL ADENOMAS IN SV40 TRANSGENIC MICE WITH PERIPHERAL NEUROPATHY. K. Dyer* and A. Messing. School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

The expression of oncogenes in transgenic mice has become a useful means for creating animal models of disease processes, including those involving systemic influences on the nervous system. One group of transgenic mice carrying the structural genes for the simian virus 40 (SV40) small and large T antigens, attached in an inverted orientation to a metallothionein-growth hormone fusion gene (gene construction #202), develops hepatocellular tumors, islet cell adenomas of the pancreas, and generalized peripheral neuropathies in which axonal degeneration may be a prominent feature. We are interested in whether the peripheral neuropathy might be secondary to SV40 gene expression and neoplasia in a remote tissue. We have investigated the tissue-specificity and developmental onset of SV40 gene expression, using immunohistochemical methods, and have identified abnormalities in serum glucose and insulin levels which correlate with the onset of SV40 gene expression in the endocrine pancreas.

Mice with the #202 gene construction (described above) invariably develop generalized weakness and die at a mean age of 130 days, when all have multiple hepatocellular carcinomas, pancreatic β-cell tumors and generalized peripheral neuropathy. Immunocytochemical localization of SV40 T antigens, using a modified avidin-biotin peroxidase procedure, showed positive cells only in hepatic and pancreatic tumors, with occasional reactivity in the exocrine pancreas. T antigen expression was not detected in the central or peripheral nervous system of mice with obvious neuropathy. In a developmental study, evaluating mice at two week intervals from 14 days post-conception through 140 days post-natally, large T was first detected in pancreatic islet cells between 70 and 84 days of age. Pancreatic tumors became grossly evident by 98 days. The earliest age that positive immunoreactivity was detected in hepatocytes was in a 98 day-old mouse. Most animals did not begin expressing the SV40 genes in the liver until 112-126 days, and liver tumors were not grossly evident until after 126 days of age. Hypoglycemia and hyperinsulinemia were found in 126 day-old animals.

Expression of SV40 large and small T antigens does not appear to take place in either the central or peripheral nervous system of #202 transgenic mice. However, abnormalities in serum insulin and glucose levels resulting from functional islet cell adenomas of the pancreas and/or liver dysfunction may contribute to the degenerative changes seen in peripheral nerve.

138.17 LESIONS PRODUCED BY INTRACEREBROVENTRICULAR INJECTIONS OF MONOCLONAL ANTI-CONJUGATED DOPAMINE ANTIBODY IN THE MOUSE. C. Messier, N. Mons*, M. Meunier*, M. Geffard and C. Destrade. Labo. de Psychophysiologie, UA CNRS n°339, Institut de Biologie Animale, Avenue des Facultés, 33405 Talence and Labo. de Neuroimmunologie, Institut de Biologie Cellulaire et de Neurochimie du CNRS, 33077 Bordeaux, France.

This study examined the long-term effects of repeated intracerebroventricular injections of a monoclonal anti-conjugated dopamine antibody in the Balb/c mouse. This monoclonal antibody was raised after immunization with dopamine-glutaraldehyde-bovine serum albumin and produced by fused spleen cells with SP20 myeloma cells (Chagnaud et al., 1987). One of the selected hybridomas gave antibodies with a good affinity (IC50= 10^{-9} M) and was used for subsequent injection. Control animals received immunoglobulins from non-immune Balb/c mice. One month before the start of the experiment, 5 to 7 months old mice were implanted with a chronic cannula in the lateral ventricule and given 3 to 4 weeks to recover. The mice received a0.2µl injection (over 10 min) of the appropriate substance. This injection was repeated twice a week for five consecutive weeks. Weights of the animals were recorded before each injection and behavioral observations were made during the five-week period. Four to five months after the last injection, the animals were sacrificed and perfused through the aorta with glutaraldehyde for dopamine immunocytochemistry or classical histology. Some of the brain sections in each animal were processed for serotonin immunocytochemistry. In all the animals that received the monoclonal anti-conjugated dopamine antibody, we observed an enlargement of the ipsilateral ventricule and a moderate to complete destruction of the lateral and basal amygdala nuclei. In some animals, these lesions were observed bilaterally. In other animals, large portions of the ipsilateral cortex were necrotic. However, in most of these animals, the nigro-striatal pathway and the paraventricular dopamine cell groups were spared. A few animals showed a moderate to severe loss of dopamine immunoreactivity in the ventral tegmental area, the substantia nigra pars compacta and the caudate nucleus.The lack of a decrease of serotonin immunoreactivity in these animals suggest that the loss of dopamine immunoreactivity was a consequence of the repeated injections of the dopamine antibody. No lesions were observed in the mice that received the immunoglobulins from non-immune mice. These results show that an immune response directed against dopamine components can produce extensive damage to dopamine neuronal systems when this response takes place inside the blood brain barrier. The results may also provide some insights into the causes of diseases that affect the dopamine systems.

138.18 GENETIC LINKAGE OF VON RECKLINGHAUSEN'S NEUROFIBROMATOSIS TO THE NERVE GROWTH FACTOR RECEPTOR GENE

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Von Recklinghausen's neurofibromatosis(VRNF) is one of the most frequent and important genetic disorders affecting the human nervous system, with an incidence of 1 in 3000. VRNF has numerous clinical manifestations including mental retardation, learning disabilities, macrocephaly, bone abnormalities,etc., as well as the formation of multiple tumors affecting various organ systems. While different cell types can be affected,the most common abnormalities are in cells of neural crest origin. The primary defect underlying VRNF is not yet known, but may relate to fundamental mechanisms controlling growth and differentiation during development of the nervous system.

We have used a molecular genetic approach with polymorphic DNA markers for genetic linkage analysis in families displaying VRNF. We find that the VRNF gene is genetically linked to the locus encoding the receptor for the nerve growth factor, on the long arm of chromosome 17 in the region 17q12-17q22. The detection of recombination events separating the two loci suggests however that the nerve growth factor receptor gene is not the site of the primary defect in this disorder,but represents a genetic marker linked to the VRNF gene. The generation of additional DNA markers for this region on chromosome 17 containing the VRNF gene should allow prenatal and presymptomatic diagnosis, and will eventually lead to cloning and characterization of the defect, based on its chromosomal localization. This might open the doors for the development of an effective therapy in this serious neurological disorder, and could provide new insights into fundamental control mechanisms of proliferation and differentiation of the nervous system.

ALCOHOL AND BARBITURATES II

139.1 SEROTONIN NEURONAL SYSTEM INVOLVEMENT IN THE DIFFERENTIAL SENSITIVITY OF LONG-SLEEP (LS) AND SHORT-SLEEP (SS) MICE TO ETHANOL-INDUCED HYPOTHERMIA AND SLEEP TIME. T.A. French* and N. Weiner. Dept. of Pharmacology, University of Colo. Sch. of Med., Denver, CO 80262.

LS and SS mice selectively bred for differences in CNS sensitivity to ethanol (ETOH) exhibit markedly different sleep times following ETOH administration (4.2 g/kg, i.p.: LS-230 min, SS-0-5 min). We have previously determined that the greater sensitivity of LS mice to the hypothermic effects of ETOH may be partially explained by ETOH's greater depressant effect on serotonin (5HT) neurons in the hypothalamus (HYP) and dorsal raphe (DR), as indicated by a greater decrease in the in vivo tryptophan hydroxylase (TPH) activity in these brain regions of LS mice. The present investigation further examines the nature of the role of the 5HT neuronal system in the ETOH sensitivity differences between LS and SS mice.

ETOH administration (LS-3.0 g/kg, SS-5.2g/kg) 60 or 120 min prior to sacrifice was found to have no effect on in vitro TPH activity from the HYP or DR in either LS or SS mice. To examine the possibility that ETOH may decrease TPH activity in vivo by affecting the availability of the substrate tryptophan (TRP), TRP (100 mg/kg, ip.) was administered prior to ETOH. This dose of TRP in control mice will result in increases in 5HT neuronal function as indicated by 2-3 fold increases in in vivo TPH activity and 5-HIAA levels and 25-40% increases in 5HT levels with a maximum effect at about 50 min. When TRP is administered 30 min prior to ETOH (3.0, 3.5 or 4.2 g/kg) to LS mice the sleep times are reduced by 25-41% and waking blood ethanol levels are 30-35% higher than control LS mice. TRP pretreatment in LS mice not only prevents the 25% decrease in in vivo TPH activity at 20 min seen with ETOH alone, but results in a 2-fold increase in TPH activity in the HYP and DR relative to untreated mice. The ethanol-induced (E-I) hypothermia in TRP-treated LS mice is attenuated by 0.8-1.3°C between 15-180 min after ETOH. TRP pretreatment in SS mice results in a 3-fold increase in in vivo TPH activity at 20 min relative to ETOH alone. However, this increase in TPH activity has no apparent effect on either E-I sleep time or hypothermia in SS mice.

The present results demonstrate that the enhanced sensitivity of LS mice with respect to E-I sleep time and hypothermia can be attenuated by raising the level of activity in the 5HT neurons prior to ETOH treatment. This is consistent with our earlier observation that the increased sensitivity to ethanol inhibition of LS 5HT neurons in the HYP and DR may contribute to the greater E-I hypothermia and now suggests that these 5HT neurons may also contribute to the greater sleep time of LS mice.
Supported by USPHS grant AA03527.

139.2 DEPLETION OF BRAINSTEM 5-HYDROXYTRYPTAMINE (5HT) SUPPRESSES THE EXCITATORY EFFECT OF SYSTEMIC ETHANOL ON INFERIOR OLIVARY NEURONS (ION). S.G. Madamba*, G.R. Siggins, E.L. Battenberg and F.E. Bloom. Division of Preclinical Neuroscience, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Previous studies in our laboratory show that systemic ethanol increases the discharge frequency of IONs in rats immobilized with halothane (1). These excitatory ethanol effects might be mediated by 5HT or a 5HT metabolite, because: 1) there is an abundant 5HT innervation of IONs; 2) 5HT can condense with acetaldehyde to form beta carbolines; 3) the beta carboline harmaline increases ION firing; 4) evidence exists for interactions between 5HT and ethanol (refs in 1). Therefore, we pretreated rats i.c.v. or intracisternally with 5,7 dihydroxytryptamine (5,7DHT; 75-150 µg in 20-50 µl) or with vehicle, after desipramine (25 mg/kg) to reduce depletion of catecholamines. After 7-10 days, animals were prepared for recording of IONs under 1% halothane anesthesia (1). Neuronal discharge rates were quantified every 10-15 min by computer before and 30-90 min after i.p. injection of ethanol 2 gm/kg. Neuronal locations were then marked with pontamine sky blue and the animals perfused with 4% paraformaldehyde. Brains were removed and prepared for routine immunohistochemistry. Serotonin immunoreactive structures (5HT-irs) were surveyed by light microscopy to classify the extent of terminal and perikaryal depletion and disruption by 5,7DHT. In separate unrecorded animals the 5,7DHT treatment was surveyed by electron microscopy to assess the probable functional status of the residual 5HT-irs. Light microscopy showed that the normal 5-HT innervation pattern of ION was greatly disrupted by 5,7DHT, with the normal moderate-to-rich innervation by fine varicose fibers severely diminished; remaining 5HT-irs were dilated and terminated bluntly. Ultrastructurally, at least 1/2 of control 5HT-irs are dendritic extensions of neurons located around or within the Olive. 5-HT reactive terminals make symmetrical synapses on the dendrites of unreactive IONs. In 5,7DHT treated animals, the remaining 5HT-irs were mainly vesicle-engorged dilated dendrites and axons. No convincing 5-HT reactive presynaptic structures were identified in samples from 5 treated rats. Firing rates in 6 neurons from 5,7DHT treated animals averaged a 12% increase after ethanol injection, compared to a 50% increase in artificial CSF-treated controls and a 68% increase in untreated controls (1). Only one neuron from 5,7DHT treated animals showed a marked increase in rate (to 170%) with ethanol, whereas one showed a marked rate reduction (to 59%). Our results suggest that ethanol-induced increases in olivary discharge rate previously reported (1) may involve intermediation by 5HT or a 5HT metabolite. Supported by NIAAA (AA-06420).

1. Rogers et al, Brain Res. 385: 253, 1986.

139.3 CYCLIC-AMP BLOCKS ETHANOL SUPPRESSANT EFFECT ON HIPPOCAMPAL NEURONAL FIRING. D.M. Benson, R.D. Blitzer and E.M. Landau. Depts. of Psychiatry, Mt. Sinai School of Medicine and Bronx VA Medical Center, New York, N.Y. 10029.

Ethanol causes a number of CNS effects, on biochemical, physiological and behavioral levels. The aim of the present study was to investigate the effects of ethanol on hippocampal pyramidal neurons in vitro , and to explore possible mechanisms underlying these effects. Hippocampal slices were prepared from adult male guinea pigs, and superfused with oxygenated artificial cerebrospinal fluid in a submerged tissue bath at room temperature. CA1 pyramidal neurons were impaled with either 3M KCl or 2M $KMeSO_4$ - filled glass microelectrodes. For voltage-clamp experiments, a single-electrode clamping amplifier was used (Axoclamp-2, switching frequency: 2 kHz).

50-100 mM ethanol produced a number of effects on the pyramidal cells. Slight membrane hyperpolarization and a small decrease in membrane resistance was typically observed. Spike threshold was increased and spike duration was shortened in most cells. Ethanol also produced an increase in the after-hyperpolarization current (I_{AHP}). The number of spikes elicited in response to a 30 second depolarizing current pulse was markedly decreased by ethanol, to 63.7 $\pm$ 15.1 % (n=22) of the control value. Ethanol also decreased the excitability produced by 1 uM carbachol. The suppressant effect of ethanol on spike rate was blocked by cyclic 3',5'-adenosine monophosphate (cAMP), when present in the recording electrode (0.1M, n=3) or applied in the bath as the 8-bromo analogue (0.5mM, n=3). Since cAMP decreases I_{AHP} , it is proposed that the AHP increase is the mechanism by which ethanol suppresses pyramidal cell firing.

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139.4 ETHANOL REDUCES SINGLE UNIT ACTIVITY AND DISRUPTS THE RHYTHMICALLY BURSTING PATTERN OF NEURONS IN THE RAT MEDIAL SEPTUM/DIAGONAL BAND. B.S. Givens and G.R. Breese. Biol. Sci. Res. Ctr. Univ. of North Carolina Chapel Hill, N.C. 27514.

Ethanol alters the activity of the septohippocampal pathway as measured by acetylcholine release and high affinity choline uptake in the hippocampus. Ethanol also reduces the occurrence of theta rhythm recorded from the hippocampus. The region of the medial septum and the vertical limb of nucleus of the diagonal band (MS/DB) contains both the cholinergic septohippocampal neurons as well as the rhythmically bursting neurons which underlie hippocampal theta rhythm. Previous work in this laboratory has implicated the medial septum in the behavioral depressant effects of ethanol. The present study was performed to test the hypothesis that systemic administration of ethanol alters neuronal activity of MS/DB bursting neurons.

Extracellular single unit activity was recorded from rhythmically bursting MS/DB neurons in urethane anesthetized Sprague-Dawley rats. Intraperitoneal administration of 10% ethanol at doses of 0.75, 1.5 and 3.0 g/kg produced a dose related inhibition (29%, 42% and 50% below control levels) of the firing rate in approximately 80% of the cells at each dose. Equal volume injections of saline did not alter neuronal activity. The decrease in unit activity by ethanol began within 5 minutes, lasted for 30-60 minutes and was usually associated with a disruption of the rhythmically bursting pattern of firing. Blood ethanol levels, determined by gas chromatography, increased exponentially during the initial 40 minutes following ethanol administration after which they remain at a constant elevated level throughout the remainder of the experiment (3 hours). While the depression of MS/DB neuronal activity lasts an average of 45 minutes, the ethanol remains elevated long after the neurons return to a normal rate and pattern of firing.

One of the controlling influences on MS/DB activity is a GABAergic input from the lateral septum. Since several reports have indicated that ethanol can potentiate the effects of GABA, we iontophoresed GABA onto MS/DB neurons before and after the administration of 0.75 or 1.5 g/kg ethanol. Ethanol administration at both doses resulted in an approximately 20% enhancement of the inhibition due to GABA in a majority of MS/DB cells. While this effect did not reach significance, there was a significant correlation between the magnitude of the enhancement and the degree of inhibition of the spontaneous firing rate due to systemic ethanol. Thus, those MS/DB neurons which were most sensitive to ethanol were the cells most likely to demonstrate a potentiated response to GABA. Supported by a fellowship from the North Carolina Alcoholism Research Authority.

139.5 EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON UNIT ACTIVITY AND POPULATION RESPONSES OF THE DENTATE GYRUS OF THE HIPPOCAMPUS. J.B. Wiesner and S.J. Henriksen. Div. Preclin. Neuroscience and Endocrinology, Res. Inst. at Scripps Clinic, La Jolla, CA 92024.

In order to determine the effects of acute ethanol administration on physiological processes of the hippocampal dentate gyrus, a structure associated with memory function, evoked field potentials and single unit activity were monitored in conjunction with administration of ethanol in halothane-anesthetized rats. Blood samples were obtained at intervals after injection of ethanol (16% w/v in saline) for determination of blood alcohol concentration (BAC).

'Population spikes' (PSs) of field potentials were evoked by electrical stimulation of the perforant path, and recurrent inhibition of the PS was measured by a paired-stimulation paradigm. Following intraperitoneal (i.p.) injection of ethanol (1-2.5 g/kg) but not saline, recurrent inhibition was enhanced in a dose-dependent fashion. At a dose of 2 g/kg (resulting in mean BAC of 196 mg%), recurrent inhibition was enhanced by 336% within 5 min of the injection and recovered with decreasing BAC by 4.5 hrs. In contrast, singly-evoked PSs (quantified by input-output analysis) or conditioning PSs (of the paired-stimulation analyses) were unaffected by doses of up to 2 g/kg, although they were depressed at the highest dose used (2.5 g/kg). Thus, recurrent inhibition of granule cell population responses is selectively sensitive to ethanol and appears to be independent of the depressant effect of higher doses of ethanol.

Spontaneous granule cell unit activity was suppressed by either i.p. (0.5-2 g/kg) or intravenous (0.25 g/kg) injection of ethanol, but not by control injection of saline. The effect of a 2 g/kg i.p. injection often consisted of two phases: (1) near total (92-100%) depression of unit activity beginning during the injection and lasting 4 to 15 min; (2) a rapid increase to a level of 40% of the baseline rate, increasing gradually over 3.5 hrs back to baseline level. The depression during the first phase did not appear to vary closely with the instantaneous BAC, suggesting to us that the early phase may be related to either (a) the rising limb of ethanol absorption, or (b) peripheral effects of ethanol. However, local electrophoretic application of ethanol (50-150 nA) also depressed spontaneous activity, indicating that depressant effects of ethanol on cell firing could be mediated centrally.

In conclusion, intoxicating levels of ethanol exert independent effects on different physiological processes of the dentate gyrus. We are currently investigating the possibility that certain of these effects, such as the enhancement of recurrent inhibition, may be mediated by an enhancement of the inhibitory action of GABA or somatostatin. (Supported by NIAAA 06420 and NIDA 03665.)

139.6 THE EFFECT OF ACUTE ETHANOL ON THE EXCITATORY AMINO ACID RESPONSES OF PURKINJE NEURONS IN CULTURE. C.L. Franklin* and D.L. Gruol (SPON: H. Neville), Div. Preclin. Neurosci. and Endocrin. and the Alcohol Research Center, Scripps Clinic and Res. Foundation, La Jolla, CA 92037.

Cerebellar Purkinje neurons (PNs) in culture are excited by the putative transmitter glutamate (Glu) and the Glu analogues Quisqualate (Quis) and Kainic Acid (KA) which activate specific receptor subtypes (Franklin and Gruol, Neurosci. Absts. 12, 1986). The excitatory responses produced by these agonists in the PN consist of an increase in simple spike firing and a period of bursting. However, the bursting component of the KA response is attenuated relative to the Glu and Quis responses. Quis is the most potent agonist. Acute ethanol (EtOH) significantly reduces the bursting component of the Glu response (Franklin and Gruol, Brain Res., 1987). In the present study, the action of EtOH on responses evoked by Quis and KA were examined and compared to that observed for Glu, to determine if the sensitivity to EtOH is localized to a particular receptor subtype. Extracellular recording techniques and exogenous application of Quis (1 μM), KA (50 μM) and Glu (50 μM) by micropressure application were used. Response characteristics were quantitated by computer. Recordings were made from PNs maintained in culture for >12 days. The cultures were prepared from the cortical region of fetal rat cerebella as previously described (Gruol, Brain Res 263, 1983). As was observed for the Glu response, acute exposure to EtOH at pharmacologically relevant doses (200 mg%) dramatically reduced the bursting component of the Quis response. Mean values for the % bursts during the Quis response were 29$\pm$5 ($\pm$SEM; N= 19, n= 9; N= number of trails, n= number of cells) under control conditions and 14$\pm$3, (N= 10, n= 4) during EtOH exposure. Mean values for % bursting during the Glu response were 39$\pm$11 (N= 13, n= 9) for control and 16$\pm$7 (N=13, n= 9) during EtOH exposure. In contrast, the bursting component of the KA response was not reduced by EtOH. Mean values for % bursting during the KA response were 10$\pm$2 for control (N= 19, n=9) and 10$\pm$5 during EtOH exposure (N= 9, n=5). Mean firing rates during the agonists responses (averaged over the duration of the response), which represent net excitation during the response, were not significantly altered by EtOH. Mean values were 12$\pm$1 Hz (N= 11), 11$\pm$1 Hz (N= 20) and 16$\pm$2 (N= 13) for Quis, KA and Glu responses under control conditions and 12$\pm$2 Hz (N=10), 11$\pm$2 Hz (N= 9) and 17$\pm$2 (N= 17) for Quis, KA and Glu responses during EtOH exposure. These data suggest that the sensitivity of the Glu response to acute EtOH may reside at the Quis receptor site or secondary sites that mediate the bursting component of the response. (Supported by NIAAA Grants AA06665 and AA06420).

139.7 ANTAGONISM OF ETHANOL-INDUCED DEPRESSIONS OF CEREBELLAR PURKINJE NEURONS IN RATS WITH THE BENZODIAZEPINE DERIVATIVE, Ro15-4513. Elizabeth A. Moore* and Michael R. Palmer. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado 80262

Ro15-4513 is a benzodiazepine derivative which has been reported to antagonize the ataxic behavioral effects of ethanol (Suzdak et al., 1987). Local applications of ethanol by pressure ejection from multibarrel micropipettes cause dose-dependent and reversible depressions of cerebellar Purkinje neuron firing rates. These depressions could be antagonized by local applications of Ro15-4513 from another barrel of the same multibarrel pipette. In contrast, Ro15-4513 did not antagonize GABA-induced depressions of the firing rates of these same neurons. Although ethanol effects consistently recovered after short-duration applications of Ro15-4513, it was very difficult to demonstrate recovery of ethanol effects even one hour after longer local applications or repeated short applications of this putative ethanol antagonist. The lack of recovery may be due to a long half-life of Ro15-4513 in the tissue caused by the lipophilicity of this compound. To date, we have been unable to prevent or reverse the antagonism by Ro15-4513 of ethanol-induced depressions with local applications of Ro15-1788, a benzodiazepine antagonist which has been reported to prevent the effects of Ro15-4513 on ethanol-induced behavior (Suzdak et al., 1987). We conclude that Ro15-4513 can antagonize ethanol-induced depressions of cerebellar neuronal activity. However, the mechanism of this antagonism might not involve a classical benzodiazepine receptor. This work was supported by AA05915 and AA03527.

139.8 Intoxicating doses of ethanol block the development of long-term potentiation (LTP) in the dentate gyrus. M.F. Yeckel* and S.J. Henriksen, Research Institute of Scripps Clinic, La Jolla, CA. 92037.

Growing experimental evidence implicates the mammalian hippocampus in information processing, most notably, its ability to demonstrate short and long-term synaptic "plasticity" in response to repetitive stimulation. These synaptic events are hypothesized to be a potential substrate for associative learning. The hippocampus is also a brain structure uniquely sensitive to ethanol (E). An association may exist between the cognitive deficits well documented in human alcohol abusers and hippocampal sensitivity to E. Although some electrophysiological events have been shown to be selectively altered by chronic E, the effects of acute E on measures of hippoocampal plasticity remain to be adequately investigated. Accordingly, we have studied the effects of acute E on the ability to evoke long-term potentiation (LTP) in the rat dentate gyrus, both in halothane anesthetized and freely-moving, unanesthetized rats. LTP was induced in halothane-anesthetized control subjects (N=11) using a standard paradigm (8 trains of 20 ms pulses at 400 Hz, 1 train/10 sec). Stimuli were delivered to the angular bundle of the perforant path. Evoked synaptic events were recorded from the granule cell layer of the dentate gyrus using glass microelectrodes. Input/output and paired-pulse curves were determined both before and following tetanic stimulation. The tetanic stimulation induced a mean increase of 190% in the half-maximal primary evoked population spike compared to baseline, as assessed 30-60 min post-tetanus. E administered intraperitoneally (2 g/kg) to anesthetized rats (N=14) 10-25 min prior to tetanic stimulation blocked the development of LTP (defined as < 50% increase in the pre-ethanol population spike). E administered intravenously (0.5 g/kg) 1-2 min prior to tetanic stimulation also blocked the development of LTP (N=5). The mean blood alcohol concentration (BAL) in each case was 130-170 mg% at the time of stimulation. However, when tetanic stimulation was presented in these same rats after BAL returned to baseline (approx. 4 hrs post i.p. and 60 min post i.v. injections), LTP was induced. In unanesthetized, freely-moving rats (N=5) E (2.5 gm/kg i.p.) exerted similar electrophysiological effects. In other control studies, when E was given after induction of LTP potentiation was not altered. These data suggest that development of synaptic plasticity in the dentate gyrus can be effected by low intoxicating doses of E. (Supported by NIAAA 06420)

139.9 RO 15-4513 AND FG 7142 ANTAGONIZE THE EEG EFFECTS OF ETHANOL BUT ALSO PRODUCE LIMBIC SEIZURE ACTIVITY. C.L. Ehlers*, R. Ian Chaplin*, G.F. Koob (SPON: D.J. Kupfer) Research Institute of Scripps Clinic, La Jolla, CA 92037

The anxiolytic actions of ethanol have been suggested to be mediated through the benzodiazepine/GABA receptor ionophore complex. The data which support this hypothesis are based on biochemical and pharmacological studies demonstrating a facilitation of GABA function by ethanol and experiments utilizing various antagonists and inverse agonists. Recent studies have reported that the imidazodiazepine RO 15-4513 can selectively block several of the actions of ethanol in rats at doses which do not produce behavioral actions when administered alone. In the present study the selectivity of the electrophysiological effects of RO 15-4513 and the β-carboline FG 7142 administered alone and in combination with ethanol was investigated. 21 Wistar rats were implanted with chronic electrodes in anterior cortical sites and in the dorsal hippocampus. Following a 7-day recovery period rats were administered one of 3 doses of RO 15-4513 (1.5, 3, 6 mg/kg IP) or RO (6 mg/kg) plus 1 g/kg IP ethanol, or one of 2 doses of FG 7142 (2.5, 5 mg/kg IP) or FG (5 mg/kg) plus 1 g/kg IP ethanol. Ten minutes following the injections EEG was monitored on paper and magnetic tape for periods of 1-1/2 hours. EEG tapes were analyzed for spectral characteristics by computer and paper records recorded in parallel were hand scored for abnormal activity. Following high dose RO 15-4513 all rats tested (n=7) demonstrated abnormal EEG activity in the form of slow sharp waves, and also displayed episodic high amplitude recurrent hippocampal seizures. These ictal episodes were 5-10 seconds in length and were not associated with behavioral convulsions. Lower doses of RO also produced seizures in 8 of the 14 rats tested. Co-administration of ethanol with RO 15-4513 significantly reduced the mean number of seizures observed to one half those seen following RO alone. FG 7142 also produced ictal episodes in all animals at both doses tested, whereas co-administration of ethanol with FG caused a complete disappearance of seizure activity in all 7 rats tested. These studies suggest that these drugs produce potent electrophysiological actions of their own, and that FG 7142 may be more selective in its interaction with ethanol (Supported by AA 06059, AA06420, and AA 00098.)

139.10 ALCOHOL AND THE AUDITORY BRAINSTEM RESPONSE, BRAIN TEMPERATURE, AND BLOOD ETHANOL CONCENTRATION: A PARADOX EXPLAINED J. A. Lee[1@], E. P. Schoener[2@], D. W. Nielsen[4], and R. F. Berman[3@]. [1]Department of Obstetrics-Gynecology, [2]Pharmacology, [3]Psychology, [@]Wayne State University, Detroit, MI, 48201 and [4]House Ear Institute, Los Angeles, CA 90057.

Squires et al. (1978) found temperature-independent effects of alcohol upon the auditory brainstem response (ABR), and Jones et al. (1980) found temperature-dependent effects. This study addressed those apparently contradictory results with an integration of results for alcohol's dose- and time-related effects upon three measures: the ABR, brain temperature, and blood ethanol concentration (BEC). In studies previously reported by this group (Lee et al., 1983, 1985), the ABR and brain temperature were measured in unrestrained, Long-Evans rats throughout the 30 min before and 2 1/2 hrs after three alcohol doses (0.5, 2.5, and 5.0 g/kg), which were administered at least one week apart. The auditory stimulus, a 0.1-msec, constant-intensity click with repetition rates of 11, 33, and 66/sec, was delivered through a miniature transducer mounted on the rat's head. Brain temperature was measured with a thermocouple implanted in the forebrain. In a separate study (Lee et al., 1984), BEC was measured at nine time points following the same three alcohol doses. Blood samples were obtained from an indwelling aortic catheter. It was concluded that alcohol has both temperature-dependent and -independent effects, which vary according to alcohol dose and BEC-curve phase. Temperature-independent effects are more likely soon after alcohol administration when BEC is rising, or when BEC is fairly high and steady following a moderate dose. Temperature-dependent effects are more likely when BEC has fallen precipitously and is comparatively low following a high alcohol dose, particularly when behavioral thermoregulation is less likely. The Squires et al. (1978) and Jones et al. (1980) studies each used a combination of procedures likely to produce the respective ABR-alcohol-temperature results. Thus, not surprisingly, the paradoxical research results were due to differing experimental procedures. Based upon statistical analyses, it was concluded that alcohol-ABR effects are primarily central, since (1) alcohol had no statistically significant effect upon ABR wave I, and (2) alcohol-ABR effects did not differ significantly as a function of stimulus repetition rate. (The research was carried out in the Otology Research Laboratories, Henry Ford Hospital, Detroit, MI 48202.)

139.11 TEMPERATURE DEPENDENCE OF LETHALITY FROM ETHANOL GIVEN ALONE OR IN COMBINATION WITH PENTOBARBITAL. R.L. Alkana, M. Bejanian, B. Jones*, P.J. Syapin(1) and D.A. Finn*. Alcohol and Brain Research Laboratory, Institute for Toxicology, School of Pharmacy and (1) School of Medicine, University of Southern California, Los Angeles, CA 90033.

The relationship between ambient temperature, rectal temperature and ethanol sensitivity has been investigated in mice and rats following sub-hypnotic, hypnotic and lethal doses. In general, sensitivity to ethanol was found to increase as intoxicated body temperature increased. Ethanol dose response studies in the lethal range found a shift to the left in the 8 and 24 hour log dose-percent mortality curves and a significant decrease in the LD50 as ambient temperature was increased from 20 to 35°C. The present study extended the above findings to include the effects of body temperature manipulation on lethality from ethanol given in combination with pentobarbital. C57 male mice were injected with 7.8 g/kg ethanol, or with a combination of 7.8 g/kg ethanol plus 20 mg/kg pentobarbital, and were individually housed within chambers at 20, 25, 30 or 35°C for 24 hours following injection. Mortality was determined at 1, 2, 4, 8 and 24 hours post-injection. Rectal temperatures were measured in the surviving animals. The results indicate that ambient temperature significantly affected lethality at all time points after injection in both the ethanol and ethanol/pentobarbital groups. Chi square analysis indicated that survival was significantly greater in both groups of animals exposed to 20 and 25°C when compared to the animals exposed to 30 and 35°C at 2, 4 and 8 hours post-injection and when compared to the 35°C exposed animals at 1 hour post-injection ($p < 0.01$). At the 24 hour measurement survival was significantly greater in the 25°C animals when compared to all other temperature groups. There were statistically significant, ambient temperature-related differences in rectal temperatures in both the ethanol and ethanol/pentobarbital groups. The 35°C exposed animals became slightly hyperthermic, while the animals exposed to 30, 25 and 20°C became hypothermic. Rectal temperatures in the 35, 30 and 25°C exposed animals remained constant across time, but body temperatures in the 20°C exposed animals continued to drop throughout the exposure period. The increase in mortality from the 8 to 24 hour measurement in the 20°C exposed animals may have been due to prolonged extreme hypothermia. The relationship between body temperature and lethality can be explained by membrane perturbation theories of anesthesia and suggests that body temperature manipulation may represent an effective means of reducing lethality from ethanol or ethanol/combination overdoses. (Supported by USPHS grant R01 AA05234 from the National Institute on Alcohol Abuse and Alcoholism).

139.12 SELECTION OF LINES OF MICE FOR ACUTE SENSITIVITY TO THE HYPOTHERMIC EFFECTS OF ETHANOL. D.J. Feller, E. Young*, B. Tam*, C. Deutsch*, and J.C. Crabbe. Veterans Administration Medical Center, Portland, OR 97201.

The mechanism by which ethanol produces its behavioral effects and the way genotypic factors modulates the response of animals to ethanol is poorly understood. One approach to studying these questions is the selective breeding of mouse lines which differ in specific behavioral responses to ethanol. Thermoregulation, which is altered by ethanol, is an interesting system for examining sensitivity and the development of tolerance to ethanol. Mouse lines sensitive (COLD) to the acute hypothermic effect of ethanol and resistant (HOT) to this effect are being produced by the method of within family selection. The selection of replicate HOT and COLD line is now into the 8th generation. The response of the lines has gradually diverged. After 7 generations the difference in maximal temperature response between HOT1 and COLD1 was 2.3°C and 2.4°C for HOT2 vs COLD2. The average realized heritability for the diverging response in both replicates at generation 5 was $h^2=0.17$. No difference has been found in ethanol metabolism, which suggests that the lines differ in neurosensitivity. Second litter males and females of the 7th generation have been tested for their sensitivity to other alcohols and to the effect of hydralazine, a drug which induces hypothermia by a peripheral mechanism of action. Mice were tested for their hypothermic response to four doses of each alcohol, including ethanol, propanol, n-butanol, t-butanol and pentanol. The dose response curves were shifted to the left as the chain length increased. COLD2 mice exhibited a greater difference in hypothermic response than HOT2 mice to the 5 alcohols. At a dose of 3 g/kg ethanol, the HOT2 and COLD2 lines differed by 2°C. Comparable changes were observed with each of the other alcohols. The effects in the HOT1 vs COLD1 lines were not as great, but may increase in later generations of the selection process. The lines were identical in their hypothermic response to hydralazine, suggesting that the divergence in response to ethanol for the lines is not mediated through peripheral vasodilation. In conclusion, the HOT and COLD mice should be useful for elucidating ethanol's mechanism of action and the biological factors regulating sensitivity to its effects. These experiments are supported by PHS-NIAAA grants AA05828, AA06243, AA06498 and by a grant from the Veterans Administration.

139.13 EFFECTS OF PENTOBARBITAL ANESTHESIA ON RAT GASTROINTESTINAL FUNCTION. John J. Stewart, Cheryl D. Curd-Sneed* and Thomas W. Woods*, Department of Pharmacology, L.S.U. Med. Ctr., Shreveport, LA 71130.

This study examined the effects of sodium pentobarbital on gastric emptying, small intestinal transit and intestinal myoelectric activity. Gastric emptying and small intestinal transit were determined simultaneously in fasted, male rats given sodium pentobarbital (40 mg/kg). Drug was administered intragastrically by means of a stomach tube in 1, 2 or 3 ml dosing volumes. Each administered solution additionally contained a small quantity of nonabsorbed radioactive marker (0.5 μCi, $Na_2{}^{51}CrO_4$). Control animals received 2 ml of water containing marker only. Animals were sacrificed at 5, 15, 30, 45, 60 and 90 mins after drug administration and the stomach and small intestine were removed. Gastric emptying was calculated from the percentage of radioactivity remaining in the stomach versus time using a curve stripping program. Small intestinal transit was determined by calculating the geometric center of distribution for radioactive chromium along the bowel. Nine animals were chronically implanted with four bipolar, extracellular electrodes placed at 10 cm intervals beginning at the gastroduodenal junction. Five days after surgical preparation, intestinal myoelectric activity was monitored for a 1 hr period before, and a 3 hr period after intragastric sodium pentobarbital (40 mg/kg). All animals lost the righting reflex within 7 mins after drug administration. Gastric emptying was biphasic, consisting of a brief rapid phase, followed by a prolonged slow phase. Time to half emptying was 47 mins for water and 122, 156 and 128 mins for sodium pentobarbital given in 1, 2 and 3 ml, respectively. In contrast, small intestinal transit was not different for animals given sodium pentobarbital and water. The migrating myoelectric complex was present in fasted animals anesthetized with sodium pentobarbital, but there was a significant decrease in slow wave frequency and increase in the duration of regular spike activity at the sites monitored. Migrating myoelectric complex cycled every 12.8 ± 1.3 mins (mean ± SEM) during the control period and every 21.8 ± 1.2 mins ($P < 0.01$) after sodium pentobarbital. The results suggest that gastric emptying is inhibited during sodium pentobarbital anesthesia, while small intestinal transit and fasted patterns of intestinal myoelectric activity are relatively normal. (Supported by a grant from the Edward R. Stiles Trust Fund.)

139.14 SODIUM PENTOBARBITAL DECREASES FACILITATION AT THE CRAYFISH NEUROMUSCULAR SYNAPSE. B.D. Winegar, S.W. Leslie, and G.D. Bittner. Department of Zoology, University of Texas, Austin, TX 78712.

Synaptic plastic events such as facilitation are of interest in accounting for plastic changes in behavior which occur as a result of experience. Since the synapse is thought to be the primary site of action of many barbiturates, it is likely that many of the behavioral plasticities (e.g., sedation, tolerance) associated with barbiturate use and abuse are due to the effects of these drugs on synaptic plasticity.

Crayfish (Procambarus clarkii) were weighed and injected at the ventrocaudal aspect of the abdomen with various concentrations of sodium pentobarbital (PB) in order to determine at what dosage PB produces behavioral plasticities. Sedation and tolerance to PB were quantified by measuring eyestalk retractions from light tactile stimuli on the carapace dorsal to the compound eye. The animals showed significantly decreased eyestalk retractions ($F=5.35$; $df=24$; $p<0.05$) up to 30 min after injection of 109 mg/kg PB (0.6 mM). Significant tolerance to PB was observed following daily injections of 90.5 mg/kg PB (0.5mM) on day two ($F=16.30$; $p<0.0005$; $df=57$), with almost complete recovery on day three.

In order to determine whether PB produces changes in various measures of synaptic plasticity that might begin to account for behavioral plasticity, PB was tested for effects on facilitation and other measures of synaptic plasticity at opener-excitor neuromuscular synapses in the crayfish claw (cheliped). This preparation was exposed to various concentrations of PB dissolved in Van Harreveld's solution (pH=7.6) for 2 min while the excitor axon was stimulated at 5 Hz with 10 V twin pulses (0.02 msec duration; 5.0 msec delay). Facilitation was calculated from averaged (N=100) excitatory postsynaptic potentials (EPSP's) produced by the first and second stimuli in the twin pulse train. PB increased EPSP time constants while producing dose-dependent decreases in facilitation and EPSP amplitudes. Facilitation was significantly reduced at a concentration of 0.1 mM PB ($F=5.59$; $p<0.05$; $df=16$). Additionally, preliminary data suggest that 0.2 mM PB produces defacilitation, which is accompanied by severe suppression of EPSP amplitudes.

The decreases in facilitation suggest a presynaptic action of PB on synaptic plasticity. Since PB decreases synaptic calcium currents in a variety of preparations, it may reduce presynaptic residual calcium at the crayfish neuromuscular synapse to decrease facilitation. Observed increases in EPSP time constants by PB suggest that the drug also increases postsynaptic membrane resistance.

139.15 THE DIFFERENTIAL EFFECTS OF LIPOPHILIC AND HYDROPHILIC ANESTHETIC AGENTS ON THE SURFACE AND INTERIOR MEMBRANE ORDER OF DIMYRISTYLPHOSPHATIDYLCHOLINE MULTILAMELLAR LIPOSOMES. R.J. Hitzemann and A. Skolnakorn*. Department of Psychiatry and Behavioral Science, S.U.N.Y. at Stony Brook, Stony brook, NY 11794, and Psychiatry Service, VA Medical Center, Northport, NY 11768.

We have recently reported that as determined by 1H-NMR, ethanol disorders the interior of synaptic plasma membranes but orders the membrane surface (Hitzemann et al., BBA 852:159, 1986). The ratio of ordering/disordering activities increases with increasing temperature due to the preferential partitioning of ethanol to the membrane surface. It has also been noted that the genetic selectivity of anesthetic response [e.g., long sleep vs short sleep mice] decreases with increasing lipid solubility of the anesthetic agent (Howerton et al., Psychophamacol. 79:313, 1983 and J. Pharmacol. Exp. Ther. 227:389, 1983 and Marley et al, personal communication). We now report that anesthetics which demonstrate genetic discrimination, and some related anesthetics share with ethanol the ability to order the membrane surface. Fluorescence polarization (FPZ) techniques were used to monitor membrane order with the probes DPH (interior) and TMA-DPH (surface) in DMPC multilamellar liposomes both above and below the main phase transition temperature. The following anesthetics were examined: ethanol (1), propanol (2), butanol (3), pentanol, (4) hexanol (5), 2-chloroethanol (6), 2,2-dichloroethanol (7), 2,2,2-trichloroethanol (8), urethane (9), ethylacetate (10), acetone (11), ether (12) and chloroform (13). With an order of potency paralleling their oil/water partition coefficients all of the compounds tested disordered the membrane interior. In contrast, compounds 1, 2, 6, 7, 9, 10 and 11 had an ordering effect on the membrane surface. Compounds 3, 4, 5, 8, 12 and 13 disordered the membrane surface, although the concentrations required were higher than those needed to disorder the membrane interior. We suggest that the membrane components associated with the regulation of the ordering of the lipid bilayer may be the same components involved in the genetic regulation of anesthesia at the membrane level. Supported in part by the Veterans Administration.

139.16 ETHANOL-INDUCED LIBERATION OF SIALIC ACID, *WITHOUT SIALIDASE ACTIVATION, IN RAT LIVER SLICES. Joy Mathew* and W. R. Klemm, Brain Research Laboratory, Department of Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

Membrane-bound glycoconjugates (glycoproteins, gangliosides) that contain sialic acid appear to be vulnerable to the action of ethanol on the brain (J. Neurosci. Res. 3: 341; 4: 371). Because the liver is the principal site of ethanol metabolism, we hypothesized that a similar phenomenon could occur in liver and may be of greater consequence.

When liver homogenates were incubated with varying concentrations of ethanol, there was no change in endogenous or exogenous sialidase activity up to 1 M, but there was a steady and marked loss of activity above 1 M. To investigate this effect under more physiological conditions, rat liver slices were incubated with varying amounts of ethanol (0, 0.1, 1, and 3 M) in circulated, oxygenated Yamamotto solution for 5 hours. There was a 48% increase in free sialic acid at 0.1 M, but a decrease at the higher, toxic concentrations. Sialidase activity, however, was not significantly affected at 0.1 M, but was inhibited at 1 M and 3 M. Alcohol-induced inhibition of sialidase was not prevented by addition of 2 mM pyrazole, a well-known inhibitor of alcohol dehydrogenase. Total liver protein was progressively decreased to 60% of control levels by increasing ethanol concentrations, and pyrazole did prevent this effect of ethanol. When ethanol was replaced by sorbitol, which mimics ethanol in shifting of the redox level, both sialidase activity and protein were decreased.

The results suggest that although sialidase may not be inhibited substantially by physiological levels of ethanol, toxic levels inactivate it in two ways: 1) direct ethanol effect, and 2) by shifting the redox level. The physiological effect of ethanol in increasing free sialic acid, even though enzyme activity seems unchanged, may indicate that the enzyme has greater accessibility to sialocompounds of intact cell membranes when the membrane is fluidized and destabilized by ethanol.

139.17 AN INTERACTION BETWEEN ETHANOL AND CYANAMIDE IN RAT BRAIN CATALASE ACTIVITY IN VIVO. C.M.G. Aragon*., L.M. Stotland* and Z. Amit. Center for Studies in Behavioral Neurobiology, Concordia Univ., Montreal, Quebec, Canada H3G 1M8.

The purpose of the present study was to further investigate the relationship between ethanol and brain catalase. Rats were pre-treated (i.p.) with different doses of ethanol (0.19 to 3 g/kg) or saline 30 minutes prior to administration of cyanamide (0.68 m mol/kg; i.p.) or saline. One hour later, under ether anesthesia, cardiac blood was collected and tissues were perfused in situ. Brain catalase activity was measured using the Clark electrode. Results confirm inhibition of brain catalase activity by cyanamide. Plasma ethanol levels were not altered by cyanamide. Ethanol protected catalase from cyanamide inactivation in a dose-related manner (maximum protection at 1.5 and 3 g/kg). In a second study the protected effect of ethanol (0, 0.37, 0.75 and 1.5 g/kg) was tested against several cyanamide concentrations. Results indicated that ethanol protection was progressively reversed by increasing concentrations of cyanamide. These data suggest a competition between ethanol and cyanamide for the catalase molecule, they confirm the presence of catalase and generation of H_2O_2 in the rat brain in vivo, and overall seem to support the notion that centrally formed acetaldehyde via brain catalase may be responsible for some of the psychopharmacological actions of ethanol.

139.18 MOTOR IMPAIRMENT PRODUCED BY ETHANOL, BARBITAL, OR LORAZEPAM IN RAT LINES SELECTED FOR DIFFERENTIAL SENSITIVITY TO ETHANOL: EFFECT OF GABAERGIC DRUGS. K. Kiianmaa* and K. Hellevuo*. (SPON: European Neuroscience Association). Research Laboratories of the Finnish State Alcohol Company (Alko Ltd), POB 350, SF-00101 Helsinki, Finland.

The AT (Alcohol Tolerant) and ANT (Alcohol Non-Tolerant) lines of rats have been selectively outbred for low and high sensitivity to motor impairment from ethanol, respectively. An acute dose of ethanol (1.25-2.75 g/kg, IP) impairs the motor performance on the tilting plane test of the ANT rats more than that of the AT rats at the same blood level. When tested over a wide range of doses, it was found that the ANT rats were also more sensitive than the AT rats to the motor impairment caused by barbital (80-160 mg/kg, IP) and lorazepam (1-7 mg/kg, IP). Since the primary target of these drugs is probably the GABA-benzodiazepine-receptor complex, we studied here the importance of GABAergic neurons in the differential sensitivity of the rat lines to ethanol. The rats were injected with the GABA receptor antagonist picrotoxin (2 mg/kg, IP), the GABA synthesis inhibitor isoniazid (250 mg/kg, IP) or the benzodiazepine antagonist Ro15-1788 (10 mg/kg, IP) followed by an injection of ethanol (2.75 g/kg, IP) or barbital (160 mg/kg, IP), doses, which caused marked motor impairment in both rat lines, or lorazepam 3 mg/kg, IP), which caused marked motor impairment only in the ANT rats. The tilting plane test was conducted 30 min later. Ro15-1788 antagonized only lorazepam-induced motor impairment. Isoniazid had no influence on the intoxication produced by any of the three drugs. Picrotoxin, however, antagonized the impairment of motor performance induced by ethanol, barbital, and lorazepam. Furthermore, there seemed to be some variability in this antagonism with the genotype: although the AT and ANT rat lines did not differ in the effect of picrotoxin on ethanol-induced intoxication, the ANT rats seemed to be more sensitive than the AT rats to the antagonism of barbital-induced motor impairment produced by picrotoxin. The results of these experiments encourage further studies on the involvement of GABA systems in the genetically-determined differences in ethanol sensitivity.

139.19 FAILURE OF A BENZODIAZEPINE PARTIAL INVERSE AGONIST Ro15-4513 TO ANTAGONIZE MOTOR IMPAIRMENT INDUCED BY ETHANOL. E.R. Korpi and K. Hellevuo*. Research Laboratories of the Finnish State Alcohol Company, Alko Ltd., POB 350, SF-00101 Helsinki, Finland.

Recently it has been reported that acute ethanol intoxication can be reversed by an imidazobenzodiazepine Ro15-4513 (1, 2, 3). We have found that the ANT and AT rat lines, selectively out-bred for high and low acute sensitivity to ethanol-induced motor impairment in the tilting plane test, respectively, also in the same way differ in motor impairment from the benzodiazepine agonist lorazepam (4). Therefore, it was very interesting to see whether Ro15-4513 would counteract the effects of ethanol in these rat lines.

Ro15-4513 was administered as a suspension in 5% (w/v) acaciae gummi (Ph.Eur.) in water, and ethanol as a 12 or 15% (w/v) solution in saline. The following experiments were conducted on the selected animal lines: 1) ANT and AT rats received ethanol (2.75 g/kg, i.p.) 15 min after Ro15-4513 (10 mg/kg, i.p.) or vehicle and were tested on the tilting plane 30 min after ethanol. 2) ANT and AT rats received ethanol (1.5 g/kg, i.p.), followed by Ro15-4513 (30 mg/kg, i.p.) or vehicle 5 min later, and were tested on the horizontal wire test at 10, 20, 30, 40, 50 and 60 min after ethanol. No differences were detected in the motor performances between the groups treated with Ro15-4513 or vehicle in either rat line.

In additional experiments, Han:Wistar rats were given ethanol (1.5 g/kg) intraperitoneally, and Ro15-4513 (2.5 to 100 mg/kg) either intraperitoneally or intragastrically at various times before and after ethanol administration, and the motor performance of the animals was measured on a Rotarod at various times after drug administrations. The effects of ethanol could not be counteracted by Ro15-4513 in these experiments, although the compound could penetrate into the brain as demonstrated by inhibition of [^{3}H]flunitrazepam binding in unwashed forebrain homogenates.

In summary, at present the benzodiazepine receptor inverse agonist Ro15-4513 in our hands has not proven to be an efficient antagonist of the motor impairment produced by moderate ethanol doses in rats.

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3) P.D. Suzdak et al., Science 234, 1243-1247, 1986.
4) K. Hellevuo et al., Psychopharmacology 91, 263-267, 1987.

139.20 GENETIC DIFFERENCES IN SUB-COLONIES OF LONG- AND SHORT-SLEEP MICE SELECTIVELY BRED FOR ETHANOL SENSITIVITY. D. Kim* and B.C. Dudek. Dept. of Psychology, SUNY at Albany, Albany, NY 12222.

Long- and Short-Sleep mouse lines (LS and SS) were bred for differences in sensitivity to the soporific effects of ethanol (ETOH). LS mice lose the righting reflex an order of magnitude longer than SS mice when given an IP dose of 3.8 g/kg. This difference has been shown to be the result of differences in nervous system sensitivity controlled by a polygenic system of at least nine genes. Two sub-colonies of these lines exist (Albany and Colorado) and have been separated for 26 generations. The Albany colony has been maintained by random mating within each line since generation 18. The original Colorado colony underwent some additional selection following generation 18 and the LS mice were tested with a lower dose of ETOH than SS mice in these generations. The present experiments directly compared the colonies on two dimensions. These were (1) the selection phenotype, loss of the righting reflex following hypnotic doses of ethanol (LRR duration) and (2) the biphasic shape of the sub-hypnotic ethanol dose response curve on locomotor activity, measured in a photocell apparatus.

Following IP administration of 3.8 g/kg ETOH, male LS mice of both colonies lost the righting reflex (LRR) for about 200 min. Female LS mice of both colonies had LRR durations of about 160 min. Albany colony LS mice regained the righting reflex at about 270 mg/sl blood ethanol levels (BEC) and Colorado colony LS mice at a surprisingly higher 305 mg/dl. A colony difference in LRR duration was seen for SS mice. Not all Colorado SS mice given 3.8 g/kg lost the righting reflex, but all Albany colony SS mice did, with a mean of about 27 min. At 4.2 g/kg ethanol, Colorado SS mice had significantly shorter LRR durations than Albany mice and BECs at waking were an average of 45 mg/dl higher in Colorado SS mice (500 mg/dl), as expected. Rectal temperatures at waking were lower in LS mice than SS mice, but this effect was similar for the two colonies. Locomotor activity effects of ethanol were biphasic in LS mice which showed activation at 1.5 g/kg but marked sedation at 2.5 g/kg. In SS mice, locomotor activity was increased by all doses up to 3.0 g/kg. No colony differences in activity dose response curves were detected. These studies thus demonstrate that added selection further increased resistance to ETOH sedation in Colorado SS mice, but did not increase sensitivity in LS Colorado mice. These changes did not extend to locomotor effects of sub-hypnotic doses, suggesting independence of activational effects of low ETOH doses and sedative effects of higher doses.

ALCOHOL AND BARBITURATES III

140.1 HYPERRESPONSIVENESS TO STRESS: DIFFERENTIAL EFFECTS OF PRENATAL ETHANOL ON MALES AND FEMALES. J. Weinberg. Department of Anatomy, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Animals prenatally exposed to ethanol are hyperresponsive to stress in adulthood, and exhibit hyperactivity and deficits in behavioral response inhibition. In view of these findings, we hypothesized that behavioral or psychological variables which are effective in reducing arousal and thus dampening the pituitary-adrenal response to stress in controls may be less effective in ethanol exposed offspring.

Ethanol was administered to pregnant females in liquid diets and both pair-fed and ad libitum fed control groups were included. Following weaning (day 22), offspring were pair-housed by litter and by sex until testing at 60-80 days of age.

We first investigated whether opportunity to perform a consummatory response can reduce the adrenocortical response to novelty in fetal ethanol exposed animals. Animals were water deprived for 24 hr and tested in one of 3 conditions: 1) removed from home cage and blood sample obtained immediately; 2) placed in novel cage with water available; blood sample obtained 30 min later; 3) placed in novel cage with no water available; blood sample obtained 30 min later. All animals showed an increase in corticoids following 24 hr water deprivation, and placement in a novel cage produced a significant further increase in corticosterone. Availability of water in the novel cage markedly attenuated the corticoid response to novelty for all males. However, fetal ethanol-exposed females showed significantly less attenuation of their corticosterone response to novelty than both pair-fed and control females.

In a second experiment, we examined habituation to a stressful stimulus. Animals were restrained in Plexiglas tubes and blood samples were obtained following 30 or 60 min of restraint. All animals showed significant corticoid elevations at 30 min. Males showed no change in corticoids at 60 min, while both pair-fed and control females showed a significant corticoid decrease at 60 min. Once again, however, fetal ethanol-exposed females were more responsive to stress and still showed elevated corticoid levels at 60 min.

These data support the hypothesis that behavioral or psychological variables are less effective in inhibiting or attenuating the pituitary-adrenal response to stress in animals prenatally exposed to ethanol than in controls. Interestingly, females were more vulnerable to these effects of ethanol than males.

Supported by a grant from the Medical Research Council.

140.2 DOES THE PRENATAL EXPOSURE TO ETHANOL AFFECT AN ADULT'S TOLERANCE TO ALCOHOL? E. Reyes, K.D. Garcia, and J. Wolfe Dept. of Pharmacology, Univ. of New Mexico Sch. of Medicine Albuquerque, NM 87131

Fetal Alcohol Syndrome (FAS) is a condition that afflicts 2.2 out of every 1000 children born in North America. FAS is the leading cause of mental retardation with a known etiology.
Past studies in our laboratory have shown that rats exposed to ethanol in-utero have an increased preference for ethanol as adults. Corticosterol levels in response to stress have also shown to be altered by the in-utero exposure to ethanol.
The present study was undertaken to determine the effects of the maternal administration of alcohol on adult ethanol tolerance. Female wistar rats were fed lab chow, or were pair-fed nutritionally adequate liquid diets (Bioserv) containing either alcohol (6.7% w/v) or isocoloric carbohydrates starting on day 1 of gestation. The litters were culled to six and the pups were placed with non-alcohol treated surrogate mothers until they were weaned. At 45 days of age or older, animals were trained to walk on a wooden rod for a period of 5 minutes. Ethanol (2.5 gm/kg) was injected intraperitoneally (IP), and the animal was placed back on the rod, and timing was started. After the animal fell off of the rod 3 times (within 5 seconds) blood was collected and the time was recorded. The blood was assayed for alcohol content by the method of Lundquest (1959) which couples ETOH conversion to acetaldehyde to NADH generation. The in-utero exposure to ethanol has a significant effect on fall time between 6% and 0% pair-fed controls. The prenatal exposure to alcohol thus appears to increase an adult's tolerance to alcohol.
(Supported in part by MBRS #RR08139 and NIAAA #AA06759).

140.3 UNDERNUTRITION AND/OR PRENATAL ETHANOL EXPOSURE DIFFERENTIALLY INFLUENCE STEREOTYPIC AND LOCOMOTOR RESPONSES TO APOMORPHINE IN RATS. M. Horner*, J. H. Hannigan, J. W. Nalwalk*, B. A. Blanchard* and E. P. Riley. Center for Behavioral Teratology, SUNY-Albany, Albany, NY 12222.

We psychopharmacologically assessed the neural basis for behavioral dysfunction in rats exposed in utero to ethanol. Dose-dependent multiple behavioral responses to the dopamine agonist apomorphine were measured. Long-Evans rats were given a nutritionally-balanced liquid diet with 35% ethanol-derived calories (EDC) on days 6 through 20 of pregnancy. Controls were given either a liquid diet with sucrose substituted isocalorically for ethanol (0% EDC), or ad lib lab chow (LC). Pups were weaned at 21 days. At 27 days of age activity, locomotion, thigmotaxia and bouts of stereotypic behavior were scored in automated activity monitors for 15 min. On the next day male and female rats were injected with apomorphine-HCl (0.02, 0.1, 2.0 or 5.0 mg/kg/ml) or saline/ascorbate vehicle and behavior was assessed again for 60 min. Although analyses showed females were more active than males overall, there were no significant interactions with sex. There were no significant behavioral effects of prenatal ethanol exposure on day 27. Under the challenge of apomorphine administration, however, 35% EDC and 0% EDC animals displayed a dose-response shift to the right for stereotypy relative to LC rats. The 35% EDC animals also tended to show a dose-response shift to the left for locomotor activity relative to both the LC and 0% EDC groups. Taken together with our previous findings of altered responses to amphetamine by 0% EDC females and 35% EDC males at 28 days of age, the present results suggest that general undernutrition due to prenatal alcohol exposure (35% EDC) or resrtricted caloric intake (0% EDC) produces dysfunction in nigrostriatal dopamine systems, perhaps to be reflected in an increased synthesis and/or storage of dopamine without a concommittant change in utilization. Similarly, prenatal ethanol exposure per se may produce a more specific disruption of activation-mediating mesolimbic dopamine systems. The presumed pattern of neural dysfunction is being studied further with dopamine antagonists and in animals at different ages.

This work was funded by NIAAA grant #06721 to JHH.

140.4 OPEN-FIELD BEHAVIORS IN RATS TREATED WITH LOW OR HIGH DOSES OF ETHANOL ON POSTNATAL DAYS 1-7 OR 8-14. T.B. Sonderegger, D. Weimer*, J. Benz*, K. Mallon*, and B. Mesloh*, Dept. of Psychol., Univ. of Nebraska-Lincoln, Lincoln, NE 68588-0308.

This study examines the effects of low (L) (4g/kg) or high (H) (7g/kg) doses of ethanol administered during postnatal weeks 1 or 2 upon open field behaviors of female and male rats. Ethanol was administered twice daily on postnatal days (1-7), Week-1, or postnatal days (8-14) Week-2, in a 30% Sustagen (Mead Johnson) vehicle using an intragastric intubation procedure designed to counteract effects of underfeeding (Sonderegger et al., Neurobehavior. Tox. and Teratol. 4:477, 1982). Doses were tapered to reach the maximum dose in Wk-1 animals on day 4 and in Wk-2 animals on day 11. On postpartum day 1, pups from 14 litters of Charles Rivers CD albino rats (10 pups per litter) were used in a split-litter design. On postpartum day 1 pups were assigned to a treatment group: Week-1 (W-1), low dose (L) ethanol (E); L Sustagen (S); Pair Fed (PF); Week-2 (W-2) LE, W-2 HE, W-2 LS, and W-2, LE. S animals received comparable volumes of isocaloric (sucrose) vehicle. Body weights were comparable on day 21; animals were weaned at that time and housed in same-sex pairs with ad libitum food and water.

On days 70-90, blocks of same-sex animals were tested for four consecutive days in the open-field during the first part of the dark cycle. Two observers made microcomputer assisted observations (Sonderegger et al. Neurobehavior. Tox. and Teratol. 6:326, 1984) using programs for real-time observations. The programs permitted recorded of traditional activity measures, timing of event onsets, frequency records of specific behaviors, records of episode sequences, and summation of event times, etc., a total of 19 variables. Data were summed over the four days and submitted to a principal component factor analysis with a varimax rotation. Four factors, accounting for 63% of the variance, were identified as: Exploratory Activity, Nonexploratory Behavior, Grooming Behavior, and Atypical Head movements. As expected, females were more active than males, and W2H E animals differed most from the controls on several variables. Manova's performed on these data and their implications will be discussed. Univ. NE-Lin Research Council, Biomedical Support Grant RR 07055.

140.5 IMPAIRED SPATIAL NAVIGATION IN ADULT FEMALE BUT NOT ADULT MALE RATS EXPOSED TO ALCOHOL DURING THE BRAIN GROWTH SPURT. S.J. Kelly, C.R Goodlett, S.A. Hulsether and J.R. West. Alcohol and Brain Research Laboratory, Department of Anatomy, College of Medicine, University of Iowa, Iowa City, IA 52242, U.S.A.

Locating a submerged platform in a white tank filled with milky water using distal extramaze cues, a task referred to as the Morris water maze, can be used to assess hippocampal function (Morris, Garrud, Rawlings and O'Keefe, Nature 1982 297:681). Alcohol exposure during the brain growth spurt has been shown to alter the hippocampus and we have recently reported that rats aged 19 to 30 days of both sexes exposed to high blood alcohol concentrations (BACs) had impaired Morris maze performance (Goodlett, Kelly and West, Psychobiology, 1987, in press). In order to distinguish between a permanent effect and a developmental delay, we now report the effects of the same patterns of alcohol exposure that we used previously on the performance of adult rats in the Morris water maze.

Two groups were given 6.6 g/kg/day of ethanol via an artificial rearing technique (feedings every 2 h) on postnatal days 4 to 10. One group received the alcohol condensed into 8 h of each day which caused cyclic BACs with peaks at 307 mg/dl whereas the other group received the alcohol uniformly over 24 h which caused stable BACs of 60 mg/dl. Two control groups consisted of rats artificially reared on milk solution alone and rats reared normally by a dam. All rats were raised to day 90 and then tested in the Morris water maze. The rats were given eight trials per day until they met a strict performance criterion. Then the platform was moved to another position and the rats were tested until criterion was met again. In order to determine whether the effects found were the result of impaired ability to use distal extramaze cues, additional groups were tested using an elevated visible platform.

In adult male rats, neither alcohol exposure nor artificial rearing had an effect on Morris maze performance. In contrast, in adult female rats, condensed alcohol exposure but not uniform alcohol exposure was detrimental to the initial learning of the Morris water maze. Female rats given condensed alcohol exposure or artificially reared on milk solution alone did not differ in their ability to locate a visible platform.

In summary, exposure to high BACs during the brain growth spurt impairs Morris maze performance in adult female rats but not adult male rats even though both sexes were equally impaired during the juvenile period. This finding suggests that alcohol alters Morris water maze performance via disruption of a still developing sexually dimorphic substrate(s). (Supported by NIAAA grant AA05523 to J.R.W.).

140.6 ACUTE AND CHRONIC ETHANOL TREATMENTS ALTER GABA RECEPTOR-OPERATED CHLORIDE CHANNELS. Andrea M. Allan and R. Adron Harris. Department of Pharmacology and Alcohol Research Center, University of Colorado Health Sciences Center, Denver, CO 80262.

The molecular mechanisms underlying the development of ethanol tolerance and dependence are still unknown. There is considerable evidence that ethanol enhances the opening of $GABA_A$ receptor-operated chloride channels. Our laboratory has previously demonstrated that in vitro additions of low concentrations(10-45mM) of ethanol increases GABA agonist mediated chloride ($^{36}Cl^-$) uptake into brain membrane vesicles (microsacs) (Allan and Harris, Pharmacologist 27:125, 1985; Allan and Harris, Life Sci. 39:2005, 1986). Here we report the effects of acute and chronic ethanol administration on muscimol- and ethanol augmentation of muscimol-stimulated $^{36}Cl^-$ flux into microsacs prepared from mouse cerebellum

DBA/2J mice (25-30g) were chronically fed ethanol (5% v/v) in a liquid diet for 7 days. Control mice were fed an isocaloric diet with sucrose subsituted for ethanol. Animals were sacrificed the morning of the 8^{th} day and cerebellar microsacs were prepared for muscimol- and ethanol potentiation of muscimol-stimulated $^{36}Cl^-$ uptake as previously described (Harris and Allan, Science 228:1108, 1985). Exposure to physiologically relevant concentrations of ethanol (10-45mM) in vitro potentiated muscimol-stimulation of $^{36}Cl^-$ flux in control (pair-fed), but had no effect on cerebellar microsacs prepared from ethanol tolerant/dependent mice. Muscimol-stimulation of $^{36}Cl^-$ flux was not different for pair-fed and ethanol treated mice.

Other mice were acutely treated with a single i.p. injection of 4 g/kg ethanol and sacrificed at either 5 min, 60 min, 24 hr post-injection and microsacs were prepared from cerebellum. Augmentation of muscimol-stimulated $^{36}Cl^-$ by in vitro ethanol was abolished by a single 4 g/kg injection of ethanol. This "rapid tolerance" occurred within 5 min and disappeared within 24hr after ethanol treatment. The reduced sensitivity of ethanol treated (acute and chronic) mice to ethanol potentiation of muscimol-stimulated $^{36}Cl^-$ uptake offers a biochemical correlate to the phenomenon of ethanol tolerance. Moreover, these findings suggest that this tolerance develops rapidly following a single hypnotic dose of ethanol. Supported by funds from the Veterans Admin. and USPHS grants AA05233, AA06399 and AA03527.

140.7 REGIONAL DIFFERENCES IN CA1 POPULATION EVOKED RESPONSES IN HIPPOCAMPAL SLICES OF CHRONIC ETHANOL-TREATED RATS.
R. A. Palovcik, B. E. Hunter, and D.W. Walker. Dept. of Neuroscience, Univ. of Fla., Coll. of Med. and V. A. Med. Ctr., Gainesville, FL 32610.

Among the long term effects of chronic ethanol treatment (CET) are changes in excitability of hippocampal CA1 pyramidal neurons to stimulation of the stratum radiatum (SR) fiber pathways. Increases in the amplitude of extracellular evoked population spikes have been demonstrated for dorsal hippocampus in vivo and in vitro with paired pulse stimulation (Abraham et al., 1981 and Rogers and Hunter, 1985). No differences between CET and controls have been found with single pulse stimulation in dorsal hippocampus. It was therefore of interest to determine whether regional differences exist in the magnitude of field potentials between dorsal and ventral hippocampus in CET and control populations. CET rats were fed a nutritionally complete liquid diet containing ethanol for 20 weeks followed by an 8 week withdrawal period. Control rats were pair fed the same diet without ethanol but with isocalorically substituted sucrose. Population evoked responses from CA1 to SR stimulation were evaluated for areas of dorsal, central, and ventral hippocampal slices by systematically varying stimulus current magnitude (input/output functions).

Control animals exhibited significantly smaller population spike amplitudes in ventral hippocampus than dorsal or central hippocampus to SR stimulation. CET animals exhibited no regional differences for SR evoked population spikes. Therefore, CET produced a significant increase in population spike amplitude in ventral hippocampal slices relative to control. No significant differences were found for EPSP amplitudes recorded from stratum pyramidale (SP) or SR between CET and control rats. However, EPSP slopes in ventral hippocampus of control rats were significantly decreased compared to CET rats and dorsal and central hippocampal areas in control rats. There were no significant differences in latency to onset of EPSP in stratum pyramidale, negative EPSP in SR or any population spikes. No significant differences in threshold were detected for elicitation of population spikes between CET and control rats.

Our results indicate a decreased population spike size in ventral hippocampus of normal rats with no concomitant changes in negative EPSP amplitude recorded in SR or positive EPSP amplitude recorded in SP. This suggests that increased population spike amplitude for CET rats in ventral hippocampus is due to decreased inhibition, consistent with previous results. Decreased inhibition may also be reflected in the increased slope of the SP extracellular EPSPs of CET rats. Population spike amplitude for central and dorsal CET hippocampus were also greater than for control hippocampus. Although these were not significant, they may reflect reduced CET effects on dorsal and central hippocampus which can only be detected with paired pulse techniques. Changes in hippocampal excitability due to CET exhibit regional specificity and appear to be greatest for ventral hippocampus.

Supported by the Veterans Administration, Grants NIAAA AA00200 and RCDA AA00065 (BEH).

140.8 ETHANOL TREATMENT ALTERS CNS PLASMA MEMBRANE COMPONENTS IN MICE. B. L. Hungund*, M. J. Modak* and S. P. Mahadik. (Spon. M. Rapport) Dept. of Analyt. Psychopharm. & Div. of Neurosci., N. Y. State Psych. Institute, & Dept. of Biochem. & Mole. Biophys., Coll. of Physicians & Surgeons of Columbia Univ., New York, NY 10032 and Dept. of Biochem. Univ. of UMDNJ, Newark, NJ 07103.

It is now accepted that ethanol exerts its pharmacological effects by altering the physicochemical properties of biological membranes. Some evidence is based on the changes in the lipids and fluidity associated with the development of tolerance to ethanol. We report here the results of the effects of chronic ethanol treatment on a) levels of fatty acid ethyl esters (FAEEs), indicators of structural change in plasma membranes; b) Na,K-ATPase, one of the major markers for plasma membrane function and c) GTP-binding proteins (G-Ps), presumed to be involved in signal (neurotransmitter or hormonal receptor-mediated) transduction across plasma membranes, in membrane fractions from whole brain and subcellular fractions of mouse. Male Swiss-Webster mice were treated with ethanol by an inhalation procedure. The animals were sacrificed by decapitation after 3 and 6 days of exposure to ethanol and the membrane fractions were prepared. The FAEEs were quantitated by gas chromatographic procedure. FAEEs (oleic, C18:1 and linoleic, C18:2 acids) were increased in whole brain membranes as well as in synaptic plasma membranes after 3 days of exposure. Na,K-ATPase activity of whole brain membranes was increased (15%) upto 3 days of treatment and then showed slight decrease after 6 days of treatment. The G-Ps were first labeled by photoaffinity using α-P32-GTP and then labeled proteins were analysed by SDS-slab gel electrophoresis followed by autoradiography (Basu & Modak J. Biol. Chem. 262:2369, 1987). A total of 18 labeled polypeptides were identified in both control as well as alcohol treated membranes. However, differences were noted in the amounts of individual G-Ps. These differences were of two types: 1. the contents of some G-Ps (70 kDa, 59 kDa & 44 kDa) were increased substantially after 3 days of treatment and then decreased after 6 days of treatment, 2. the extractibility of G-Ps with Triton X100 was reduced in alcohol treated membranes compared with controls. It is possible that after alcohol administration, the turnover of these proteins is altered either by their increased synthesis (which is unlikely since overall protein synthesis is shown to be decreased) or by their decreased loss from membrane. Alternately, the level of GTP incorporation is altered as a result of alteration of membrane structure. It is suggested that the chronic alcohol treatment leads to a significant changes in FAEEs which probably contribute to changes in activities of membrane proteins.

140.9 EFFECTS OF ETHANOL ON ARRAYS OF SINGLE NEURONS RECORDED OVER SEVERAL DAYS IN THE RAT CORTEX Chapin, J.K. and Patel, I.M.* Hahnemann University, Philadelphia, PA 19102

Traditional methods of using single unit recording to assess drug effects on the brain are often limited by: 1- the presence of anesthetics, 2- the inability to hold the units long enough to observe a recovery, or long term effects, and 3- the high degree of variance associated with single unit discharge. To alleviate these problems we have implemented the technique of recording many units simultaneously through chronically implanted microwire electrodes (stainless steel, .001", teflon coated). Well isolated cortical neurons may be continuously recorded through such electrodes for periods of up to 21 days in awake, behaving animals. This is advantageous in drug studies because it allows: 1- units to be recorded to full recovery, 2- chronic effects of drugs to be observed, 3- an increased data yield per experiment, 4- internal controls for several different types of experimental variation, and 5- a view of drug effects on the "ensemble" properties of neural networks.

In each of several animals we have implanted bundles of up to eight microwires in either the somatosensory or motor cortex of rats. In experiments, ethanol was administered intraperitoneally 3-4 times over a three-week period. Experimental monitoring of the units' activity was typically done every day whether or not drugs were being administered. Each day the spike waveforms, discharge characteristics, and sensorimotor response properties of each unit were thoroughly scrutinized to determine whether they were the same cells as were recorded the previous day. These units were recorded for one or several hours while the rat alternately ran and rested on a computer controlled treadmill (5 sec ON, 5 sec OFF cycle). A precise record of this motor behavior was obtained by video taping the experiments. Each video frame was automatically labelled with the time in 10 msec increments, allowing post-hoc frame-by-frame analysis for correlation of behavior with the activity of all recorded units.

Use of these techniques allowed the following observations: 1- cortical neurons exhibit a high degree of variability in their response to ethanol. Some cells were virtually unaffected while others recorded simultaneously in the same animal virtually ceased their activity for more than an hour, finally recovering to normal only after up to 8 hours. 2- Tolerance of these single cells to ethanol developed after a single injection. Supported by AA-06965 and AA-00089.

140.10 EFFECT OF CHRONIC ETHANOL TREATMENT ON DOPAMINE RECEPTOR SUBTYPES IN RAT STRIATUM. L. Lucchi*, S. Govoni, M.R. Moresco*, S. Bergamaschi* and M. Trabucchi^. (SPON: N. Brunello) Istitute of Pharmacological Sciences, University of Milan and ^Chair of Toxicology, 2nd University of Rome, Italy.

The chronic treatment with ethanol modifies the postsynaptic transducing mechanisms linked to dopamine receptors. In particular, the adenylate cyclase displays an increased basal activity and a reduced responsiveness to dopamine.

The recent progress in the understanding of adenylate cyclase regulation at striatal level indicates the existence of subclasses of dopaminergic receptors which have an opposite action on cyclic AMP formation (D1 stimulatory and D2 inhibitory).

Along this line, we have revised the effect of chronic ethanol administration (6% in the drinking water for 21 days) on adenylate cyclase activity by measuring the dopamine promoted cyclic AMP formation and the ability of selective D2 agonists to inhibit adenylate cyclase. In addition, the effect of chronic ethanol treatment on dopamine recognition sites was assessed by means of the measurement of the binding of specific ligands. SCH 23390 was used as selective D1 dopamine receptor probe. Dopamine D2 recognition sites were measured using tritiated spiperone as ligand and sulpiride as displacer. This technique labels D2 sites functionally correlated with the dopamine-inhibited adenylate cyclase (Govoni et al., Brain Res. 381:138,1986).

Ethanol treatment affected both the dopamine stimulated and the dopamine inhibited cyclases. The ability of dopamine to stimulate cyclic AMP was less than half in homogenates prepared from treated animals (80% in controls, 30% in ethanol treated). Bromocriptine inhibited cyclic AMP formation by 37% in homogenates prepared from control striata and by 14% in treated animals.

In striatal synaptic membranes prepared from ethanol treated rats the sulpiride displaceable tritiated spiperone binding was reduced by 27% and tritiated SCH 23390 by 25%.

The data show that striatal adenylate cyclase is less stimulated by dopamine and less inhibited by bromocriptine in homogenates prepared from rats chronically treated with ethanol. The binding results indicate that both dopamine-receptor types are reduced by the chronic ethanol treatment paralleling the functional results on cyclase activity.

140.11 CHRONIC ETHANOL TREATMENT REDUCES CHOLERA TOXIN MEDIATED [^{32}P]ADP-RIBOSYLATION OF 46 KILODALTON PROTEIN IN CEREBRAL CORTICAL MEMBRANES OF C57BL MICE. P. T. Nhamburo, P. L. Hoffman and B. Tabakoff. (SPON: C. Rabe). Lab. of Physiologic and Pharmacologic Studies, NIAAA, Bethesda, MD 20892

Receptor regulated adenylate cyclase (AC) system consists of three components; receptor, catalytic unit and guanine nucleotide binding proteins (G). The alpha subunits of the inhibitory (α_i) (Mol. Wt. ranging 39-41 Kd) and stimulatory (α_s) (Mol. Wt. ranging 42-52 Kd) G-proteins contain a site which is ADP-ribosylated by pertussis (islet-activating protein) and cholera toxins, respectively. Chronic administration of ethanol to mice in vivo in a paradigm that produced tolerance to and physical dependence on ethanol has been reported to cause significant decreases in the Gpp(NH)p-dependent AC activity and in the apparent coupling of β-adrenoceptors to G_s. To elucidate the mechanism of these changes, we have therefore, measured [^{32}P]ADP-ribosylation catalysed by either cholera or pertussis toxin in ethanol-treated animals. Male mice (C57BL/6) were fed a liquid diet containing either 7% (v/v) ethanol or an equicaloric amount of sucrose (controls) for 7 days. Cerebral cortical membranes from naive (chow-fed), control and ethanol-treated mice were incubated with 100 μM [α-32P]NAD for 60 minutes at 30°C, in the presence or absence of either preactivated toxin. [^{32}P]ADP-labelled proteins were then separated by SDS-polyacrylamide gel electrophoresis, and ^{32}P estimated by autoradiography. Although several protein bands were [^{32}P]ADP-ribosylated by cholera toxin, only the 46Kd band was signficantly decreased (30-50%) following ethanol treatments in vivo compared to those from either controls or naive mice. There was no significant change in [^{32}P]ADP-ribosylation of pertussis toxin substrate (40 Kd band). The decrease in ADP-ribosylation of the 46 Kd protein which shares the same molecular weight as that reported for G_s, could indicate a reduced amount of this protein, or structural changes or defects in this protein so that it is altered as a substrate for ADP-ribosylation by cholera toxin.

140.12 DO GLUCOCORTICOIDS PLAY A ROLE IN HIPPOCAMPAL CELL LOSS DURING CHRONIC ETHANOL TREATMENT? G. Rachamin*, W.G. Luttge, B.E. Hunter and D.W. Walker. Department of Neuroscience, College of Medicine, University of Florida, and VA Medical Center, Gainesville, Fl 32610.

Both chronic exposure to ethanol and aging result in loss of hippocampal pyramidal neurons. Recent reports indicate that corticosterone (CORT) receptors and CORT-concentrating cells are depleted in the hippocampus of aged rats, due to cumulative exposure to glucocorticoids over the life span. We have investigated the hypothesis that glucocorticoids play a similar role in the production of hippocampal cell damage during chronic ethanol treatment (CET), thus leading to selective losses of glucocorticoid receptors and glucocorticoid-concentrating cells.

Liquid diet containing ethanol (36% of total calories as ethanol) was administered to male Long-Evans rats for 20-24wks (starting at 3mo of age), a period of CET previously found in our laboratory to produce hippocampal neuronal cell loss. Control rats were pair-fed with identical diets, except for isocaloric substitution of sucrose for ethanol. Morning blood ethanol concentrations in the ethanol-fed rats averaged 149±20mg%. At the end of CET animals were adrenalectomized, and 24hrs later specific binding to cytosolic glucocorticoid receptors in the hippocampus was determined. Specific binding of [^{3}H]dexamethasone to Type II receptors was not significantly different between the two groups (Ethanol=236±10 and Sucrose=285±48 fmoles/mg protein). Similarly, no significant differences in [^{3}H]aldosterone specific binding to Type I receptors (in the presence of RU26988 to block binding to Type II receptors) were observed between ethanol and control animals (53±8 and 71±12 fmoles/mg protein, respectively). Basal morning plasma CORT concentrations in the ethanol rats were within the normal physiological range, while those in control rats were significantly elevated (5.61±2.19 versus 59.41±12.15ng/ml, $p<0.001$). Since our CET studies were conducted in Long-Evans rats we also measured adrenocorticosteroid binding in young (3-5mo) and old (24-26mo) male Long Evans rats. There was no significant influence of age on binding to Type II (Young=237±32 and Old=245±13 fmoles/mg protein) nor to type I (Young=70±8 and Old=60±6 fmoles/mg protein) receptors in these animals. In conclusion, the data do not appear to support the idea that glucorticoids play a role in the production of hippocampal neuronal damage during CET or aging in the Long Evans strain of rats. However, the possibility that alcohol may have a differential effect on glucocorticoid receptors in the left and right hippocampus or in different regions within it can bot be excluded.

This research was supported by the Vetrans Administration and Grants AA00200, AA05793, and RCDA AA0065 (B.E.H.) from the NIAAA

140.13 EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON [^{3}H] INOSITOL TRISPHOSPHATE SPECIFIC BINDING IN MOUSE CEREBELLUM. T.L. Smith and S. Battiste-Milton. Veterans Administration Medical Center, Tucson, AZ 85723 and Dept of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Inositol 1,4,5 trisphosphate (IP_3), a product of receptor-mediated phosphoinositide hydrolysis, has been identified as a second messenger which mobilizes intracellular Ca^{2+}. Specific saturable binding of radiolabeled IP_3 has been demonstrated in adrenal cortex, hepatocytes, neutrophils, and more recently in rat brain. Since ethanol is known to affect neuronal Ca^{2+} disposition, it was of interest to determine 1.) whether specific binding sites can also be observed in mouse brain and 2.) to determine what effects ethanol added in vivo and in vitro may have on this system. Membrane fragments from discrete brain regions of C57/BL mice were suspended in 20 mM TRIS (pH 7.7) containing NaCl, 20 mM; KCl, 100 mM; EDTA, 1 mM. Tissue samples (250-600 ug protein) were incubated for 10 mins at 0° in the presence of 2.5-80 nM [^{3}H]IP_3 (3.5 Ci/mmol) in a final volume of 0.5 ml. The reaction was stopped by rapid vacuum filtration and the filters rinsed with three 3 ml aliquots of ice-cold 50 mM TRIS (pH 7.7). Differences between measurements in the absence and presence of 10uM IP_3 were used to define specific binding. The rank order of [^{3}H]IP_3 specific binding capacity was: cerebellum > hippocampus > cortex > midbrain. Scatchard analyses of the binding data for cerebellum yielded K_D and Bmax control values of 62 nM and 3.86 pmoles/mg protein, respectively. Ethanol, at or above 100 mM, enhanced [^{3}H]IP_3 binding by c.a. 15% in control mice. In contrast, chronic consumption of an ethanol-containing liquid diet (7%, v/v) for 8 days resulted in a significant decrease ($P < 0.05$) in specific binding: 2.42 ± 0.60 pmoles/mg protein vs 3.89 ± 0.77 pmoles/mg protein for pair-fed controls in the presence of 50 nM [^{3}H]IP_3. It is concluded that IP_3 binding varies greatly in different brain regions and that ethanol given in physiological concentrations has a modest effect on this system. (supported by a Vet. Admin. Medical Research grant.)

140.14 CHANGES IN THE DENTATE FASCIA FOLLOWING CHRONIC ETHANOL EXPOSURE IN LS AND SS MICE. A. J. Scheetz, J. A. Markham and E. Fifkova. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Short-Sleep (SS) and Long-Sleep (LS) mice differ in the duration of acute ethanol induced narcosis. The SS mice are relatively unaffected by acute doses of ethanol that are sufficient to induce several hours of sleep in the LS mice. We have previously shown that chronic ethanol exposure results in a decrease in the frequency of basket cells subjacent to the granule cell layer of the dentate fascia in the ethanol sensitive LS mice but not in the ethanol insensitive SS mice (Scheetz et al., Brain Res., 403:151-154, 1987). The present report details information regarding the specificity and time course of this effect. Mice were grouped into one of three conditions: Ethanol, nonethanol liquid diet and standard lab chow receiving groups. At the end of the 3 months of ethanol exposure animals were perfused with glutaraldehyde and embedded in epon. Five 1 μm sections, 60 μm apart, were obtained from each tissue block. The sections were mounted and stained with toluidine blue. We exposed another group of SS and LS mice to 20 days of ethanol with the concentration of ethanol gradually increased from 11.5% ethanol derived calories (EDC) to 23% EDC to 35% EDC over a period of 2, 7, and 11 days respectively. The perfusion and tissue preparation was the same as in the preceding experiment. Basket cells were identified according to the criteria of Ribak and Anderson (J Comp. Neurol., 192:903-916, 1980). The frequency of basket cells was defined as the number of basket cells per unit length of the granule cell layer. The density of granule cells was computed by dividing the total number of granule cells by the total area of the granule cell layer. In these two experiments there was no significant difference in the density of granule cells between LS control and LS ethanol animals. Likewise, no differences in density of granule cells were found between SS control and ethanol mice. In the short-term exposure experiment there was a clear, but insignificant, trend in the LS groups for the ethanol treated animals to have a lower frequency of basket cells. Because glial proliferation is known to precede neuronal degeneration, we counted the density of glial cells in these slides. It was found that the density of glial cells in the LS ethanol animals was significantly higher than in the LS control group. The density of glial cells in the two SS groups was not significantly different. No such glial proliferation was observed in the tissue from the 3 month exposure experiment, indicating that a majority of the cell loss has occurred before 3 months. The fact that we found the glial proliferation after only 20 days of ethanol exposure indicates that the effect of ethanol on the loss of basket cells may be occurring during the first month of chronic ethanol exposure. None of these effects is accompanied by the loss of the principal neurons in this region. Supported by NIAAA grant AA06196.

140.15 THE EFFECT OF ETHANOL WITHDRAWAL ON GABA FUNCTION IN THE IN VITRO HIPPOCAMPUS. T.J. Chesnut. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY, 12201.

Changes in gamma-aminobutyric acid (GABA) mediated systems in the central nervous system have been suggested to be involved in ethanol withdrawal induced hyperexcitability. The in vitro brain slice preparation of rat hippocampus was used to determine the effects of ethanol withdrawal on the GABA-mediated recurrent inhibitory pathway of area CA1. Wistar rats were rendered ethanol dependent by feeding them ethanol in a liquid diet (Bio-Serve, Inc.) at a concentration supplying 35% of the total calories in the diet. Rats treated thusly for 9 days became highly ethanol dependent as documented by the behavioral criteria of Majchrowicz (Psychopharmacol., 43:245-254). Blood alcohol levels (BALs) were measured in these animals and indicated that the BALs at 9 days averaged 50 mM. This information was used to develop an "in-chamber" withdrawal technique by assuming that the cerebrospinal fluid ethanol concentration was also 50 mM (Newlin et al, Brain Res., 209:113-128).

Transverse slices 375 um thick were cut from hippocampi obtained from animals rendered ethanol dependent as indicated. All slices were incubated at 32-33°C in oxygenated artificial cerebrospinal fluid (ACSF) constantly perfused through the experimental chamber. The moment of ethanol withdrawal was controlled by preparing and incubating the slices in ACSF containing 50 mM ethanol. Withdrawal could then be initiated when desired by changing the perfusate to ethanol-free ACSF. Comparison of measurements obtained both before and after such "in-chamber" withdrawal can therefore indicate changes at the moment of withdrawal under conditions that can be directly related to behavioral dependence.

Surprisingly, intracellular measurements obtained from 13 pyramidal cells indicated no withdrawal-induced hyperexcitability except for one cell that displayed spontaneous depolarizing potentials lasting up to 10 seconds in length upon which were superimposed action potentials. Changes in the post-synaptic potential (PSP) recorded due to activation of the GABA-mediated recurrent inhibitory pathway were measured by obtaining current-voltage curves, both in the presence and in the absence of the GABA-mediated response. No withdrawal-induced changes in the resting input conductance of the cell or in the reversal potential of the PSP were observed. The effects of withdrawal on the conductance due to the PSP have not yet been determined due to the small number of cells. The studies do indicate, however, that the described method of "in-chamber" withdrawal permits detailed investigation of the effects of ethanol withdrawal on GABA function under conditions directly related to behaviorally-induced ethanol dependence.

This work supported by NINCDS grant #NS22231.

140.16 **INTENSITY OF PENTYLENETETRAZOL-LIKE DISCRIMINATIVE STIMULUS PRODUCED BY ETHANOL WITHDRAWAL DEPENDS ON DOSE AND DURATION OF ETHANOL GIVEN IN NUTRITIONALLY BALANCED DIET.** D. Benjamin*, C.M. Harris, S. Bhadra, M.W. Emmett-Oglesby, and H. Lal. (SPON: L.J. Achor) Dept of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth TX 76107.

Ethanol withdrawal produces anxiety which may motivate continued ethanol abuse. We have developed the pentylenetetrazol (PTZ) discrimination paradigm (Lal and Emmett-Oglesby, Neuropharmacology 22:1423, 1983) as a bioassay (Lal and Fielding, Drug Dev. Res., 4:3-21, 1984) to investigate anxiogenic treatments, including ethanol withdrawal. PTZ is anxiogenic in humans and produces an interoceptive discriminative stimulus (IDS) in rats that is mimicked by anxiogenic drugs and antagonized by anxiolytic drugs. Rats trained to discriminate PTZ and given ethanol by gavage exhibit a PTZ-like IDS during withdrawal (Lal et al., Fed. Proc. 46:1301, 1987); however, the only source of nutrition in the previous experiments was the ethanol or sucrose solution. To test if the PTZ-like withdrawal stimulus could be demonstrated when rats are adequately nourished during ethanol treatment, a nutritionally complete diet was used as the vehicle (Benjamin et al., Fed. Proc. 46:712, 1987) for ethanol. The rats were given the ethanol-diet mixture for 4 days, followed by a terminal dose (3 g/kg) of ethanol given by gavage, and tested for blood ethanol concentrations or lever-selection. Blood ethanol peaked at 3 mg/ml, at 3 h after gavage, and declined to 0.6 mg/ml by 12 h after the final dose, at which time 90% of ethanol-treated rats selected the PTZ-appropriate lever. The percent of rats selecting the PTZ-lever declined to baseline values within 72 h. This withdrawal IDS was dose-dependently antagonized by ethanol or diazepam. When the duration or dose of the ethanol treatment was varied, the percent of rats selecting the PTZ-appropriate lever during withdrawal varied accordingly. The intensity of the PTZ-like IDS was maximal after a daily ethanol dose of 12.5 g/kg, given for 3 days. These results suggest that withdrawal from ethanol given in a nutritionally complete diet produces a discriminative stimulus comparable to the stimulus produced by anxiogenic drugs. In addition, the intensity of the withdrawal stimulus varies directly with the dose and duration of ethanol treatment. (Supported by NIAAA grant #R01-AA06890)

140.17 EFFECT OF CHRONIC ETHANOL WITHDRAWAL ON SEIZURE RESPONSES TO INFERIOR COLLICULUS MICROINJECTIONS OF BICUCULLINE METHYLIODIDE, PICROTOXININ AND KAINIC ACID. Gerald D. Frye. Dept. of Medical Pharmacology, Texas A&M University College of Medicine, College Station, TX 77843.

Susceptibility to sound-induced wild running, clonic-tonic seizures is a well documented characteristic of physical dependence on ethanol in rats undergoing withdrawal. This susceptibility to seizures can be blocked by microinjection of gamma-aminobutyric acid (GABA) agonists into the inferior colliculus. An adaptive reduction in GABAergic inhibition in the inferior colliculus may be responsible for sound seizure susceptibility since similar wild running, clonic-tonic seizures can also be induced in ethanol-naive rats by microinjections of GABA antagonists, bicuculline methiodide (BMI) and picrotoxin into the inferior colliculus (Frye, G.D., J. Pharmacol. Exp. Ther. 237:478, 1986). The present experiments compared the sensitivity of inferior colliculi in ethanol-dependent and ethanol-naive rats to bilateral microinjections of BMI, picrotoxinin (PIC) and the excitatory amino acid agonist, kainic acid (KA; infusion rate = 0.5ul over 5min). The ED_{50}s for wild running seizures in ethanol-naive rats were approximately, BMI (10-pmol), PIC (11.0pmol), KA (17.0pmol). ED_{50} estimates for wild running seizures in withdrawing ethanol-dependent rats were not different from those of ethanol-naive animals for any of the agents. However, when more severe clonic seizure responses were considered, ED_{50}s in withdrawing ethanol-dependent rats were significantly lower than those for ethanol-naive animals for all three convulsants. These results suggest that ethanol dependence increases susceptibility to clonic seizures that may be due to enhanced rostral seizure spread from the inferior colliculus, while susceptibility to wild running responses that originate more directly from the inferior colliculus are unaltered. However, ethanol dependence does not appear to evoke significant changes in the relative sensitivity of the inferior colliculus to seizures caused by GABA antagonists when compared to excitatory amino acid agonists, as might be expected if GABAergic adaptation had occurred. This work was supported in part by Public Health Service Grant AA06322.

140.18 ETHANOL WITHDRAWAL IN WSP AND WSR SELECTIVELY BRED MICE: MEASURES ACROSS GENERATIONS. A. Kosobud, E. Young*, B. Tam*, C. Deutsch*, and J.C. Crabbe. Departments of Medical Psychology and Pharmacology, Oregon Health Sciences University and Veterans Administration Medical Center, Portland, OR, 97201.

Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) lines of mice have been selectively bred for differences in withdrawal following chronic inhalation of ethanol (EtOH) vapor. Mice are selected on the basis of severity of handling-induced convulsions (HIC) during withdrawal. In the experiments presented here, withdrawal was assessed in WSP, WSR and WSC (a non-selected control) lines of mice between the 10th (S_{10}) and 17th (S_{17}) selected generation, and following three generations of relaxed selection (S_{24}). Blood ethanol concentrations (BEC) were measured during induction of physical dependence and during withdrawal. During withdrawal, HIC and body temperature were recorded, and mice were observed for other signs of withdrawal including tremor, straub tail and backward walking. Comparisons of earlier selected generations (Kosobud and Crabbe, J. Pharmacol. Exp. Ther., 238:170-177, 1986) showed that WSP and WSR mice reached similar BEC during intoxication, and eliminated EtOH at a similar rate. During withdrawal, WSP mice consistently showed more severe HIC and tremor, and tended to show more straub tail than WSR mice. In the present experiments, WSP mice showed more severe HIC, tremor and straub tail during withdrawal than WSR mice, while WSC mice were intermediate. In S_{17}, the amount of hypothermia seen during withdrawal was similar in WSP and WSR mice, but in S_{24}, hypothermia was greater in WSP than WSR mice. BEC were similar in WSP, WSC and WSR mice in the early generations, but in later generations WSP mice reached higher BEC during intoxication, and appeared to eliminate ethanol more slowly, than WSR mice. In summary, WSP and WSR mice also differ in severity of signs of EtOH withdrawal other than HIC, confirming that the selection has altered some general properties of EtOH withdrawal. Three generations of relaxed selection did not result in a large reduction in selection response, suggesting that many of the genes responsible for the withdrawal difference in these lines are fixed. A small difference in EtOH metabolism is present in the most recent generations of these lines, and is probably making some contribution to the difference in withdrawal severity seen in WSP and WSR mice. These experiments are supported by PHS-NIAAA grants AA05828, AA06243, AA06498 and by the Veterans Administration.

140.19 GABA MODULATION OF ELECTRICAL SEIZURE DISCHARGE DURING ETHANOL WITHDRAWAL L.P. Gonzalez and J.F. Czachura* . Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Previous reports from this laboratory have suggested that GABAergic neurons of the substantia nigra may be regulators of ethanol (EtOH) withdrawal seizures and related symptomatology. In those studies, we observed a significant suppression of induced motor convulsions following intranigral administration of the GABA agonist muscimol or of the benzodiazepine flurazepam. In order to further evaluate the role of GABA-receptive neurons in the EtOH withdrawal syndrome, we examined the effects of local injections of muscimol on EEG spikes and paroxysmal activity observed in cortical and subcortical sites during withdrawal. Sprague-Dawley rats received chronic bilateral implants of guide cannulae placed just above the substantia nigra zona reticulata (SNR). Monopolar, semi-microelectrodes were implanted within the amygdala, hippocampus, inferior colliculus (IC), and SNR, and bilateral electrodes over visual cortex. One week after surgery the animals received chronic EtOH exposure in EtOH-vapor inhalation chambers. Following sixteen days of chronic exposure, the animals were removed from the chambers and were observed for evidence of withdrawal hyperexcitability. Eight hrs. after removal from the chamber, 30 min. of EEG activity was recorded from the implanted electrodes. Animals then received bilateral injections (0.5 ul) into SNR of either muscimol (15, 30, or 60.0 ng per side) or vehicle. Twenty min. after injection, EEG activity was again observed and recorded for an additional 30 min. Four to six hrs. after injection, the animals were tested for susceptibility to audiogenic seizures. Control subjects received the same handling and treatment, but no exposure to EtOH.

EtOH withdrawal was accompanied by the occurrence of EEG spikes at all of the sites observed, but most consistently in SNR and in IC. Intranigral muscimol injection resulted in a significant decrease in spike activity in both SNR and in IC, but had no effect on cortical spikes; effects at other subcortical sites were inconsistent. Although several interpretations of these results are possible, they suggest that nigral GABA regulation of EtOH withdrawal seizure activity may be due to effects on IC, which has been implicated in the mediation of audiogenic seizures, and perhaps also on influences of SNR on descending motor pathways. Further, it appears that nigral GABA neurons may not be involved in regulation of forebrain seizure activity during EtOH withdrawal.

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TRANSPLANTATION III

141.1 PHYSIOLOGICAL COMPARISON OF NORMALLY DEVELOPING STRIATAL NEURONS AND TRANSPLANTED STRIATAL NEURONS IN RATS. J.P. Walsh, F.C. Zhou, C.D. Hull, R.S. Fisher, M.S. Levine and N.A. Buchwald. Mental Retardation Research Center. University of California, Los Angeles. 760 Westwood Plaza, Los Angeles, CA 90024.

Intracellular techniques were used in brain slices to study transplanted striatal neurons (TSNs), neonatal striatal neurons and adult striatal neurons. To produce TSNs, fetal striatal tissue (E14) was implanted as a dissociated cellular suspension into the striatum of adult rats. Intracellular records were obtained 2-6 weeks after transplantation. The graft was easily identifiable in each brain slice study as a separate nonmyelinated structure within the host striatum. Differences in biophysical, synaptic and morphological properties were found between TSNs and adult striatal neurons. These dissimilarities appeared to be related principally to the lower developmental age of the TSNs versus the host striatal neurons. To test this possibility, striatal neurons from rat pups, age-matched with the TSNs, were also examined. The results indicated a general agreement in neuronal properties between the TSNs and rat pup neurons.

Responses of neurons to hyperpolarizing current pulses were used to determine input resistance. The input resistance of TSNs (20 to 27 days after transplantation) was 30.7±1.13 mΩ (n=6), while age matched pups (10-16 days postnatal) had an input resistance of 26.5±4.8 mΩ (n=7). By contrast, the input resistance for adult striatal neurons was 14.5±1.5mΩ (n=11).

Stimulation of adjacent host striatum elicited both excitatory and inhibitory postsynaptic potentials (PSPs) in TSNs (20-38 days post transplant). Similarly, striatal neurons of age matched pups displayed combined excitatory and inhibitory PSPs in response to local stimulation. In adult striatal neurons, by contrast, only excitatory PSPs were evoked.

The morphology of individual neurons was examined following intracellular injection of the fluorescent dye, lucifer yellow. TSNs characteristically had varicose swellings along dendritic shafts and a relatively sparse distribution of spines. These finding were similar to those in striatal neurons of neonatal rats. Medium-sized spiny neurons of the adult striatum, however, lacked dendritic varicosities, had a very dense distribution of dendritic spines and tended to have more complex dendritic fields. Each of the properties examined in this study suggests that TSNs follow the usual developmental pattern expected for maturing medium-sized spiny neurons of the striatum. Supported by HD-07032.

141.2 LONG TERM SURVIVING EMBRYONIC GRAFTS OF STRIATAL TISSUE IN RAI BRAINS. F.C. Zhou, N.A. Buchwald, C.D. Hull, R.S. Fisher and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024.

Embryonic brain tissue has been grafted into host brains in many species of animals. In almost all cases the graft survived for a relatively short period of time as compared to the life span of the species involved. The short period of graft survival leads to two as yet, unanswered questions. First, will brain grafts survive long enough to be considered as "permanent" constituents of the recipients brain? Second, if a humoral and/or connective interaction occurs between the graft and the host brain, is this interaction "durable" as compared to the life-span of the species involved. We investigated these questions by grafting neo-striatal tissue into brains of 2-month old Sprague-Dawley rats and allowing the recipients to survive for 17 months (the average life-span of rats is 2-3 years and they are considered to be aged at 17-18 months).

Dissociated striatal cells from 14 day embryonic rats were grafted into striatum (n=7) and substantia nigra (n=3) of the 2 month old recipients. Eight animals survived for seventeen months. Of these, 6 had sizeable (dia. >0.7mm) transplants (TP) [striatum, n=4; nigra, n=2]. Immunocytochemical staining showed that a large population of neurons staining positive for gamma aminobutyric and existed in the TPs. Acetylcholinesterase (AcHE) staining showed that AcHE-positive cells were scattered throughout the TPs.

In general, neurons in the TP seemed to be normal at least in terms of nuclear position. Staining with an antiserum to the catecholaminergic enzyme, tyrosine hydroxylase (TH), showed that TH-positive fibers from the host had penetrated into the grafts and formed dense terminal boutons. These TH-positive terminal boutons existed in a patchy pattern similar to that which occurs at 6-7 weeks of graft survival. In the long term survivals however, the bouton density of TH fibers appeared greater than in the 6-7 week grafts. These results indicate that grafted neurons can survive through the adult life-span of the recipient species, and that host TH-positive fiber ingrowth to the TP is not a transitory phenomenon. Supported by USPHS Grant HD05958.

141.3 **EFFECTS OF A UNILATERAL ASPIRATIVE LESION OF THE FIMBRIA-FORNIX ON HIPPOCAMPAL ELECTROENCEPHALOGRAPHIC AND GLUCOSE METABOLIC ACTIVITY.** R.W.Jonnson*, R.D.Vingan*, D.L. Dow-Edwards, T.H. Milhorat, and J.L. Rubie. Laboratory of Cerebral Metabolism, Department of Neurosurgery and Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, N.Y. 11203

Adult female Long-Evans rats were implanted with unilateral hippocampal chronic recording electrodes. Baseline hippocampal theta activity generated within Ammon's horn and dentate gyrus was obtained for each rat. Animals were then divided into two groups: (1) unilateral fimbria-fornix lesion and, (2) sham operation. The lesion consisted of aspiration of the fimbria-fornix and overlying neocortical tissue ipsilateral to the recording electrode. Sham operated rats underwent identical operative exposure of neocortex without lesioning of the intracranial contents.

At 1 week intervals following surgical intervention, theta wave activity was recorded during treadmill walking and in the anesthetized state (using ethyl ether).

After 3 months, each rat was prepared for measurement of cerebral glucose metabolic activity according to the [^{14}C] 2-deoxyglucose protocol of Sokoloff. Under halothane anesthesia each rat (375-425g) received a femoral arterial and a venous catheter which were threaded subcutaneously and externalized at the dorsum of the neck. The rats were allowed to awaken for a minimum of 2 hours. [^{14}C] 2-deoxyglucose was administered as an intravenous pulse (125 uCi/kg). Each animal was freely moving on a treadmill apparatus and timed arterial samples were then taken for determination of the time course of the arterial plasma concentrations of [^{14}C] deoxyglucose and glucose. At 45 minutes the animals were sacrificed with sodium pentobarbital. The brains were removed, frozen and later sectioned for autoradiography. Sections were also taken for acetylcholinesterase staining and thionin staining. Autoradiographs were analyzed densitometrically to determine the local concentrations of ^{14}C in the tissues. From the local tissue ^{14}C concentrations and the time courses of the plasma [^{14}C] deoxyglucose and glucose concentrations, local rates of glucose incorporation were calculated by the operational equation of the method.

Results demonstrate that complete unilateral fimbria-fornix lesions lead to sustained loss of ipsilateral hippocampal theta activity as well as loss of acetylcholineserase staining. Glucose metabolic activity is also diminished in several regions of the ipsilateral hippocampus.

141.4 **RESTORATION OF HIPPOCAMPAL THETA RHYTHM AND GLUCOSE METABOLISM BY EMBRYONIC SEPTAL CELL SUSPENSION GRAFTS FOLLOWING UNILATERAL FIMBRIA-FORNIX LESION.** R.D. Vingan*, D.L. Dow-Edwards, T.H. Milhorat, L.A. Freed* and S.E. Fox. Laboratory of Cerebral Metabolism, Department of Neurosurgery and Department of Physiology, SUNY Health Science Center @ Brooklyn, Brooklyn, N.Y. 11203

Electroencephalographic patterns, glucose metabolic activity and cholinergic neurochemical anatomy were analyzed as indices of hippocampal activity in adult female Long-Evans rats following unilateral fimbria-fornix lesions and subsequent transplantation of embryonic septal cell suspensions.

Each rat received a unilateral hippocampal chronic recording electrode for determination of baseline theta wave activity in the walking and anesthetized states. Animals were divided into 2 groups: (1) unilateral fimbria-fornix lesion only (control), and (2) lesion with embryonic day 15-16 septal cell suspension transplants (experimental). The lesion consisted of aspiration of the fimbria-fornix and overlying neocortex ipsilateral to the recording electrode. Septal transplant tissue (5 ul) was injected over a 1 minute period into the lesion cavity of the experimental group immediately after the lesion was completed.

At 1 week intervals following surgical intervention, hippocampal theta activity was recorded during treadmill walking and in the anesthetized state (using ethyl ether).

After 3 months, each rat was prepared for measurement of cerebral glucose metabolic activity according to the quantitative [^{14}C] 2-deoxyglucose protocol of Sokoloff. Under halothane anesthesia each rat (375-425g) received a femoral arterial and a venous catheter which were threaded subcutaneously and externalized at the dorsum of the neck. The rats were allowed to awaken for a minimum of 2 hours. Each animal was freely moving on a treadmill apparatus and timed arterial samples were then taken. At 45 minutes the animals were sacrificed with sodium pentobarbital. The brains were removed, frozen and later sectioned for autoradiography. Sections were also taken for acetylcholinesterase staining and thionin staining.

Results indicate that embryonic day 15-16 septal cell suspension implants restore activity to the denervated hippocampus with normalization of EEG patterns, glucose metabolism and cholinergic neurochemistry. Work on the cholinergic relationship to glucose metabolism and EEG activity is currently being analyzed with the use of eserine (i.p. 0.1-0.15 mg/kg).

141.5 PROTECTION OF CHOLINE ACETYLTRANSFERASE- AND NERVE GROWTH FACTOR RECEPTOR- IMMUNOREACTIVE NEURONS IN NUCLEUS BASALIS MAGNOCELLULARIS BY EMBRYONIC CORTICAL TRANSPLANTS INTO DEVASCULARIZED RAT NEOCORTEX.
E.P. Pioro* and A.C. Cuello (SPON: B.E. Jones). Dept. of Pharmacology and Therapeutics, McGill University, Montreal, PQ, H3G 1Y6, Canada.

Choline acetyltransferase (ChAT) immunoreactive neurons in the rat nucleus basalis magnocellularis (NBM) project to the cortex (Rye, et al., Neuroscience, 13: 627, 1984) and also display nerve growth factor receptor (NGFr) immunoreactivity (Taniuchi, et al., PNAS, 83: 1950, 1986). We have previously shown that protection from retrograde degeneration of these neurons in response to a devascularizing neocortical lesion can be achieved with pharmacological means, such as gangliosides (Cuello, et al., Brain Res, 376: 373, 1986). A morphological study was undertaken to determine if embryonic cortical cells implanted into devascularized rat neocortex could similarly prevent retrograde neuronal degeneration in this nucleus as has been shown with kainic acid-induced cortical lesions (Sofroniew, et al., Brain Res, 378: 409, 1986).

Cell suspension preparations of cortex obtained from rat embryos were injected into previously devascularized neocortex of 3 month old rats. After a 6 month post-transplantation survival, rats were perfusion-fixed and brains were processed for peroxidase immunocytochemistry using monoclonal antibodies against ChAT (provided by F. Eckenstein and H. Thoenen) and rat NGFr (provided by E. Johnson). The lesioned cortical area which formed a cavity in untreated rats was replaced in some rats receiving cortical implants by proliferated neural tissue. Retrograde neuronal degeneration as revealed by ChAT and NGFr immunostaining was noted in the NBM ipsilateral to lesioned neocortex in untreated animals. However, in rats receiving implants, these neurons showed less degenerative changes which will be discussed. In addition, the NBM neurons ipsilateral to the transplant demonstrated an abundant plexus of NGFr immunopositive neurites.

The findings demonstrate survival of embryonic cortical cells implanted into previously devascularized rat neocortex and at least a partial protection from retrograde degeneration of ChAT and NGFr immunoreactive neurons in the NBM.

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141.6 TRANSPLANTS OF CELL SUSPENSIONS FROM FETAL TECTUM INTO ADULT RAT.
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Embryonic tectal tissue was implanted into normal adult hosts as a step in establishing a model for neural transplantation. The study had two related objectives: to determine the viability of transplanted embryonic tectal cells, and to evaluate the efficacy of True Blue, a flourescent dye, in labeling transplanted cells.

Developing tecta were dissected from Sprague-Dawley (S-D) rat fetuses at 16 days of gestation (E16). The tissue was processed into a cell suspension with a modification of standard procedure (cf. Bjorklund et al., 1983, Acta Physiol. Scand. Supp., 522: 1-10). At the incubation phase E16 tecta were suspended in 2% (w/v) True Blue (Sigma) in a .1% trypsin in .6% D-glucose medium. After one hour exposure to True Blue the tissue was rinsed, resuspended in D-glucose solution, and injected into S-D adult male rats. Host animals received bilateral 10 µl injections of the tissue suspension. Implants to the cerebellum were placed stereotaxically just caudal to the inferior colliculi. Adult control rats received bilateral 10 µl injections of 2% True Blue in D-glucose solution at similar sites. After four weeks survival animals were sacrificed and perfused with saline and buffered formalin. Every fourth section was saved for Nissl processing and stained with thionin.

In Nissl material host animal transplant sites were highly vascularized, densely packed, heterogeneous cellular areas in the folia white matter. Small, darkly stained, disc shaped and slightly larger spherical cells predominated; the former possibly small collicular stellate cells and the latter cerebellar granule cells. Normal cerebellar medium size, violet, fusiform Golgi cells and large, opaque violet Purkinje cells were also seen. Atypical to cerebellum were green-blue stained, rectangulated, polygonal cells similar in size to the medium multipolar cells of dorsal cortex and central nucleus of inferior colliclus. Observations of Nissl sections in controls revealed less densely packed and vascularized injection regions predominated by green-blue stained cerebellar Golgi, Purkinje, and molecular layer spheroidal cells. In controls green-blue stained cells were found throughout a greater region of cerebellum than in hosts. Control granule cells were darkly stained but less opaque than putative small tectal stellate cells found in host cerebellum. In host animals trails of flourescent cells were observed along cannula tracts in the cerebellum with prominent clusters of cells seen at the top and bottom of injection sites. In contrast, flourescent cells in controls followed Purkinje cell layers through much of the cerebellum. While flourescence sections did show retrograde labeled brain stem cells in all animals, it was apparent that transplanted tectal cells were present in host brains.

141.7 CROSS-SPECIES TRANSPLANTS OF FLOW CYTOMETRY SORTED VENTRAL MESENCEPHALIC BOVINE CELLS. J.J. Lopez-Lozano. Dept. of Neurology and Dept. of Experimental Surgery. Clinica Puerta de Hierro. Universidad Autonoma. 28035-Madrid. Spain.

Our research group is interested in analyzing and isolating specific populations of CNS cells to be used for in vitro (i.e: cell culture) or in vivo (i.e: cell transplant) studies. Laser flow cytometry has been found to be an appropriate tool for the analysis and isolation of large numbers of cells and specifically for discrimination and separation among heterogeneous cell populations. Since we reported that relatively pure Tyrosine hydroxylase (TH)-containing neurons from neonatal ventral mesencephalon could be obtained by flow cytometry and cell sorting techniques (FCACS) (Lopez-Lozano et al., Soc. for Neuroscience, 1985) and that FCACS-cells from the anterior hypothalamus survive, migrate and express vasopressin after being implanted into neurohypophysectomyzed adult rats (Lopez-Lozano et al., Anat. Rec. 214:75,1986), we continue studying the short-term effects that the laser and hydrodynamic sheer forces used in cell sorting procedures may have on a population of fetal ventral mesencephalic bovine cells. The purpose of the present communication is to examine the morphological and immunocytochemical behavior of TH-containing ventral mesencephalic FCACS neurons from bovine embryos after being implanted into unilaterally denervated striatum of adult Sprague-Dawley rats (200 gr.). Ventral mesencephalon (containing substantia nigra) from 3-4 month-pc old bovine animals were quickly dissected and cells were dispersed by an enzymatic-mechanical procedure (0.1% trypsin) (Notter et al., Brain Res. Bull, 12: 307, 1984). Viability was better than 92%. The suspension of ventral mesencephalic cells was then analyzed separately in an EPICS C (Coulter Electronics, Madrid, España) multiparameter flow cytometer system and relatively enriched by flow cytometry and cell sorting techniques. Later, the recovered suspension of sorted ventral mesencephalic cells was implanted into the dorsal striatum of 6-OHDA lesioned rats. In order to describe the migration and differentiation of the transplanted cells, the recovered suspension was previously labelled with 0.03% fast blue. At time periods of 4 days to 4 weeks, animals were intracardially perfused with appropriate fixative. Implanted cells were studied by Fluorescence and phase contrast microscopy, catecholamine-containing histofluorescence and TH-immunocytochemistry. The data demonstrate that FC-sorted fetal ventral mesencephalic cells can survive and develop after being cross-species implanted into adult host striatum and are similar to the results obtained with unsorted dissociated cells. Also, the results of this study indicate that neurons maintain their phenotype and express TH. This study provides further evidence that flow cytometry and cell sorting techniques can be used to provide a source of donor tissue for transplantation. (Supported by FIS 86/680 , 87/687 and SEN 86 to JJLL)

141.8 THALAMIC STIMULATION EVOKES SINGLE-UNIT ACTIVITY IN FETAL NEOCORTICAL TISSUE GRAFTED INTO THE CEREBRAL HEMISPHERE OF NEWBORN RATS. E.J. Neafsey, J.C. Sørensen*, N. Tønder* and A.J. Castro. Dept. of Anatomy, Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153 and Instit. of Anat. B., Univ. of Aarhus, Denmark.

Several studies involving fetal neocortical transplants placed within the newborn cerebral hemisphere have demonstrated extensive host-transplant interconnections. In light of data demonstrating host thalamic inputs to these grafts, the present study was initiated to examine thalamo-transplant connections electrophysiologically. Accordingly, unilateral neocortical lesions were made by aspiration just rostral to the coronal suture in 0-1 day old, hypothermic-anesthetized black-hooded rats. Immediately after lesion placement, blocks of fetal (E15-16) neocortex were placed into the lesion cavity. The grafts were held in place by a bone flap. At 4-5 months of age, host animals were re-anesthetized with ketamine (100 mg/kg) and secured in a stereotaxic instrument. A large craniotomy was made and a stimulating electrode was inserted into the ventral thalamus ipsilateral to the transplant. A glass-insulated tungsten recording microelectrode was used to sample single unit activity within the graft while electrically stimulating the thalamus with 0.1-0.2 msec negative single pulses of 300-500 uamps. In several animals, the stimulation evoked single or multiple orthrodromic spikes at a latency of 5-6 msec; this activity was usually followed by a long lasting (200 msec) cessation in the cell's activity. In one instance the single unit appeared to be driven antidromically by the stimulation since the cell responded at a constant 2 msec latency. At selected points along each electrode tract, small electrolytic (10 uamps DC for 10 sec) marking lesions were made and their locations within the transplant and ventral thalamus were subsequently verified histologically. The patterns of the observed responses resembled electrophysiological findings in normal animals. These results provide electrophysiological confirmation of transplant afferents arising from the host thalamus. (Supported by NIH Grant NS 13230 and funds from the Danish MRC.)

141.9 CONNECTIONS OF NEOCORTEX XENOGRAFTS IN THE RAT BRAINSTEM. D. W. Marion,* and R. D. Lund° (SPON: R. G. Selker), Dept. of Neurological Surgery and °Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

Previous studies have shown that retinal ganglion cell grafts placed in the rat midbrain send projections only to appropriate subcortical visual centers. To determine if this specificity is unique to retinal transplants or if it occurs with other types of CNS grafts, we studied the projections of mouse sensorimotor (SM) and occipital (Occ) cortex, transplanted into neonatal rat brainstem. Neocortex was dissected from E13 CD-1 mice and placed into various brainstem regions of one-day-old Sprague-Dawley rats. At 3-5 weeks of age horseradish peroxidase (HRP) was injected into the cervical spinal cord. Thirty-six hours later the animals were perfused with 4% paraformaldehyde. Serial coronal brainstem sections 30μm thick were stained for cell morphology, fibers, or HRP reaction product. Graft projections were also demonstrated with a monoclonal antibody to a mouse neuronal cell-surface antigen.

Transplants within the aqueduct or substance of the dorsal midbrain sent projections to the superior colliculus with specific fields of termination: Occ cortex to the superficial layers, and SM cortex to the intermediate gray. Grafts in the ventral midbrain projected to the substantia nigra, grafts in the dorsal pons projected to the periaqueductal gray and the region of the dorsal raphe nucleus, and surface grafts along the ventral lateral pons projected into the spinal cord. Projections to the substantia nigra, periaqueductal gray, region of the dorsal raphe nucleus and spinal cord were found from SM and Occ cortex grafts. HRP reaction product was found only in cells of ventral lateral pontine grafts. Thus, while SM and Occ cortex grafts placed near the superior colliculus projected to specific and appropriate targets, grafts placed in the ventral midbrain and pons projected to nuclei not considered normal targets for neocortical projections.

We conclude that specificity of projections of neocortex xenografts is influenced by the region of rat brainstem into which the graft is placed. Cortical grafts respond to both specific and non-specific cues which may be target derived, and regional location is an important factor in determining graft innervation patterns. Supported by NIH grant EY05283.

141.10 DEVELOPMENT OF GABA NEURONS IN CULTURES AND GRAFTS IN THE ADULT RAT HIPPOCAMPUS.

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The intrahippocampal GABAergic system mediates a particularly powerful inhibition which plays a central role in preventing paroxysmal discharges. Using antibodies directed as GABA, we have examined the development of GABA labeled neurons in cultures and in intrahippocampal grafts.

The hippocampus taken form E19 rat embryos was dissociated into a cell suspension and grafted into the hippocampus of adult rats. Animals were examined at various time intervals (up to 3 months) following transplantation. The grafts were localized either in the stratum radiatum of CA1 or the molecular layer of the fascia dentata. The implanted tissue was capable of survival and growth without exhibiting a laminar organization. Golgi silver staining allowed the visualization of various morphological neuronal types : large spiny, small aspiny pyramidal neurons and bipolar cells. Granule neurons were rarely encountered. Conventionnals electron-microscopy revealed various types of symmetric and asymmetric synapse in the grafts. With an antibody directed againts GABA, there was a substantial proportion (around 20 %) of GABA immunoreactive neurons within the graft, this proportion is higher than that found in the host. Moreover a dense labelling of the neuropil was observed, this allowed a clear distinction between the grafted area and the host.

A substantial proportion of labeled neurons (both with the GABA antibodies and with 3H GABA uptake) was also found in cultures of the cell suspensions. The morphological patterns were similar to those seen in the grafts with a majority of pyramidal shaped GABA neurons.

In conclusion dissociated hippocampal neurons taken from embryos survive in the adult rat hippocampus and express a high potential for growth and sprouting in vitro and in vivo. This is particularly conspicuous for GABA immunoreactive neurons.

141.11 FETAL HIPPOCAMPAL CELLS TRANSPLANTED INTO THE ISCHEMICALLY DAMAGED CA1 REGION DEMONSTRATE NORMAL CHARACTERISTICS OF ADULT CA1 CELLS. L.A. Mudrick, K.G. Baimbridge and J.J. Miller. Dept. of Physiology, U. of British Columbia, Vancouver, B.C., V6T 1W5

Previously we have demonstrated that transplanted fetal hippocampal cells can survive and mature in brain regions irreversibly damaged by cerebral ischemia. We have now examined the properties of these transplanted cells and observe that there is some degree of selectivity for the survival of CA1-like neurons. This may have resulted from the judicious selection of fetal hippocampi at an appropriate developmental stage.

Consistent, highly selective damage was produced in the dorsal CA1 region of the hippocampus by combining haemorrhagic hypotension to a MAP of 30mmHg with bilateral carotid occlusion for 20 minutes. In an attempt to reconstruct the necrotic area, one week following ischemia, the region was repopulated with five rostro-caudal injections of dispersed E18 hippocampal neurons. In one series of experiments the cells were labelled with ^{3}H-Thymidine to confirm the presence of transplanted cells. After one to eight months the brain tissue was fixed, 20µm serial sections were made and the following anatomical techniques were undertaken on groups of adjacent sections. One set of sections was stained with thionin for quantification and morphological analysis. A second set of sections was histochemically processed to visualize AChE-containing axons in order to assess the integration of the transplanted cells into the host tissue. Lastly, immunohistochemical techniques were used to localize calbindin D_{28K} (a protein specific for CA1 hippocampal pyramidal cells, parvalbumin (a protein localized in GABA containing neurons, and ChAT to characterize the intrinsic properties of the transplanted cells.

Histological examination revealed that the grafted cells were morphologically similar in size and appearance to adult CA1 hippocampal pyramidal cells. A network of AChE-containing fibres were seen throughout the grafts possibly representing neurite extensions of the original medial septal-diagonal band afferents and formation of new synaptic contacts. Calbindin and parvalbumin were detected within subpopulations of the grafted neurons. No ChAT positive cells were observed. The immunoreactivity also revealed that the transplanted cells had formed extensive neurite networks.

The results indicate that CA1 hippocampal cells, taken from 18-day old fetuses, may be at a developmental stage which permits their selective survival after dissociation and transplantation. When placed in a suitable environment, they integrate into the host tissue, appear morphologically similar and express the same neuron specific markers as the adult cells they have replaced.
(Supported by a Canadian MRC Program Grant to J.J.M. and K.G.B.)

141.12 EFFECTS OF TOLUENE EXPOSURE ON DEVELOPING PURKINJE NEURONS: EVIDENCE FROM INTRAOCULAR CEREBELLAR TRANSPLANTS USING BIOCHEMICAL AND ELECTROPHYSIOLOGICAL TECHNIQUES. A-Ch. Granholm, G. A. Gerhardt, M. Eriksdotter-Nilsson*, A-C. Hansson*, A. Henschen*, G. Hoglund*, P. Nylen*, M. Palmer, L. Olson* and B. Hoffer, Depts. Pharmacol. and Psychiat., U. of Colo. Hlth. Sci. Ctr., Denver, CO 80262, Depts. Histol. and Physiol., Karolinska Institute, Box 60400, 10401 Stockholm, Sweden and Dept. Occupat. Neuromedicine, Natl. Board Occupat. Safety Hlth, Res. Dept., 17184 Solna, Sweden.

The purpose of this study was to investigate the temporal and regional effects of the solvent toluene in the CNS. Isolated cerebellar transplants in oculo were studied using electrophysiological and biochemical techniques. Fetal rat cerebellar anlage (E 13-14) were grafted to adult hosts which were then exposed to a dose of 1000 ppm gaseous toluene 21 hrs per day for 9 weeks. Controls were cerebellar anlage from the same litter transplanted to recipients which were exposed to room air during the same interval. Growth of cerebellar grafts, monitored by repeated stereomicroscopic observations through the cornea, was moderately reduced in the toluene-treated animals. Extracellular recordings of the spontaneous activity of Purkinje neurons in the cerebellar grafts were performed within 10 days after the last exposure in both groups. Purkinje neurons in the toluene-treated recipients showed a slightly decreased spontaneous discharge rate as compared to the controls (mean discharge rates were 22 $\pm$ 1.5 Hz (n=27) vs. 29 $\pm$ 1.2 Hz (n=6), $p<0.05$). Postsynaptic sensitivity of the intraocular Purkinje neurons to norepinephrine (NE) was evaluated by superfusions with known concentrations. NE elicited a dose-dependent slowing of the discharge rate in both groups, however, the toluene-exposed Purkinje neurons were significantly supersensitive to NE as compared to the control group (EC50 0.35 uM, 95 % confidence limit 0.31-0.39 uM (n=19) vs. EC50 15.9 uM, 95 % confidence limit 2.4-100 uM (n=27)). Hill plots demonstrated that the dose-response relationships in both groups were linear and parallel to one another. The tissue levels of NE in the toluene-treated grafts were approximately 2-fold greater than in the control transplants. Moreover, the ratios of NE to free (3-methoxy-4-hydroxyphenyl)ethylene glycol (MHPG) were significantly reduced in both transplant and host cerebellum after toluene exposure. These data indicate that toluene treatment during development causes decreased spontaneous activity of isolated Purkinje neurons as well as a marked alteration in pre- and postsynaptic NE mechanisms. (Supported by USPHS grants ES02011 and AA05915).

141.13 EFFECTS OF FETAL SUBSTANTIA NIGRA TRANSPLANTS ON LESION-INDUCED DEFICITS IN TRAINED CIRCLING BEHAVIOR. J.B. Richards and C.R. Freed., Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

Rats with unilateral lesions of the nigrostriatal dopamine (DA) system will circle in a direction ipsilateral (ipsi) to the lesioned side when injected with amphetamine (AMPH). Unilateral transplantation of fetal substantia nigra DA neurons into the DA deficient striatum reduces or even reverses the direction of AMPH circling because of release of DA from the transplant and receptor supersensitivity on the same side. Unilateral DA lesions also cause performance deficits in rats trained to circle for reward (Dunnett and Bjorklund, Neurosci. Letters 41: 173, 1983). We have found these rats have great difficulty in turning contralateral (contra) to the lesioned side and show moderate deficits in turning ipsi to the lesioned side. We have studied whether unilateral transplantation of fetal substantia nigra neurons can restore trained circling behavior in rats with unilateral nigrostriatal lesions. Male Sprague-Dawley rats 300-400 gm were trained to circle both to the left and to the right for a water reward. After performance stabilized, rats were lesioned with unilateral injections of 6-hydroxydopamine (8 µg in 4 ul of 0.2 mg/ml ascorbate-saline) in the medial forebrain bundle (AP. 4.4 caudal to bregma; L. 0.9 from midline; V. 7.5 below dura; nose bar 2.3 below interaural line). Three to four weeks after lesioning, animals were tested for AMPH-induced circling with a dose of 1.5 mg/kg i.p. Rats which failed to reach a criterion of 150 net turns ipsi to the lesion during a 2 hr period were eliminated from the study. Their ability to circle for a water reward contra and ipsi to the side of the lesion was also measured. Rats were divided into a tranplant group and a control group. The transplant group was injected with a minced suspension containing DA cells from the ventral mesencephalon of 14-16 day old rat embryos. Injections of 3 µl were made along 5 tracks in the nucleus accumbens and striatum (Site 1: AP +3.4, L 2.0; Site 2: AP +2.4, L 2.0; Site 3: AP +2.4, L 3.5; Site 4: AP +1.4, L 3.5; Site 5: AP +0.2, L 4.0; nose bar set at 5.0 above the interaural line). Control rats received saline. After transplantation the rats were assessed for AMPH-induced circling and trained circling at 4 week intervals. Following behavioral experiments, animals were sacrificed and transplant survival verified by immunohistochemical staining for tyrosine hydroxylase. Preliminary results have shown that rats transplanted with fetal cells (n=6) reversed their circling direction after AMPH (439 net ipsi turns/2 hr to 220 net contra turns/2 hr) but showed only slight and not significant improvement in the trained circling task.

141.14 TRANSPLANTATION OF FETAL CORTEX INTO ADULT RAT BRAINS WITH MIDDLE CEREBRAL ARTERY INFARCTIONS. M. F. Gonzalez, T. J. Mampalam*, J. E. Loken*, A. Poncelet* and F. R. Sharp. Departments of Neurology and Neurological Surgery, V.A. Medical Center and University of California, San Francisco, CA 94121.

Recently it has been demonstrated that transplantation of fetal neural tissue can be used to treat the symptoms and signs of degenerative or inherited brain disorders. The present study extends the uses of transplantation by showing that parietal fetal cortex transplants survive in infarcted regions of adult rat brains produced by middle cerebral artery (MCA) occlusions.

Adult (250-300 g) Sprague-Dawley rats were anesthetized and their MCA occluded using the technique of Zea Longa et al. (Stroke, in press). Briefly, a 4-0 monofilament nylon suture was threaded up the common carotid and internal carotid arteries into the MCA until resistance was felt. Following occlusion of the MCA for 3 hours the suture was withdrawn. One to two weeks later 12 surviving subjects were reanesthetized and received transplants of neocortex from 16-17 days old embryos. A month later these subjects were perfused. Coronal sections throughout the infarcted area and surviving transplants were Nissl stained and reacted for NADPH-d and acetylcholinesterase (AChE) histochemistry.

Fetal cortex transplants survived in infarcted or ischemic areas of the brains of 9 of 12 subjects. The size of the surviving transplants varied considerably, and they filled infarcted cavities, formed bridges between surviving host cortex, or displaced non-infarcted but ischemic host cortex. Nissl stain confirmed the presence of transplants and the demarcation of the transplant/host border. The number of neurons in transplants was similar to or greater than in surrounding host cortex, but cells were not arranged in laminae. Normal NADPH-d stained neurons were found in transplants as previously reported in frontal cortex transplants (Gonzalez and Sharp, J. Neurosci., in press). All surviving transplants also contained normal appearing AChE positive neurons which were either similar to or greater in number than in surrounding host cortex. AChE positive fibers were also found in all transplants but their density was usually less than that of normal host cortex. Both, NADPH-d and AChE positive fibers crossed host/transplant interfaces. These results demonstrate that it is possible to successfully transplant fetal tissue into ischemic and infarcted areas of adult mammalian brain.

141.15 INTRODUCTION OF NEW GENES INTO ADULT RAT BRAIN VIA GRAFTED CELLS. M.B. Rosenberg[1*], J.A. Wolff[1*], J.-K. Yee[1*], Li Xu[1*], C. Shultz[2], T. Friedmann[1*] and F.H. Gage[2]. Departments of [1]Pediatrics and [2]Neurosciences, School of Medicine, Univ. Calif. San Diego, La Jolla, CA 92093.

We are developing methods for introducing new functions into the mammalian CNS by implantation into the brain of cells that have been genetically modified in vitro to express foreign genes (transgenes). Continuous rat fibroblast lines, primary cultures of rat skin fibroblasts and glia from neonatal rat neocortex were infected with replication-incompetent, helper-free retrovirus vectors expressing either the human hypoxanthine phosphoribosyltransferase (HPRT) gene alone or the bacterial neomycin resistance gene together with either the firefly luciferase gene or the E. coli beta-galactosidase gene. The cultures were grown in media containing hypoxanthine/aminopterin/thymidine (HAT) or the neomycin analog G418 to select for $HPRT^+$ or neomycin-resistant infected cells, respectively, and the resulting selected cells were then injected intracerebrally into rats. The grafted cells survived for at least three months, as indicated by immunohistochemical detection of the fibroblast and astrocyte marker proteins fibronectin and glial fibrillary acidic protein. Furthermore, the transgenes continued to be expressed, as determined by measurement of HPRT and luciferase enzyme activities in extracts prepared from dissected tissue or by histochemical detection of beta-galactosidase activity in situ. These experiments indicate that genetically modified cells survive and express new functions after implantation into rat brain and suggest a new approach to the therapy of some CNS disorders.

141.16 NORADRENERGIC NEURON TRANSPLANTS: ELECTROPHYSIOLOGICAL UNIT ACTIVITY OF INTRASTRIATAL LOCUS COERULEUS GRAFTS IN FREELY MOVING CATS. M.E. Trulson. Dept. Anat., Coll. Med., Texas A&M University, College Station, TX 77843.

A great deal of attention has been devoted recently to transplanting fetal brain donor tissue into adult host brain. Much of this work has been done with monoamine-containing neurons. We have previously studied transplanted serotonergic (Brain Res. Bull. 17, 1986, 461) and dopaminergic (Life Sci., in press) neurons. In the present study we examined the electrophysiological activity of individual noradrenergic (NE) neurons transplanted from cat fetal brain tissue to adult host cat brain. Forty-day old cat fetus served as the source of immature NE-neurons. Pregnant females were anesthetized by intravenous sodium pentobarbital (dose as needed to maintain surgical anesthesia) and prepared for caesarian section. The embryos were surgically removed from the uterus under sterile conditions and their brains removed and the dorsal medulla containing the LC was dissected free. The grafting procedures were essentially those described by Bjorklund and his colleagues (Acta Physiol. Scand. Suppl. 522, 1983, 1). The female host cats were anesthetized with ketamine (10 mg/kg) and xylocine (0.4 mg/kg) and placed in a stereotaxic instrument. Injections were made through a small burr hole with a sterile 100 µl Hamilton syringe with a 23 gauge needle. The 5 µl suspensions were deposited at the rate of 1 µl/min and 5 min were allowed for diffusion prior to removing the needle. Macroelectrodes for recording the electroencephalogram (EEG) electromyogram (EMG) and eye movement potentials (EOG) were implanted according to standard procedures. Following recovery from surgery electrophysiological recordings were made using procedures previously described (Brain Res. Bull. 17, 1986, 461). Transplanted LC neurons displayed the slow (2-3 spikes/sec) and regular spontaneous activity during periods of quiet waking, as previously described by Rasmussen et al. (Brain Res. 371, 1986, 324). However, in contrast to the profound decrease in LC unit activity that occurs in intact cats during sleep, transplanted neurons maintained the same discharge pattern and rate across sleep stages. Furthermore, transplanted LC neurons were unresponsive to simple auditory and visual stimuli in contrast to their sensitivity to such stimuli in intact, freely moving cats (Rasmussen, et al., Brain Res. 371, 1986, 324). Transplanted LC neurons were inhibited by clonidine; however, the dose required to inhibit the neurons was significantly higher than that in intact animals. These data suggest that NE neurons can be transplanted into a host brain and display spontaneous electrophysiological activity. However, the electrophysiological and pharmacological properties are very different from those in intact animals.

141.17 FETAL HIPPOCAMPAL GRAFTS REDUCE INCREASED OPEN FIELD ACTIVITY PRODUCED BY ADMINISTRATION OF TRIMETHYLTIN TO ADULT RATS. M. L. Woodruff, R. H. Baisden, D. L. Whittington,* and S. Wray# Department of Anatomy, East Tennessee State University, Quillen-Dishner College of Medicine, Johnson City, TN 37614, and #NIH, NINCDS, Bethesda, MD 20892.

Exposure to trimethyltin (TMT) is known to produce behavioral changes related to TMT's effect on the hippocampus (e.g. Dyer et al., Neurobehav. Tox. Teratol., 4, 1982). The purpose of the present experiment was to determine whether grafts of normal fetal hippocampal tissue could ameliorate one of the behavioral changes associated with exposure to TMT. Thirty nine adult male Long-Evans rats were included in the experiment. Twenty three rats were gavaged with an aqueous solution containing 7 mg/kg (expressed as the free base) of TMT. Seven rats (Group TMT) received no further treatment. Forty five days after gavage eight rats were given bilateral lesions of the hippocampus (Group THL) and eight received bilateral lesions of the hippocampus followed immediately by implantation of fetal brain tissue (Group TTR) into the lesion cavity. Of the remaining rats eight served as normal control subjects (Group N), and eight were given bilateral lesions of the hippocampus (Group HL). All surgery was performed while the rats were anesthetized with sodium pentobarbital. Counts of line crossings in an open field during three consecutive daily 10 min sessions was used to assess activity begining eighty days after surgery. A two-factor ANOVA with a repeated measure on the within (days) factor was used to evaluate the data. Significant groups ($F(4,34) = 10.16$, $p < .01$) and days effects ($F(2,68) = 4.10$, $p < .05$) were found. The interaction was not significant. Post hoc Newman-Keuls tests indicated that Group TTR did not differ from Group N on any of the days, but did cross significantly fewer lines than Groups TMT, THL, and HL. Histological evaluation using Nissl, Bodian, or immunocytochemical procedures was conducted following behavioral testing. The transplants were found to occupy fifty percent or more of the lesion cavity. Nerve fibers were seen to cross between the host and the transplant. Choline acetyltransferase- and vasoactive intestinal polypeptide-positive cells and fibers and substance P- and tyrosine hydroxylase (TH)-positive fibers were seen within all transplants. Some of the transplants also contained a few TH positive cells. These data indicate that fetal brain grafts can develop in TMT-damaged host brains, obtain innervation from the host and reduce at least one toxicant-induced behavioral deficit. (Supported by USPHS Grant # ES04070 to MLW.)

142.1 THE EFFECT OF DEXAMETHASONE ON THE BINDING OF ^{125}I-NERVE GROWTH FACTOR TO PC12 CELLS. M. D. Tocco,* M. L. Contreras, and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, Bethesda, MD 20892

PC12 is a cell line developed from a rat pheochromocytoma that exhibits sympathetic neuron-like properties in response to nerve growth factor (NGF) and some chromaffin cell-like properties when treated with corticosteroids. The responses of the cells to nerve growth factor have been explored in some detail; the responses to corticosteroids not so thoroughly. Corticosteroids prevent the neurite outgrowth produced by nerve growth factor in normal chromaffin cells from neonatal animals. Although no such inhibition of neurite outgrowth has been seen in PC12 cells, it seemed reasonable to explore the possibility that NGF and corticosteroids display some type of competitive interaction in this model. Accordingly, we have studied the effects of dexamethasone on the binding of ^{125}I-NGF to PC12 cells.

PC12 cells were grown as monolayers in Dulbecco's modified Eagle's medium with 6% CO_2 and 15% serum. The cells were treated for various times with 10^{-6} to 10^{-11} M dexamethasone. After the treatment the cells were washed and exposed to ^{125}I-NGF. After 60 minutes the cells were washed thoroughly and dissolved in 0.5 M NaOH. Binding was evaluated by counting a portion of the NaOH and assaying another portion for protein. Specific binding was calculated by comparing the binding with tracer alone with that in the presence of 2.5 µg/ml of unlabeled NGF.

Our results indicate that dexamethasone causes a time- and concentration-dependent decrease in the binding of ^{125}I-NGF to the cells. This decrease begins after 1 day of treatment and is maximal after 5 days. The maximal decrease is frequently as much as 70%. A maximal decrease can be seen at concentrations of dexamethasone as low as 10^{-8} M. The decrease appears to involve primarily the low affinity sites. Since chronic treatment of PC12 cells with NGF has been shown to induce a long-term, several-fold increase in the binding of ^{125}I-NGF, we examined the effect of dexamethasone treatment on this apparent up-regulation of the NGF receptor. Our data indicate that simultaneous treatment of PC12 cells with NGF and dexamethasone completely prevents this up-regulation.

The mechanisms by which dexamethasone decreases the binding of ^{125}I-NGF are unknown. We are inspecting the suggestion that dexamethasone selects for a receptor-low population of PC12 cells. Alternatively, we are pursuing the possibility that dexamethasone alters the rate of biosynthesis of the NGF receptor. These studies may shed light on the interactions of nerve growth factor and corticosteroids in the phenotypic determination of normal chromaffin cells and sympathetic neurons.

142.2 **VARIANT PC12 CELLS WHICH FAIL TO RESPOND TO NERVE GROWTH FACTOR POSSESS HIGH AFFINITY NGF RECEPTORS** D.D. Eveleth* and R.A. Bradshaw. Dept. of Biol. Chem., College of Medicine, Univ. of California, Irvine, CA 92717.

The rat pheochromocytoma cell line PC12 is a widely used in vitro model for the nerve growth factor (NGF) induced differentiation of neural crest derivatives into mature sympathetic neurons. PC12 cells as well as other NGF responsive cells express two classes of NGF receptors that are distinguished primarily by their affinity for NGF. More recently, Green et al. (Green, S.H., Rydel, R.E., Connolly, J.L. and Greene, L.A., J. Cell Biol., 102:830,1987) isolated variant lines of PC12, designated nnr, which fail to respond to NGF, apparently lack the high affinity receptors for NGF and do not internalize NGF in the usual fashion. To determine whether the two classes of receptors are encoded by different genes and to clarify their roles in the NGF induced response, we have reexamined the variant, PC12nnr5, (kindly provided by L. Greene) and found that it expresses high affinity NGF receptors. These receptors sequester NGF bound to the cell surface and are depleted as a result. In PC12 cells, internalized NGF is degraded into amino acids; the PC12nnr5 cells do not degrade the sequestered NGF, indicating that the defect preventing the PC12nnr5 cells from responding to NGF is not a lack of appropriate receptors but an inability to properly process the bound NGF (and their receptors) once sequestered. These results suggest internalization is a required part of the signalling mechanism that leads to the differentiated state. The PC12nnr5 cell line also fails to respond to basic fibroblast growth factor (bFGF), which elicits a response very similar to NGF in PC12 cells. Since the PC12nnr5 cell line is competent to extend neurites in response to other stimuli, the defect in PC12nnr5 cells lies in a pathway common to the NGF and bFGF signaling systems. Supported by the American Cancer Society (BC273) and the USPHS (NS19964).

142.3 DETECTION OF A NERVE GROWTH FACTOR RECEPTOR ON FETAL HUMAN SCHWANN CELLS. J.G. Assouline and N.J. Pantazis. Dept. of Anatomy, Univ. of Iowa Medical College, Iowa City, IA 52242.

Several types of neural crest-derived cells, including Schwann cells, possess nerve growth factor (NGF) receptors. The possibility that fetal human Schwann cells in culture have an NGF receptor was examined in this study. Primary Schwann cell cultures were established from peripheral nerves (both thoracic and sciatic) obtained from 16-21 week human fetuses. After dissociation of nerve tissue in a trypsin-collagenase mixture, Schwann cell cultures were established by two different procedures. In one procedure, cells were seeded in a feeding medium (FM) consisting of Ham's F12/Dulbecco's modified minimal essential medium (DMEM), with 10% fetal calf serum and ascorbic acid for 48 hrs. The cells were then treated (24 hrs) with FM supplemented with cytosine arabinoside, followed by incubation in FM containing cholera toxin and pituitary extract. In an alternative procedure, the dissociated cells were seeded in a medium containing DMEM with insulin, transferrin, hydrocortisone, biotin, sodium selenite, ascorbic acid as well as cholera toxin and pituitary extract (48 hrs). Cells were then switched to a similar medium which lacked the cholera toxin and pituitary extract.

Cell identity was established by morphological and immunological criteria. Schwann cells were spindle shaped and contained laminin as indicated by immunofluorescence studies; whereas fibroblasts were flat, polygonal and contained fibronectin. Furthermore, monoclonal anti-leu 7 (HNK-1) antibody which binds to myelin associated glycoprotein in neuroectodermal tissues also labelled the Schwann cells.

The NGF receptor was detected by several techniques including light microscopic autoradiography (ARG), immunofluorescence, and transmission electron microscopy (TEM). For ARG, cells were incubated with ^{125}I-NGF and covered with photographic emulsion. The silver grains were localized predominantly to the Schwann cells. Immunofluorescence (IF) was performed in two ways. In one method the cells were incubated with unlabeled NGF followed by anti-NGF primary antibody and visualized with a fluorescent second antibody. In another method a monoclonal antibody to the human NGF receptor (gift of M. Bothwell) was used. With both IF methods, an NGF receptor was seen on the Schwann cells. The anti-NGF receptor antibody was also used in TEM and in these studies, the NGF receptor was localized to the plasma membrane of the Schwann cells.

In conclusion, various techniques have established that Schwann cells from human fetuses ranging from 16 to 21 weeks expressed an NGF receptor in culture. The NGF receptor is a useful marker for Schwann cells. Future study will examine whether human Schwann cells in vivo have an NGF receptor. (Supported by an NIH grant, GM28644, to NJP).

142.4 BIOCHEMICAL CHARACTERIZATION OF THE NERVE GROWTH FACTOR RECEPTOR ON FETAL HUMAN SCHWANN CELLS. N.J. Pantazis and J.G. Assouline. Dept. of Anatomy, Univ. of Iowa Medical College, Iowa City, IA 52242.

Fetal human Schwann cells in culture have a nerve growth factor (NGF) receptor (Assouline and Pantazis, abstract this meeting). In this study the molecular weights (M_r) of the NGF receptor proteins were determined by immunoprecipitation of the receptor and examination of the receptor proteins on sodium dodecyl sulfate (SDS) polyacrylamide gels.

Experimentally, primary cultures of fetal human Schwann cells were established from peripheral nerves from 16-21 week fetuses (Assouline and Pantazis, abstract this meeting). The Schwann cells were incubated with ^{125}I-NGF (2 nM) which was crosslinked to the NGF receptor with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC). The crosslinked ^{125}I-NGF-receptor complex was solubilized with η-octyl glucoside and was immunoprecipitated using a primary monoclonal anti-human NGF receptor antibody (gift of M. Bothwell) followed by a second antibody which is directed against the primary antibody. The precipitated receptor was solubilized in SDS sample buffer, run on an SDS gel and the positions of the NGF receptor proteins were determined with X-ray film.

Two protein bands with M_r of 190 and 90 kDa were seen on the gel. Possibly the 90 kDa protein is a fragment of the 190 kDa protein or alternatively the 190 kDa protein may be an aggregate of the 90 kDa protein. Both possibilities are under investigation.

Several observations suggest that these proteins are the NGF receptor proteins. First, monoclonal antibody was used for immunoprecipitation. Second several control experiments indicate that these are the NGF receptor proteins. For example, neither protein was seen on the gel when the antibody was omitted from the procedure. In another control experiment, a monoclonal antibody to rat NGF receptor which does not crossreact with human NGF receptor was used, and again neither protein was evident. When the immunoprecipitation procedure was performed on human melanoma cells which possess an NGF receptor, the 190 and 90 kDa proteins were observed. Third, proteins of similar M_r were observed by other investigators who have examined the NGF receptor on SDS gels.

In conclusion, an NGF receptor was detected on fetal human Schwann cells in culture and the M_r of the receptor proteins were 190 and 90 kDa. This immunoprecipitation procedure is being used to determine whether freshly dissected nerve which has not been placed in culture contains an NGF receptor. Both low and high affinity NGF receptors have been described and whether human Schwann cells have one or both receptor types is being investigated. Although the significance of an NGF receptor on Schwann cells is unexplained, it may play an important role in neuronal development. (Supported by an NIH grant, GM28644, to NJP).

142.5 CULTURED NEURAL CREST CELLS EXPRESSING A NON-NEURONAL ANTIGENIC DETERMINANT RECOGNIZED BY A MONOCLONAL ANTIBODY, G1N1, POSSESS RECEPTORS FOR NERVE GROWTH FACTOR (NGF). P. Bernd. Department of Anatomy, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029.

This laboratory has previously demonstrated that a subpopulation of long-term cultured neural crest cells (1 to 2 weeks past explantation) have receptors for Nerve Growth Factor (NGF) and exhibit specific binding of iodinated NGF (^{125}I-NGF). These cells are likely to be targets of NGF during the early stages of embryonic development. Further co-localization studies revealed that neuron-like cells possessing tyrosine hydroxylase-like, serotonin-like, or vasoactive intestinal polypeptide-like immunoreactivity did not have receptors for NGF. Cultures were prepared from the trunk region of 48 hr quail embryos, and maintained in complete medium containing 5% chick embryo extract (CEE) and 15% fetal bovine serum.

It has been previously shown (Barbu et al., J. Neurosci., 6:2215, 1986) that 25% of migrating neural crest cells carry an antigenic determinant recognized by a monoclonal antibody, G1N1. This determinant was also demonstrated to be present in virtually all satellite cells of peripheral ganglia, all Schwann cells of peripheral nerves, and in subpopulations of sensory and autonomic neurons. Non-neuronal precursor cells, recognized by G1N1, were identified immunocytochemically in our neural crest culture system using an avidin-biotin-peroxidase technique (G1N1 antibody, courtesy of Dr. N.M. Le Douarin, used at a dilution of 1:100 or 1:500). We also observed that a subpopulation of cells was G1N1 positive. These cells were either spindle-shaped or flattened in appearance.

To determine whether the G1N1 positive cells have NGF receptors, co-localization studies were performed. Cultured neural crest cells were rinsed with CEE-free tissue culture medium (2 hr; 37°C), incubated with ^{125}I-NGF (5 ng/ml, 100 cpm/pg; 1 hr; 37°C), rapidly rinsed six times with ice-cold phosphate buffered saline, fixed (4% paraformaldehyde in 0.1M sodium phosphate buffer), and processed for immunocytochemistry. The cultures were subsequently embedded in epon and sectioned (1 μm). Examination of light microscopic radioautographs revealed that the majority of G1N1 positive cells specifically bound ^{125}I-NGF. It is likely, therefore, that the non-neuronal precursor cells, present in neural crest cultures, are responsive to NGF. The nature of this response remains to be determined. Unstained neural crest cells, labelled by ^{125}I-NGF, were also observed, indicating that additional cell types are NGF targets and need to be identified. Supported by grants from the NSF (#BNS-8600256), March of Dimes (#1-1011), and the Dysautonomia Foundation.

142.6 LOCALIZATION OF NGF RECEPTOR IMMUNOREACTIVITY ON SPECIFIC EPITHELIAL CELL POPULATIONS. S.L. Patterson*, G.C. Schatteman*, S.J. Thompson*, and M. Bothwell (SPON: M. Liebowitz). Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

We have employed monoclonal antibodies to human NGF receptor (Marano et al., J. Neurochem. 48: 225-232 1987) and to rat NGF receptor (Chandler et al., J. Biol. Chem. 259: 6882-6889 1984) to immunocytochemically localize NGF receptors in human, monkey, and rat tissues. In addition to the expected immunostaining of elements of the sensory and sympathetic nervous system, we were suprised to detect immunoreactivity on certain epithelial cells.

Most epithelia, including epidermis of finger tip and of hairy skin and epithelia of the GI tract, were devoid of NGF receptor immunoreactivity except for that on nerve fibers. However NGF receptor immunoreactivity was detected on basal epithelial cells of some hair follicles, of some papillae of dorsal tongue, of some regions of lip, and of the junctional epithelium of gingiva. In addition, myoepithelial cells of a variety of exocrine organs, including mammary gland, salivary gland and sweat gland were specifically immunostained.

The basal epithelial cell populations which bear NGF receptor immunoreactivity typically are in regions which receive unusually abundant sensory innervation. In addition, myoepithelial cells, with the exception of those of mammary gland, receive sympathetic innervation. However the immunoreactivity we detect associated with epithelial cells is apparently intrinsic to the cells since it is too extensive to represent merely closely associated nerve fibers.

We have not yet confirmed by independent means that the immunoreactivity observed corresponds to authentic NGF receptors. However, if these are functional NGF receptors, their functional role is obscure. As most of these epithelial cell populations are targets of sensory or sympathetic innervation, they presumably produce NGF. In this sense, they may be likened to Schwann cells which may express both NGF and NGF receptors, for reasons which are not yet understood.

142.7 LOCALIZATION OF NGF RECEPTOR mRNA AND PROTEIN INPRIMATE SENSORY NEURONS. J.G. Heuer*, G.C. Schatteman* and M. Bothwell. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

Mammalian dorsal root ganglionic (DRG) neurons require NGF for survival during embryonic development and retain some responsiveness in adults. While the mechanism of action of NGF remains obscure, cell surface receptors for NGF have been extensively characterized. This laboratory and its collaborators have isolated monoclonal antibodies against the human NGF receptor, and cDNA clones encoding the human NGF receptor. Using these tools, we have been characterizing the distribution of receptor expression in developing and adult primates. Immunostaining with monoclonal antibody NGFR5, using an avidin-biotin horseradish peroxidase system, has been employed to localize sites of NGF receptor expression in monkey and human dorsal root ganglia. In both adult and embryonic DRG, NGF receptor immunoreactivity was heterogeneously distributed among sensory neuronal cell bodies. Some cell bodies stain strongly, while staining of others was barely greater than in controls with irrelevant antibodies. There was no apparent correlation of level of receptor immunoreactivity with size of cell bodies or position within the ganglia. Immunocytochemical results were confirmed by localization of NGF receptor mRNA utilizing in situ hybridization histochemistry with an NGF receptor cDNA probe. In DRG of adult monkey, we detected extensive hybridization to some neuronal cell bodies, while hybridization to other cell bodies was only slightly above background. These results are consistent with the results of Richardson et al., (J. Neurosci. 6: 2312-2321) showing that a subpopulation of rat DRG neurons bind NGF with high affinity. We find that immunostaining of NGF receptors in central projections of DRG neurons to spinal cord is predominantly restricted to Rexed lamina 1 and the dorsal margin of lamina 2 where small unmyelinated (C) fibers predominate. Peripheral projections of sensory neurons to cutaneous tissue also show NGF receptor immunoreactivity restricted predominantly to small unmyelinated axons. These results are consistent with the observed preverential loss of small unmyelinated axons in sciatic nerve in fetal guinea pigs immunochemically deprived of NGF (Johnson et al., Neurosci. 8: 631-642 1983).

142.8 AN IMMUNOHISTOCHEMICAL STUDY OF THE NERVE GROWTH FACTOR (NGF) RECEPTOR IN DEVELOPING RATS. Qiao Yan and E.M. Johnson, Jr. Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

NGF receptor expression was studied in rats between embryonic day 11 (E11) to postnatal day 10 (PND-10) by using a monoclonal antibody, 192-IgG, which specifically recognizes rat NGF receptor.

Sympathetic ganglia were positive for NGF receptor immunoactivity (NGFRI) at all ages. The staining was dense and uniform. As early as E11, NGFRI was found in the trigeminal ganglia, rostral dorsal root ganglia (DRG), and potential caudal DRG region, which suggests that the migrating neural crest cells may already be NGFRI positive. In DRG, NGFRI was relatively low at E13, had increased by E18 and decreased thereafter. Scattered cells, apparently neuroblasts, were NGFRI positive in E13 gut, but were not seen at other times. All embryonic peripheral nerves were very densely stained which dramatically decreased postnatally.

In spinal cord, NGFRI first appeared in the rostral portion of ventral, but not in the caudal, spinal cord as early as E11. By E13 NGFRI staining in ventral spinal cord was very dense; the caudal portion of E13 spinal cord showed an NGFRI staining pattern similar to rostral E11 spinal cord, indicating there is a rostral to caudal gradient of expression of NGFRI. At E15 the staining was much reduced in ventral spinal cord, both in terms of density and area. Between E18 and PND-0, some motoneurons in the lateral motor column appeared NGFRI positive. In dorsal spinal cord, NGFRI expression began at the dorsal root entry zone of E13, became very prominent in the whole dorsal horn at E18, and continued through postnatal stages. Area in brain stem innervated by the trigeminal ganglia central processes was NGFRI positive as early as E11 and persisted into the postnatal period. Large cell bodies in the basal forebrain region were NGFRI positive starting at about E15 and remained so postnatally. The cell surface of cerebellar Purkinje's cells including dendrites, were NGFRI positive starting at about PND-6 and very prominent at PND-10. Staining was also present on external granular cells. NGFRI disappeared in cerebellum around PND-15.

NGFRI staining in pia matter was very dense. Dental papilla also showed very distinctive staining. Both of these structures are known to originate from neural crest. Muscle components, limb buds and somites, were NGFRI positive starting at E11; the staining on myotubes became very dense at E15 and E18 and largely disappeared after E20.

The changes in NGFRI staining seen in this study suggest that NGF may have broader effects during development than previously thought.

142.9 DISTRIBUTION OF NGF-RECEPTOR-LIKE IMMUNOREACTIVITY IN THE POSTNATAL RAT BRAIN. F.P. Eckenstein, Dept. of Neurology and VIABR, Oregon Health Sciences University, Portland, OR 97201

Soluble neurotrophic factors that promote survival and differentiation of specific neuronal cell types are likely to be involved in the regulation of development in various neuronal systems. The so far best studied example is Nerve Growth Factor (NGF) and its action on sympathetic and sensory neurons. More recently, evidence has accumulated showing that NGF is also appears to be important for the development of cholinergic neurons in the mammalian basal forebrain (BFB). NGF has been shown to act on responsive cells through binding to a specific receptor. Monoclonal antibodies recognizing the receptor have now become available, and it is possible to use these antibodies to identify potential sites of NGF-action in central nervous system (CNS).

This report describes a survey of the spatial and temporal pattern of expression of NGF-receptor-like immunoreactivity (NGFR-LI) in the postnatal rat CNS. Immunohistochemical techniques (both PAP and immunofluorescence) using the monoclonal NGFR-antibody produced by Chandler et al. (J. Biol. Chem. 259: 6882-6889, 1984; a gift of Dr. E. Johnson, St. Louis) were employed for the study.

Intense, specific staining was observed in fibers in the olfactory bulb (OB); neuronal cell bodies in the BFB; non-neuronal cells in the median eminence (ME); fibers in the thalamus (THAL); fibers and possibly cell bodies in the cerebellum (CERB); fibers in the spinal cord (SC). The following table indicates the subjectively scored staining intensity for the NGFR in these areas at specific postnatal developmental stages:

	day 1	day 5	day 12	adult
OB	nd	nd	nd	+++
BFB	+	+++	++	++
ME	-	-	+	++
THAL	+	+++	+	-
CERB	nd	+++	+++	-
SC	+++	+++	+++	+++

Using double-staining techniques for the localization of the NGFR and the cholinergic marker choline acetyltransferase in the same section it was found that the markers label identical cells in the septal area and the nucleus basalis. In the caudate only very few neurons stained faintly for the NGFR. The number of stained neurons was considerably smaller than that of ChAT-positive neurons in that area, and the faint NGFR-staining did not allow unambiguous double-staining.

Interestingly, NGFR-LI was found to be transiently expressed in structures for which there is no demonstrated biological effect of NGF: the thalamus and the cerebellum. This might suggest that NGF has a specific developmental function in these areas, but the value of such a suggestion clearly depends on demonstrating that the staining observed represents true NGF-receptors and not immunologically similar material. It will also be important to identify the neuronal cell type expressing NGFR-LI in these areas. Experiments addressing these questions are in progress.

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142.10 DISTRIBUTION OF NGF RECEPTORS IN THE RAT BASAL FOREBRAIN: AN IN VITRO AUTORADIOGRAPHIC STUDY. Henry. K. Yip, Dept. of Anatomy, University of Utah, School of Medicine, Salt Lake City, Utah 84132.

We have used a monoclonal antibody 192-IgG to localize the NGF receptor in the basal forebrain of the rat by in vitro autogradiographic technique. Brain tissues were fixed in 0.1% formaldehyde and frozen sections of forebrain were incubated with (0.02 -2 nM) ^{125}I-192 -IgG or ^{125} I-NGF. Alternate serial sections were also incubated in the same media, with the addition of 1000-fold excess of unlabelled 192-IgG or NGF, or with equimolar of ^{125}I-cytochrome C, to produce autoradiograms representing nonspecific binding. Autoradiograms were generated by apposition of the labelled tissue sections to either emulsion - coated coverslip or LKB ultrofilm in X-ray cassettes. After 2- to 4- day exposure period, the coverslips and the films were developed.

The distribution of NGF receptors in the rat basal forebrain exhibits marked regional heterogenity. The binding pattern of ^{125}I-192-IgG and ^{125}I-NGF was similar but they differ in binding density. High concentrations of NGF receptors were found in the cortex, with the highest concentration in lamina VI and parts of lamina IV. The cingulate, entorhinal and primary olfactory cortices contain, in general, higher concentration of NGF receptors than did the other cortices. Very high density of NGF receptors were also seen in ventral and ventroposterior thalamus, olfactory tubercle and habenular nucleus. Moderate amount of NGF receptors were observed in association with the medial septal nuclei, diagonal band of Broca, hippocampus. lateral preoptic area, caudate-putamen nucleus, ventral pallidum. Low concentration of NGF receptors were found in amygdala and globus pallidus.

This finding indicates that in vitro receptor autoradiography of ^{125}I-192-IgG or ^{125}I-NGF binding to rat brain sections provides a sensitive method for the localization of NGF receptor in well-defined, highly circumscribed areas. These results have demonstrated that in addition to the localization of NGF receptors on the cholinergic neurons, NGF receptors can also be found on the other types of neurons in the rat basal forebrain.

TROPHIC AGENTS: NERVE GROWTH FACTOR III

143.1 CO-TREATMENT OF RAT PC12 CELLS WITH NGF AND BASIC FGF RESULTS IN COOPERATIVITY OF NEUROTROPHIC RESPONSES. R.E. Rydel and L.A. Greene, Department of Pharmacology, New York University School of Medicine, New York, N.Y. 10016

Fibroblast growth factors are well-characterized peptides that have potent angiogenic activity and which are mitogenic for many cell types. We have previously demonstrated that PC12 cells respond to FGFs by conversion to a neuronal phenotype in an almost identical fashion as that promoted by NGF (R.E. Rydel and L.A. Greene, *Soc. Neurosci. Abst.* 11:935, 1985). In the present study, we have extended these findings to determine the effects of simultaneous treatment of PC12 cells with both NGF and basic FGF (bFGF). The responses we analyzed were long-term, transcription-dependent responses of PC12 cells which are associated with their conversion to a neuronal phenotype, and which are highly specific for NGF and FGFs. These include neurite initiation, induction of phosphorylated, high molecular weight microtubule-associated protein 1.2 (MAP1.2), and elevation of AChE activity.

While both NGF and bFGF promote neurite initiation from PC12 cells, the rate of initial process outgrowth in serum-containing medium is always greater in the presence of NGF. Moreover, co-treatment with both factors results in a rate of neurite initiation of greater magnitude than that generated by either factor alone. In one experiment, after 6 days of treatment 53% of the cells possessed processes greater that 3 cell diameters in length with NGF, while neuritic processes were evident on only 24% of the cells in the presence of bFGF. However, simultaneous treatment of PC12 cell cultures with both NGF and bFGF resulted in 94% of the cells extending neurites. NGF or bFGF treatment also brings about an elevation of AChE activity. After 3 days of treatment, optimal doses of either factor will produce an approximately 2-fold increase in AChE activity. At sub-optimal doses, simultaneous treatment of PC12 cells with NGF and bFGF enhance AChE activity in an additive fashion. At optimal doses of both factors, the elevation of AChE activity was greater than that produced by either factor alone, but was less than additive. With regard to induction of phosphorylated MAP1.2, NGF and bFGF individually induce an approximately 15-fold increase when added to cultures for 13 days. Similar treatment with optimal doses of both factors added together results in an approximately 30-fold increase.

This work indicates that exposure of PC12 cells to an additional neurotrophic factor can enhance the maximal response to a single neurotrophic factor. Thus, co-expression of neurotrophic pathways during development can permit supra-maximal responses. Thus, in the presence of either sub-optimal or optimal levels of a given neurotrophic factor, further modulation of responses is still possible. Supported in part by NIH Research Grant NS-16036. R. E. Rydel was supported in part by NIH Predoctoral Training Grant GM-07827.

143.2 NERVE GROWTH FACTOR MODULATION OF NEUROPEPTIDE LEVELS IN ADULT SENSORY NEURONS IN CULTURE. R.M. Lindsay, C. Lockett and J. Winter Cell Biology, Sandoz Institute for Medical Research, London, U.K. (SPON: M. Gaze)

In contrast to the essential survival factor role of nerve growth factor (NGF) during sensory neuron ontogeny, the requirement of adult sensory neurons for NGF has not been well defined, although we have recently shown that adult rat dorsal root ganglion (DRG) sensory neurons can survive idefinitely *in vitro* in the absence of NGF (Neurosci. Soc Abs. 12, 299.13, 1986). Using immunofluorescence and radioimmunoassay, we have now examined the influence of NGF on the expression of certain neuropeptides in adult rat DRG neurons in culture. When pooled ganglia from lumbar, thoracic and cervical levels were cultured the percentage of CGRP (20-25%), substance P (12-20%) and somatostatin (<10%) containing neurons was found by immunofluorescence labelling to be essentially the same after 2 - 12 days in culture in the presence of NGF, and within the range of reported values for these ganglia *in vivo*. For a more detailed comparison between *'in vivo'* and *'in vitro'*, lumbar ganglia L3-L5 from the left side of one animal were sectioned, stained with anti CGRP and substance P, while the corresponding right-side ganglia were cultured and similarly stained after 2 and 7 days in culture. Similar percentages of CGRP (30-40%) and substance P (15-25%) positive cells were found in each case, indicating that the ratio of sub-types of DRG neurons which survive in culture is representative of the *in vivo* population and that the expression of neuropeptides by DRG neurons is probably restricted to a defined sub-population. Although NGF did not influence the number of DRG neurons which expressed specific neuropeptides, the level of substance P in adult DRG neuron cultures was found to be greatly increased (6 - 10-fold, cf. controls) when NGF was continuously present for 10 - 14 days. This marked elevation of substance P levels could be obtained even when the addition of NGF to cultures was delayed for 6 or more days. We conclude that NGF is not required for survival of peptidergic adult DRG neurons but does regulate the level of neuropeptide within these cells.

143.3 EFFECTS OF NGF AND INSULIN ON TUBULIN mRNA LEVELS IN SYMPATHETIC NEURONS. P. Fernyhough and D.N. Ishii (SPON: J.F. Masken) Physiology Department, Colorado State University, Fort Collins, CO 80523.

Like nerve growth factor (NGF), insulin and IGFs can induce neurite outgrowth and support survival of sympathetic neurons (Recio-Pinto et al., J. Neurosci. 6: 1211, 1986). These factors may provoke the increased assembly of tubulin into microtubules, which are important structural elements of neurites. Tubulin mRNA levels are elevated by NGF in pheochromocytoma PC12 cells (Fernyhough and Ishii, Neurochem. Res. in press, 1987) and by insulin and IGF-II in human neuroblastoma SH-SY5Y cells (Mill et al., 1985). The elevation of tubulin mRNA may be a common prelude to neurite formation under the direction of neuritogenic polypeptides. A critical test of this hypothesis requires that NGF and insulin can modulate tubulin mRNA levels in normal neurons. Purified RNA was analyzed by the Northern and dot blot methods using as probes for alpha- and beta-tubulin mRNAs the cDNA clones pKα1 (contains all of the coding region) and pDβ1 (contains all of the coding region except 21 bases from the 5' end), respectively. Equivalent amounts of RNA were analyzed in each experiment. NGF (4 μg) or vehicle were administered s.c. daily for 1 week to neonatal rats. Relative α-tubulin mRNA levels were increased 49 and 27% in RNA from sympathetic chain ganglia (two experiments). To determine whether these were direct effects on neurons, we continued the study in cultured 13-day-old embryonic chick sympathetic neurons under conditions in which the non-neurons were essentially eliminated (Recio-Pinto et al., 1986) on Primaria (Falcon) dishes. NGF and insulin increased both α- and β-tubulin mRNA levels within 2-4 hr. The maximum response (2-3 fold increase) was reached in about 8 hr with NGF and about 24 hrs with insulin. The response to NGF was transient, as previously observed for NGF in PC12, and insulin in SH-SY5Y, cells. It was not determined whether insulin's response in sympathetic neurons was also transient. The half-maximally effective concentration was about 10 pM for NGF, which is consistent with activity being mediated through the high affinity Slow NGF receptors. Insulin was active at concentrations below 1 nM, indicating it was acting through insulin receptors, rather than Type I IGF receptors. The mechanism for the elevation of tubulin transcripts may involve stabilization, as shown for NGF in PC12 cells (Ferynhough and Ishii, 1987). These results show NGF and insulin can elevate tubulin transcripts in sympathetic neurons, and provide critical support for the hypothesis. (Supported in part by NIH grant RO1 NSDK 24327 and an award from the Diabetes Research and Education Foundation).

143.4 NERVE GROWTH FACTOR DEPRIVATION RESULTS IN THE REACTIVATION OF LATENT HERPES SIMPLEX VIRUS IN SYMPATHETIC NEURONS IN VITRO. C.L. Wilcox* and E.M. Johnson, Jr. (SPON. D.B. McDougal, Jr.) Department of Pharmacology, Washington University School of Medicine., St. Louis, MO 63110.

Little is known about the mechanisms involved in the establishment and reactivation of latent herpes simplex virus (HSV). Neurons of neural crest-derived sensory ganglia and sympathetic ganglia are the predominant sites of latent HSV. These are nerve growth factor (NGF)-dependent neuronal cell types. We have determined that NGF is important in the maintenance of the latent state of HSV in sympathetic neurons in vitro.

Dissociated sympathetic neuronal cultures were prepared from fetal rat superior cervical ganglia. For 10 days after plating, the cultures were treated with 20 μM fluorodeoyuridine which reduced the non-neuronal cell population to less than 5%. Inoculation with 1 plaque-forming unit of HSV-1 per cell in the presence of either 10μM aphidicolin or 50 μM acyclovir resulted in 100% survival of the cultures. The drug was removed 7 days after inoculation with HSV. We detected no evidence of productive HSV infection, determined by assaying either cell lysates or culture media for infectious virus. Using immunohistochemistry, there were no detectable HSV antigens. Cultures have been maintained for up to 5 weeks postinoculation without any evidence of productive viral infection. When the cultures were deprived of NGF by treatment with antibodies to NGF, 100% of the cultures produced infectious HSV. The first cells that developed positive immunohistochemical staining for HSV antigens were neurons, indicating that the neuron was the site of latency. Latent cultures treated with nonimmune serum did not result in the reactivation of HSV. In addition, we have determined that 5 μg/ml 6-hydroxydopamine and 0.1 mM colchicine resulted in reactivation of latent HSV.

In summary, we have established an in vitro model of HSV latency which duplicates accurately viral latency in vivo. NGF deprivation resulted in the reactivation of latent HSV. These data suggest a physiologically important role of NGF in the maintenance of HSV latency. The data also indicate that the replication of HSV DNA was not necessary for the establishment of latency, as latency was achieved in the presence of drugs which block viral DNA replication.

143.5 NERVE GROWTH FACTOR PREVENTS VINBLASTINE-INDUCED SYMPATHECTOMY IN A DOSE-DEPENDENT FASHION IN NEWBORN RATS: EFFECT OF GM1 GANGLIOSIDE. G. Vantini, M. Fusco*, N. Schiavo*, D. Benvegnù* and A. Leon. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

We have utilized the model of vinblastine (VNB)-induced sympathectomy to quantitatively assess in vivo: 1) the activity of nerve growth factor (NGF; from male mouse submaxillary glands), and 2) the capability of GM1 ganglioside to modulate NGF effects. GM1 has been reported to facilitate NGF effects in cultured PC12 pheochromocytoma cells and neurons obtained from chicken embryo sensory and sympathetic ganglia.

The degree of sympathectomy induced by a single injection of VNB (0.15 mg/Kg, subcutaneously) given on postnatal day 3 was evaluated by measuring the noradrenaline (NA) content in sympathetically innervated organs (e.g. heart, spleen) of 6-day-old animals. Rat pups were treated s.c. on postnatal days 3, 4 and 5 with different doses of NGF, ranging from 0.01 to 0.5 mg/kg and/or GM1 (30 mg/kg). NGF was able to antagonize the NA decrease in a dose-dependent manner. The NGF dose-response curve obtained for the heart exhibited a different profile as compared to that for the spleen, thereby reflecting a different sensitivity of the corresponding sympathetic innervation to VNB and/or NGF treatments. Furthermore, GM1, but not asialo GM1, was able to potentiate the NGF effects in preventing the VNB-induced neurotoxicity. Thus, this model of VNB-induced sympathectomy appears to be suitable for evaluation of the activity not only of NGF(s) or NGF-like substances obtained from different sources, but also of agents capable of modulating NGF effects.

143.6 STUDIES OF GRAFTED ADRENAL MEDULLARY CELLS, EFFECTS OF NGF. I. Strömberg*, T. Ebendal*[1] and A. Hultgård*[2] (SPON: A. Seiger). Dept. of Histology and [2]Medical Cell Genetics, Karolinska Institute, 104 01 Stockholm, Sweden, and [1]Dept. of Zoology, Uppsala University, 751 22 Uppsala, Sweden.

Adrenal medullary tissue pieces, transplanted to the anterior chamber of the eye, can reinnervate a sympathetically denervated iris. In order to see which cell type(s) in the adrenal medulla that can produce processes, cell suspensions were injected into the anterior eye chamber. Using fibronectin, many single cells attached to the surface of the iris. In iris whole-mounts of such irides, a change was suggested from adrenaline-producing cells to noradrenaline-producing cells using antibodies raised against adrenaline (A) and dopamine β hydroxylase (DBH). Six days postgrafting, almost all cells showed A-like immunoreactivity and were negative with DBH, while after 1 month all cells were DBH-positive. One to three months postgrafting some single cells were elongated and had thick, short processes. When occasionally a network of nerve fibers was seen, it originated either from a cluster of cells, rather than from single chromaffin cells, or from single sympathetic ganglion-type cells. The reinnervated area was very small compared to the area that adrenal medullary tissue pieces can innervate in the same length of time. One factor that influences the outgrowth from these grafts is nerve growth factor (NGF). Although a sympathetically denervated iris has increased endogenous levels of NGF, this amount of NGF does not seem to be enough for single cells to produce nerve terminals, except for the few ganglion cells that produced large and varicose nerve plexuses resembling normal sympathetic nerves. When increasing the levels of NGF further by weekly injections into the anterior eye chamber for 3 weeks, there was a clear increase in number of cells that produced processes. The innervated areas were much larger, and the nerve plexuses had a more normal appearance as opposed to the short and thick processes that elongated chromaffin cells produced without added NGF. The number of ganglion cells had increased remarkably, and a transitional cell type with a cell body as large as a ganglion cell but with a chromaffin-like strong fluorescence intensity was also found. We conclude that large numbers of sympathetic neuron-type cells result when adult adrenal medullary cell suspensions are treated with NGF in oculo.

143.7 NGF AS A NEUROTROPHIC FACTOR IN THE BASAL FOREBRAIN: EVIDENCE FROM TISSUE TRANSPLANTED IN OCULO. S. Skirboll*, M. Eriksdotter-Nilsson*, T. Ebendal*[1], L. Hersh[2], J. Massoulié*[3], L. Olson*. (SPON: L. Skirboll). Dept. of Histology, Karolinska Institute, Stockholm, Sweden; [1]Dept. of Zoology, Uppsala Univ., Uppsala, Sweden; [2]Univ. of Texas Health Science Ctr., Dallas, TX; [3]Lab. de Neurobiologie, ENS, Paris, France.

In the present study, in order to further discern the role of NGF in the central nervous system, we have attempted to employ a unique in vivo model. Fetal (E17) rat basal forebrain tissue was transplanted to the anterior chamber of the eye, utilizing a treatment protocol of pre-grafting incubation and weekly 5 ul intraocular injections of either NGF (10,000 BU/ml) or saline. In vivo measurements of the transplants reveled a significant ($p < 0.05$) increase in the size of the NGF-treated grafts. Following pretreatment with diisopropylfluorophosphate (DFP), the transplants were processed for acetyl cholinesterase (AChE) histochemistry, and choline acetyltransferase (CAT) and recently developed AChE immunocytochemistry. The NGF-treated transplants demonstrated an increase in the number of specifically stained neurons in all three histological analyses, of which the most pronounced effect was seen with AChE immunocytochemistry ($p < 0.05$). The number of cholinergic neurons per unit volume of transplant, however, was not significantly increased with NGF treatment. The density of nerve fiber outgrowth, as visualized by AChE immunocytochemistry (unaffected by prior DFP) was only slightly enhanced. In addition, preliminary results from transplants treated instead with polyclonal antibodies to NGF have proved inconclusive, which may reflect the rapid turnover of such antibodies in the anterior chamber of the eye.

Thus, we have demonstrated that in oculo transplantation of basal forebrain tissue is a useful model for examining the in vivo effects of NGF on central cholinergic function. Basal forebrain tissue can be influenced to survive and mature at different rates in oculo, and our results suggest that NGF may act as a neurotrophic factor for noncholinergic as well as cholinergic neurons of the basal forebrain. Additional studies are in progress to determine the turnover of NGF in oculo, optimal dosage and treatment schedules, and to identify which noncholinergic cell populations may be the most susceptible to the effects of NGF.

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143.8 NERVE GROWTH FACTOR (NGF) IS PRESENT IN HYPOTHALAMIC OLIGODENDROCYTES: IMMUNOCYTOCHEMICAL EVIDENCE FROM PRIMARY CULTURES IN SERUM-FREE MEDIUM. D. Gonzalez*, W.L. Dees* and S.R. Ojeda. Neuroscience Division, Oregon Regional Primate Research Center, Beaverton, OR 97006 and Department of Veterinary Anatomy, Texas A & M University, College Station, TX 77843.

Although the presence of NGF in brain is well documented little attention has been given to its presence in the hypothalamus or to the cell type that contains NGF. The present investigation was undertaken to examine this issue. Hypothalamic cells from 19-day-old rat fetuses were mechanically dispersed and seeded in 35 mm culture dishes at 0.8×10^6 cells/plate onto coverslips precoated with 10% fetal bovine serum (FBS). The cells were cultured for 4 days in F12: Dulbecco's medium (1:1) containing 0.7% FBS, 5 µg/ml insulin, 100 µM putrescine, 30 nM sodium selenite, and 100 µg/ml transferrin. At the end of this period the FBS-containing medium was removed and the cells were cultured two more days in serum-free medium. They were then fixed in Zamboni's fixative (2 h) or 3.7% formaldehyde (10 min) and processed for immunohistofluorescence analysis. All primary antibodies were prepared in rabbits and were kindly supplied to us by Dr. J. deVellis, J. Bottestein and E. Tiffany-Castiglioni; an antirabbit IgG coupled to fluorescein isothiocynate was used as the secondary antibody. Cells containing immunoreactive NGF were scattered over a layer of other cells which had attached to the coverslip surface. Both the body and processes of the positive cells displayed strong NGF immunoreactivity. Absorption of the antiserum with NGF (20 µg/ml) completely eliminated the immunoreactivity. Astrocytes, identified by the presence of glial fibrillary acidic protein (GFAP) had a morphological aspect completely different from that of NGF-containing cells and did not display NGF immunoreactivity following elution of the GFAP antibody. In contrast, cells identified as oligodendrocytes (because they express the membrane galactosphingolipid galactocerebroside and the cytosolic enzyme glycerol 3'-phosphate dehydrogenase) had a morphological aspect identical to NGF-containing cells. Upon their mechanical removal from the coverslip, the NGF immunoreactivity disappeared indicating the NGF is contained in oligodendrocytes. The results suggest that the hypothalamus has the capability of expressing the NGF gene and that this capacity is limited to cells of the oligodendroglial cell lineage. Supported by NIH grants HD-09988, Project IV and NIAA07216.

143.9 NERVE GROWTH FACTOR-LIKE IMMUNOREACTIVITY IN THE DEVELOPING RAT COCHLEA. Gerard Despres* and Raymond Romand. Université de Clermont II. Laboratoire de Neurobiologie, Ensemble Scientifique des Cezeaux, B.P. 45, 63170, AUBIERE (France)

The presence of nerve growth factor-like protein have been investigated on cryostat sections from Sprague Dawley rat cochleae of various stages(0,2,6,7,8,10,15,30 days old and adults) by indirect immunofluorescence technique. Sheep polyclonal anti serum against 2.5S NGF (a gift of H. Thoenen) has been tested for specificity to NGF firstly on sections of mouse submaxilary glands. Secondly, absence of fluorescent staining was observed when anti-NGF antiserum was omitted or when normal sheep serum was used instead of anti-NGF or when anti-NGF antiserum was previously immunoabsorbed with pure 2.5S NGF. NGF-like immunoreactivity was clearly present in the organ of Corti from birth to postnatal day 6 and localised in the hair-cells. A gradient of staining was visible between basal and apical turns at birth and at 2 days where the basal was most intensely labeled. From postnatal day 7 the gradient of staining was inverted, the basal turn becoming less reactive. Immunostaining completely disappeared around postnatal days 9-10 and was also non-existant in 15 days, 1 month old and adults rats. The immunofluorescence is essentially localised at the highest third part of the hair-cells and seem to coincide with the Golgi complex network. No immunostaining could be registered in the spiral ganglion cells. Control experiments allow to postulate that immunostaining in the hair-cells probably results from the interaction of NGF-antibodies with an endogenous NGF-like material. Synaptogenesis being not completed at birth in the rat, it is possible that this substance is, up to postnatal days 7-8, one of the trophic factors suspected in the rat cochlea. Spiral ganglion cells may require other neurotrophic factor during development.

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143.10 NGF IN TESTIS AND EPIDIDYMIS: LOCALIZATION OF MESSAGE AND PROTEIN AND MEASUREMENT OF BIOLOGICAL ACTIVITY. L. Olson*, G. Ayer-LeLievre*, H. Persson*[1], T. Ebendal*[2] (SPON: G. Kindr[3]) Dept. of Histology, Karolinska Institute, 104 01 Stockholm, Sweden; [1]Dept. of Medical Genetics, [2]Dept. of Zoology, Uppsala University, 751 22 Uppsala, Sweden, and [3]Dept. of Neurosurgery, Univ. of Colo. Med. Ctr., Denver, CO 80262.

The presence of high levels of nerve growth factor (NGF) in the male mouse submandibular glands remains enigmatic, although it may represent one example in which NGF's primary function is not associated directly with the nervous system. Several observations in the literature suggest that another such instance may be the presence of NGF in the male reproductive system. However, there have been no direct studies of the rodent testis and epididymis to address this question. Using affinity-purified antibodies raised against mouse salivary gland β-NGF, we found immunoreactivity in rat and mouse testis. All cells of the germ cell line except spermatogonia were strongly immunoreactive in both species. Immunoreactivity was also seen in residual bodies in testis and epididymis. Using antibodies directed against specific peptide sequences within NGF and pro-NGF, the pattern of NGF-like immunoreactivities could be further subdivided; immunoreactivity with a high degree of regional specificity was localized to defined portions of the epithelium of ductus epididymidis throughout the various portions of caput and in corpus and cauda. Presence of NGF protein was also shown by a two-site enzyme immunoassay indicating about 10 and 70 ng NGF per gram tissue in mouse testis and epididymis, respectively. Extracts from these organs stimulated fiber outgrowth in cultured sympathetic ganglia, an effect which was blocked by anti-NGF antibody. Blot analysis of RNA prepared from testis and epididymis demonstrated the presence of 1.3 kB and 1.5 kB NGF message except in one case, the rat testis, where only the 1.5 kB message was found. In situ hybridization also localized the NGF message to the germ cell line excluding the spermatogonia, late spermatides and spermatozoa in the seminiferous tubules, and to the epithelium of ductus epididymidis. Leydig and Sertoli cells appeared negative using both immunohistochemistry and in situ hybridization techniques. We conclude that five types of analysis -- blot and in situ hybridization, enzyme immunoassay, immunohistochemistry and bioassay -- all strongly indicate production of NGF in the male rodent reproductive system, and suggest a non-neuronal role for NGF possibly relating to sperm maturation and/or motility.

143.11 IMMUNOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF IMMUNOAFFINITY PURIFIED FAB FRAGMENTS AGAINST MOUSE NERVE GROWTH FACTOR. L. Callegaro*, A. De Martino*, S.D. Skaper, D. Benvegnù*, G. Vantini, C. Triban*, G. Toffano and A. Leon. (SPON: S. Mazzari). Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

Intracerebral injections of antibodies to nerve growth factor (NGF) provide one experimental mode to verify whether endogenously occurring NGF exerts an influence on the cholinergic neurons of the basal forebrain in vivo. Since whole antibodies, due to their size, may not penetrate well the neonatal and/or adult brain, we have purified and characterized anti-NGF Fab fragments.

Polyclonal antibodies against male mouse submaxillary gland ß-NGF were raised in rabbits. The specific antibody fraction was isolated from rabbit antiserum utilizing immunoaffinity chromatography and proteolyzed by papain digestion. The resulting Fab fragments were then purified on a Protein A-Sepharose column and subsequently isolated by immunoaffinity chromatography using ß-NGF linked to Sepharose 4B. Elution of the immunoreactive fraction was accomplished by high salt concentration.

Chemically, the purity of the isolated IgG and its Fab fraction was evaluated by SDS-gel electrophoresis and fast protein liquid chromatography; specificity was assessed utilizing Western blots. In addition, the biological activity of both the purified IgG and its Fab fragment was tested by the ability of these fractions to block the survival-promoting activity of NGF for embryonic (E8) chick dissociated dorsal root ganglionic neurons. Both fractions displayed a high activity, with 5 ng/ml of ß-NGF being fully blocked by 0.5 ug/ml or less of the specific Fab fragments. Fab fragments prepared from preimmune serum IgG were inactive up to 80 ug/ml. Experiments are currently in progress to assess the ability of these Fab fragments to function in vivo by inducing immunosympathectomy.

SPINAL CORD I

144.1 **A SIMPLE METHOD OF COMBINING AUTORADIOGRAPHY AND HRP-TMB HISTOCHEMISTRY ON THE SAME TISSUE SECTION.** K.N. Nandi*, J.A. Beal and D.S. Knight* (SPON: J.E. Penny). Department of Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

In order to determine the sequence of development of various types of spinal neurons defined by their projection we have developed a method for combining tritiated thymidine autoradiography for birthdate determination with the demonstration of retrogradely transported horseradish peroxidase (HRP). Because of its greater sensitivity, TMB is the chromogen of choice for the demonstration of HRP. However, the HRP-TMB reaction product is unstable and lost when the tissue is processed for autoradiography. By reversing the process, i.e. developing the tissue for autoradiography and then reacting with TMB, we were able to preserve some HRP-TMB reaction product but results were inconsistent. However, the use of osmium as a postreaction stabilizing agent (1% osmium tetroxide in 0.1M phosphate buffer, pH 6.0 at 45°C for 15-30 minutes) preserves the HRP-TMB reaction product in the form of a brownblack precipitate which is not destroyed when the tissue is subsequently processed for autoradiography. Although some reaction product appeared to fade from secondary and tertiary dendrites, no detectable loss was noted in the nerve cell bodies. As an additional advantage we found that background levels of the autoradiographs stabilized with the osmium procedure are extremely low.

In conclusion, osmication of HRP-TMB reaction product results in a stable compound which is not destroyed by autoradiographic processing and enables us to demonstrate tritiated thymidine autoradiography and HRP-TMB reaction product on the same tissue sections.

144.2 **DETERMINATION OF THE SEQUENCE OF PROLIFERATION OF MARGINAL (LAMINA I) NEURONS IN THE SPINAL CORD OF THE RAT.** J.A. Beal, D.S. Knight* and K.N. Nandi* Department of Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

The present study is an attempt to determine if different types of neurons within lamina I proliferate in a specific pattern or sequence. Separate groups of marginal neurons were defined on the basis of size and dendritic architecture using the Golgi technique. This method was combined with tritiated thymidine autoradiography to determine each neuron's time of origin or "birthdate". Pregnant rats of the Wistar strain were injected (i.p.) with tritiated thymidine (5.0µCi/g body wgt., spec. act. 6.7 Ci/m mole) on one of embryonic (E) days E13 through E18. Pups were delivered and later sacrificed on postnatal day P30. Tissue from the lumbar enlargement was processed via the Golgi technique and impregnated neurons were drawn with the aid of a Leitz microscope with drawing tube attachment. Sections were then remounted in JB-4 plastic, sectioned at 3.5 microns, and processed for autoradiography. Nuclei of impregnated neurons were then examined for radioactive label. In animals treated on day E13 all neurons of the marginal layer are labelled with tritiated thymidine. In animals treated on E14, most marginal cells are still labelled, but all of the large and some of the small to medium size pyramidal and multipolar shaped neurons are now unlabelled. In animals treated on E15 approximately half the neuronal population of the marginal zone is unlabelled. Most of these unlabelled neurons are pyramidal, multipolar or flattened shaped cells which display a variety of dendritic patterns. On the other hand most labelled cells at E15 are round or fusiform shaped. Some of these labelled neurons have longitudinal arbors restricted to lamina I, while others have delicate ventral dendrites which arborize in laminae II and III. A few of these neurons are labelled in animals treated as late as day E17 and are amongst the last neurons of the spinal cord to complete cell division. In conclusion, the results show that marginal neurons proliferate in a definite sequence, but there is overlap between the proliferative periods of the various cell types.

144.3 **MET-ENKEPHALIN IMMUNOREACTIVITY IN THE LAMINA X REGION OF PRIMATE SPINAL CORD.** C.M. Kocol*, and C.C. LaMotte, Sec. of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510.

We determined met-ENK like immunoreactivity (MELI) in the lamina X region of the primate (Macaca fascicularis) spinal cord (segments C6-7) using the PAP method. Heavy concentrations of this peptide were found in terminal fields dorsolateral to the central canal. Lesser amounts of MELI were distributed in the dorsal grey and the ventral grey commissures, as well as the pericanal area. Labelled met-ENK neurons were identified lateral to the central canal.

With electron microscopy several MELI terminal types were found throughout lamina X. The most prevalent type contained both granular and agranular vesicles; however there were also some MELI terminals containing only clear, round vesicles. In most cases, these terminals formed either asymmetrical or symmetrical synaptic junctions with various sizes of unlabelled dendrites. These dendrites, in turn, were commonly also postsynaptic to unlabelled R-terminals. MELI terminals also were found to synapse with unlabelled somata in the lateral regions of lamina X. Axo-axonic junctions were observed between MELI and unlabelled R terminals, in which the MELI terminal was presynaptic. Occasionally MELI terminals were observed to be postsynaptic to an unlabelled glomerular terminal in the most lateral region of lamina X. Lateral to the canal, MELI neurons and their dendrites received input from both MELI and unlabelled R terminals; a recent report has shown some spinoreticular neurons in X to contain ENK (Nahin and Micevych, Neurosci. Lett. 65:271).

When compared with our past studies of the ultrastructure of MELI in the monkey dorsal horn, some differences are evident; for example, terminals contacting spines were common in the dorsal horn but rarely observed in lamina X. Also, it appears that MELI terminals containing large granular vesicles are more frequent in lamina X, whereas R type MELI terminals were more common in laminae I and II. Since local ENK neurons are considered to be the major source of ENK terminals in both areas, the differences in terminal types may reflect differences in the character or function of those cells in each region, or possibly differences in colocalization of other neurochemicals. **(Supported by NIH grant NS13335)**

144.4 ENKEPHALINERGIC TRIGEMINAL AND SPINAL NEURONS PROJECTING TO THE RAT THALAMUS. J.A. Coffield* and V. Miletic, (SPON: P. Bach-Y-Rita), Dept. of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

Although much is known about the anatomy, physiology and pharmacology of trigeminothalamic (TTT) and spinothalamic tract (STT) cells, little information is available regarding their putative neurotransmitter content. In order to determine whether any TTT and STT cells exhibit enkephalin immunoreactivity (ENK), we have combined the techniques of retrograde labeling and immunocytochemistry.

Unilateral injections (50-100nl) of the retrograde tracer Fluoro-Gold (FG, 4%) were made stereotaxically into the medial thalamus (MT) of 12 rats and lateral thalamus (LT) of 7 rats. After 3-8 days, the animals were deeply anesthetized and perfused with 4% buffered paraformaldehyde. In some rats, colchicine (10μg/ml) was injected intrathecally over the lumbar spinal cord eight hours before sacrifice. Vibratome sections of the thalamus (100μm), and of the brainstem and spinal cord (30μm) were examined with a fluorescent microscope. The trigeminal and spinal sections were then processed for ENK with rhodamine immunofluorescence.

MT injection sites (anteromedial, central medial, mediodorsal, reuniens, rhomboid, and submedius nuclei) were exclusive of LT injection sites (the lateral dorsal, lateral posterior, ventroposteromedial, and ventroposterolateral nuclei, and the posterior nuclear group). FG-labeled neurons were distributed throughout the trigeminal and spinal tissue, although MT injections labeled mostly cells in laminae VI and VII, and LT injections neurons in laminae I and V.

After immunocytochemical processing, single-labeled ENK neurons paralleled the distribution of the retrogradely-labeled cells. However, only 10% of the MT-projecting, and 14% of the LT-projecting trigeminal and spinal neurons were enkephalinergic. The MT-projecting ENK neurons originated from laminae VI and VII. The LT-projecting neurons were located in the white matter just dorsal to lamina I and in lamina V.

These data indicate that, in the rat, a small number of TTT and STT neurons are enkephalinergic. These data also suggest that the MT and LT-projecting ENK neurons have differential laminar origins, and distinct roles in thalamic sensory processes.

144.5 ORIGIN OF SPINAL NERVE TERMINALS CONTAINING A NOVEL NERVE TERMINAL PROTEIN: CONTRIBUTION OF DESCENDING PATHWAYS. S.S. Kanekar, T.C. Ritchie and J.D. Coulter. Neuroscience Program and Dept. of Anatomy, The Univ. of Iowa, Iowa City, IA 52242.

The monoclonal antibody S-7B8 recognizes an integral membrane protein in selected populations of nerve terminals in the central nervous system. The S-7B8 antigen appears to be associated with synaptic vesicle membranes. In rat spinal cord, S-7B8 immunoreactive nerve terminals are concentrated in the superficial dorsal horn (laminae I and IIi). Less dense staining extends ventrally through laminae V, and the ventral horn contains some sparse staining. Primary afferent neurons give rise to most of the stained terminals in laminae I and II, and a portion of those in the deeper laminae of the dorsal horn. Current efforts are focused on localizing the cells of origin of the remaining S-7B8 positive nerve terminals in the spinal cord.

In the developing nervous system, the S-7B8 antigen is contained in certain fiber tracts, but becomes concentrated in nerve terminals during and after synaptogenesis. Since the antibody densely stains the developing corticospinal tract, this system is a possible source of S-7B8 positive nerve terminals in the adult spinal cord. Double-label studies were carried out using HRP injections into the lumbosacral spinal cord to retrogradely label corticospinal neurons, followed 3 days later by topical application of colchicine to the sensory/motor cortex to allow immunocytochemical localization of cell bodies containing the S-7B8 antigen. Numerous double-labelled pyramidal cells were evident in layer V of the cerebral cortex, indicating that in adult, as well as developing rats, corticospinal neurons do contain the S-7B8 antigen. S-7B8 immunoreactive cell bodies have also been observed in certain brainstem nuclei following application of colchicine, suggesting that additional descending pathways to the spinal cord may contain the S-7B8 antigen. Using the double-labelling method described above, cells giving rise to the lateral vestibulospinal tract have been shown to contain the S-7B8 antigen and are a likely source of the S-7B8 positive nerve terminals present in the spinal ventral horn.

These results indicate that the corticospinal tract, and possibly other descending systems, contribute to the distinctive, highly localized pattern of S-7B8 immunoreactivity in the spinal cord. From these studies, it appears that the S-7B8 antibody will be a useful probe for examining the synaptic connections and development of descending spinal pathways.

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144.6 Occurrence of substance P-like immunoreactivity in synaptic glomeruli of the spinal cord and trigeminal nucleus caudalis of the rat. A. Ribeiro-da-Silva*, P. Tagari* and A.C. Cuello. Department of Pharmacology and Therapeutics, McGill University, 3655 Drummond Street, Montreal, P.Q. H3G 1Y6, Canada.

The present study provides a characterization of the substance P-like immunoreactive central boutons of synaptic glomeruli in the superficial dorsal horn of the spinal cord and trigeminal nucleus caudalis of the rat.

A comparative study of the light microscopic and ultrastructural distribution of substance P-like immunoreactivity in laminae I-II of the spinal cord and trigeminal nucleus caudalis was carried out using a bispecific anti-substance P/anti-HRP monoclonal antibody (P4C1) (Suresh, M.R. et al., Proc. Natl. Acad. Sci. USA, 83:7989, 1986). Ultrathin sections of laminae I-II of the spinal cord and trigeminal nucleus caudalis were obtained from five blocks per animal. The immunoreactive axonal boutons and all synaptic glomeruli were counted on the electron microscope screen. The features of immunoreactive and non-immunoreactive glomeruli were analyzed with an automated image analysis system (Quantimet 920).

In lamina II, of the spinal cord, a significant number of the labelled varicostities were central boutons (C_I) of type I synaptic glomeruli. They could be recognized as such by the scalloped contour, types and number of peripheral profiles, reduced density of mitochondria and localization in the dorsal horn. However, these immunoreactive glomerular C_I boutons (about 10% of the total number of C_I) differed from the prevailing population of type I glomeruli because they established less synapses, only rarely had peripheral axonal boutons and contained more than three dense-core vesicles per profile.

In the trigeminal laminae I and II, the substance P fibres and varicosities had a plexiform orientation, at the light microscopic level, that contrasted with the mainly rostro-caudal orientation of the plexus of the spinal cord. By electron microscopy, the number of immunoreactive profiles was lower, but the main ultrastructural findings were rather similar, in spite of the different orientation of the neuropil. However, many immunoreactive varicostities were confluent and axosomatic contacts were more frequent than in the spinal cord.

In contrast with current thinking, this demonstrates that substance P immunoreactivity occurs in a considerable number of type I synaptic glomeruli, morphologically distinct from those that have fluoride-resistant acid phosphatase activity. It also reinforces the concept that the gelatinosa of the spinal cord and the trigeminal nucleus are homologous structures.

(Dr. A. Ribeiro-da-Silva acknowledges a grant from the Gulbenkian Foundation, Lisbon).

145.1 FUNCTIONALLY IDENTIFIED LOCAL CIRCUIT NEURONS IN RAT TRIGEMINAL SUBNUCLEUS INTERPOLARIS. M.F. Jacquin, M. Barcia*, J. Golden* & R.W. Rhoades. Dept. Neurosci., NY Coll. of Osteo. Med., Old Westbury, NY 11568 & Dept. Anat., NJ School of Osteo. Med., Piscataway, NJ 08854.

Intracellular recording, electrical stimulation, receptive field (RF) mapping, and HRP injection techniques were used to determine the morphology, response properties, and collateral projections of 18 local circuit (LC) neurons in trigeminal (V) subnucleus interpolaris (SpVi). None were antidromically activated from thalamus, tectum, or cerebellum, and their axons could be seen terminating locally. All exhibited narrow, biphasic action potentials following gentle displacement of 1, and only 1, facial whisker. More forceful deflection of adjacent whiskers often activated LC cells, though this reflected artifactual displacement of the 1 whisker RF. They responded within 1.34±0.28ms (mean±S.D.) of ipsilateral V ganglion shocks, and 16 gave a phasic discharge to whisker deflection without directional sensitivity or spontaneous activity. The other 2 cells were tonically activated and 1 of these exhibited spontaneous discharges which could be inhibited by deflection of adjacent whiskers. The morphology and topography of these 18 cells were remarkably consistent. Small round to ovoid shaped multipolar somata (area: 183±78 um^2) were located 0.4-1.6mm rostral to the SpVi-caudalis border, each within the confines of the longitudinal column made by the arbors of primary afferent axons with the same 1 whisker RF. Axons of LC neurons (diameter:1.69±0.66 um) had a transverse trajectory, giving off 2.7±1.3 recurrent collaterals which arborized extensively in the region of the soma with both terminal and en passant boutons. After proceding to an SpVi region remote from the soma, the parent axon always bifurcated. One branch traveled rostrally, within either a nuclear axon bundle or the V spinal tract, to end in subnucleus principalis, while the other branch traveled caudally to end in caudalis. Each branch gave off intermittent non-recurrent collaterals which arborized in topographically appropriate regions of each of the 4 V brainstem subnuclei. The total # of collaterals per subnucleus was variable (e.g. 4.5±1.8 in SpVi). These cells had 4.1±1.2 proximal dendrites which extended 440±140um rostrocaudally and occupied 2.0±1.3 of 4 transverse quadrants surrounding the soma. Thin sinuous dendrites branched extensively within a narrow area adjacent to, or overlapping with, the soma, and rarely gave rise to spines. Rather, they ended in multi-lobed, bulbous, grapelike swellings, connected by slender processes. The dendritic tree had a transverse perimeter of 459±226um and a form factor of .61±.15; neither differed from that of their presumed presynaptic primary afferent arbors (379±87um; .55±.11). An unusual feature of the SpVi LC cell is its extensive internuclear connections. Its somadendritic form, however, is similar to that of inhibitory LC cells in cat lateral geniculate (Hamos et al. Nature 317:618, 1985). SpVi LC cells may control the gating of V primary afferent inputs to projection cells in SpVi and other V subnuclei. Support: DE07662,DE07734,DE06528,BNS8517537, AOA.

145.2 MORPHOMETRIC COMPARISON OF FUNCTIONALLY IDENTIFIED TRIGEMINOTHALAMIC AND TRIGEMINOCEREBELLAR NEURONS IN TRIGEMINAL SUBNUCLEUS INTERPOLARIS. J. Golden*, M. Barcia*, R.W. Rhoades & M.F. Jacquin (SPON: H.P. Zeigler). Dept. Neurosci., NY Coll. of Osteo. Med., Old Westbury, NY 11568 & Dept. Anatomy, NJ Sch. of Osteo. Med., Piscataway, NJ 08854.

A prior study (Woolston et al., J. Neurophys. 48:160, 1982) in rat trigeminal (V) subnucleus interpolaris (SpVi) has shown that thalamic- (VB) projecting neurons have larger receptive fields (RF) than cerebellar- (CB) projecting cells, and that both groups have larger RF's than cells which do not project to either of these targets. In the present study, intracellular recording, electrical stimulation, RF mapping, and HRP injection procedures were used to determine if the structure of these cells differed. 21 VB-, 12 CB-projecting, and 32 local circuit neurons were studied. Most of these (18, 9, and 18, respectively) responded only to whisker deflection and these results are summarized below (Mean ± S.D.; see Jacquin et al., this volume, for data on local circuit neurons):

	Thalamic	Cerebellar
Latency to V ganglion shock	1.32 ± 0.22	1.54 ± 0.34 ms
Latency to antidromic activation	1.12 ± 0.75	0.61 ± 0.09 ms
# of whiskers comprising RF	11.6 ± 5.7	4.4 ± 4.9
Range of # of whiskers	3 - 25	1 - 15
Adaptation properties	all phasic	8 phasic, 1 tonic
mm rostral to caudal SpVi border	0.9 - 1.9	0.8 - 1.6
Soma transverse area	453 ± 198	351 ± 149 um^2
# of proximal dendrites	5.1 ± 1.8	5.0 ± 1.2
Modal dendritic ending	spines	spines
* Dendritic transverse perimeter	1363 ± 557	888 ± 273 um
* Dendritic transverse area	82,670 ± 46,930	48,210 ± 31,760 um^2
Dendritic shape: form factor	0.56 ± 0.20	0.70 ± 0.16
* Rostrocaudal dendritic extent	606 ± 189	412 ± 35 um
Parent axon diameter	2.80 ± 1.22	2.53 ± 1.17 um
* # of axon collaterals in SpVi	1.7 ± 1.9	0

(* - significant difference between groups, $p<.05$)

Transverse and rostrocaudal dendritic extent correlated significantly with RF size both within and between VB- and CB-projecting groups. Moreover, local circuit neurons each responded to only 1 whisker and they had significantly smaller dendritic trees than that of projection neurons. These data suggest that dendritic extent is a mechanism controlling RF size in whisker-sensitive SpVi neurons. The nature of the primary afferent convergence expressed in SpVi projection neurons merits further study. Such cells have multiple whisker RF's, yet they do not respond to deflection of intervening guard hairs or hairy skin, therein resulting in discontinuous RF's. Since these receptor types are innervated by axons which project to whisker regions of SpVi, a mechanism must exist to account for such selectivity in primary afferent convergence.

Support: NIH (DE07662, DE07734, DE06528), NSF (BNS8517537), AOA.

145.3 RECEPTIVE FIELD SYNTHESIS IN TRIGEMINAL SUBNUCLEUS INTERPOLARIS. I. CORTICAL INPUTS. K.L. Rossner & M.F. Jacquin. Dept. of Neuroscience, New York College of Osteopathic Medicine, Old Westbury, NY 11568.

Rat trigeminal (V) subnucleus interpolaris (SpVi) receives inputs from both low and high threshold primary afferents, ipsilateral V subnucleus caudalis and amine containing nuclei, and contralateral somatosensory cortex. The resultant receptive field (RF) properties of SpVi cells can be summarized: local circuit neurons have small low threshold RF's, each reflecting input from 1 class of receptor organs (e.g. 1 whisker); thalamic- (VB) or cerebellar- (CB) projecting SpVi cells have larger low threshold RF's, yet convergence is restricted to 1 class of receptor organs (intramodality; e.g. multiple whiskers). RF size correlates with dendritic tree area, though it remains to be determined why nociceptive inputs are not widely reflected in SpVi RF's, and why multi-whisker-sensitive projection neurons do not respond to intervening guard hairs or skin. Since these receptor organs are innervated by axons which project to SpVi whisker regions, primary afferent segregation cannot account for these 'discontinuous', modality-specific RF's. To determine the role of cortical input in the synthesis of SpVi RF's, right somatosensory cortex was removed in 7 adult rats. 4-34 days later, single unit recording, electrical stimulation, and RF mapping techniques were used to study the responses and projections of 235 cells in left SpVi. In 13 cells, projection status was verified by intracellular HRP staining. Physiological data are summarized below, compared with that of 208 cells from 27 normal rats:

	Cortex Ablated	Normal
Mean latency (±SD) to V ganglion shk.	1.43 ± 0.41 ms	1.43 ± 0.57
Mean # of spikes/ganglion shk. at 2XT	1.93 ± 1.24	1.88 ± 1.20
% VB- vs CB-projecting vs local circ.	17.0,11.5,71.5	28.8,10.6,60.6
% best-responsive to noxious stimuli	2.5	2.4
% with intermodality convergence	7.7	0.0
% unresponsive to any stimulus	7.2	0.5
% whisker(s)-sensitive	55.7	63.5
Mean # of whiskers (VB-project)	7.12 ± 4.98	9.23 ± 5.41
Mean # of whiskers (CB-project)	2.56 ± 2.18	3.11 ± 2.45
Mean # of whiskers (local circuit)	1.83 ± 2.01	1.04 ± 0.27
% local circuit with > 1 whisker RF	27.5	2.9
% with spontaneous discharges	26.8	17.3
% phasic vs tonic evoked responses	77.9 vs 14.9	91.3 vs 8.2
% with directional sensitivity	12.3	5.3
% with inhibitory surround	5.1	3.4
% with split or cervical RF's	0.8, 0.4	0.0, 0.0

The RF's of 41 V primary afferents were normal, as was the topographic organization of SpVi. Qualitative effects include the incidence of multiple-whisker-sensitive local circuit, and intermodality convergent, cells. The relative #'s of nociceptors were unchanged. Thus, cortical inputs contribute to RF size and convergence in a small # of low threshold SpVi neurons. Other quantitative effects remain to be substantiated. Support: NIH (DE07662, DE07734), AOA.

145.4 RECEPTIVE FIELD SYNTHESIS IN TRIGEMINAL SUBNUCLEUS INTERPOLARIS. II. INPUTS FROM SUBNUCLEUS CAUDALIS. B.H. Hallas & M.F. Jacquin. Dept. of Neuroscience, N.Y. Coll. of Osteo. Med., Old Westbury, NY 11568.

In an accompanying abstract (Rossner & Jacquin, this volume), we described the effects of cortical ablation upon the receptive field (RF) properties of neurons in trigeminal (V) subnucleus interpolaris (SpVi). Cortical inputs were shown to contribute to RF size, and the type of afferent inputs expressed (modality convergence), in a small # of low threshold SpVi neurons. The relative # of nociceptors was unchanged. A similar experimental paradigm was used here to clarify the role of V inter-subnuclear connections in the synthesis of SpVi RF's in the barbiturate-anesthetized rat. Intra- and extracellular recording, electrical stimulation, and RF mapping were used to study the responses and projections of 403 SpVi cells in 11 rats, 39-113 days after surgical isolation of V subnucleus caudalis from SpVi. Transverse hemisections were made through the lateral half of the left medulla just caudal to the obex. Since this lesion severs V primary afferents caudal to SpVi, as well as postsynaptic inter-subnuclear axons traversing to and from SpVi, kainic acid was used to selectively remove caudalis inputs to SpVi. 5 rats received kainic acid injections into caudalis 4-21 days prior to providing similar data on 294 SpVi cells. Projection status was verified in each of 13 cells stained with HRP. Physiological data (mean ± SD; %) are summarized below and compared with that of 386 cells from 9 normal adult rats:

	Hemisect	Kainic Acid	Normal
Latency to V ganglion shock (ms)	1.52 ± 1.2	1.64 ± 1.3	1.46 ± 0.5
% VB-project vs interneurons	15.9, 84.1	16.7, 83.3	26.0, 74.0
% nociceptive-biased	3.72	4.76	3.15
% with intermodality convergence	4.96	12.58	0
% unresponsive to any stimulus	16.62	12.24	1.02
% whisker(s)-sensitive	36.97	37.75	35.12
# of whiskers in RF (VB-project)	6.4 ± 2.4	8.6 ± 2.9	5.1 ± 2.3
# of whiskers in RF (interneur.)	3.1 ± 1.4	2.8 ± 1.3	1.0 ± 0
% interneur. with > 1 whisker RF	33.89	34.09	0.0
% with spontaneous discharges	31.91	34.78	9.13
% phasic vs tonic evoked resp.	59.3, 24.1	59.2, 28.6	88.0, 11.0
% with directional sensitivity	8.91	6.13	4.27
% with split or cervical RF's	3.0, 2.2	5.4, 2.0	0.0, 0.0

V primary afferents rostral to hemisection had normal RF's (N=67) and collateral arbors (N=11). Cell topography was also normal. Kainic acid and hemisection produced significant #'s of local circuit neurons with multi-whisker RF's, as well as cells expressing intermodality convergence (e.g. whisker plus guard hair). Significant #'s of spontaneously active and unresponsive neurons were also recorded, while the nociceptor encounter rate was unchanged. Thus, caudalis inputs contribute to RF size, modality convergence, responsiveness, and baseline activity in a substantial # of SpVi cells. These effects are qualitatively similar to those observed following lesions of somatosensory cortex. Support: NIH (DE07662, DE07734), AOA.

145.5 PROJECTION PATTERNS FROM PRIMATE AND FELINE DENTAL STRUCTURES TO CENTRAL TRIGEMINAL NUCLEI. L. E. Westrum[1], L. R. Johnson*[2], M. A. Henry[3], and R. C. Canfield*[4]. (SPON: R. Haschke). Depts. of Neurosurg.[1,4], Biol. Structure[1], and Restorative Dentistry[4], Univ. of Wash., Seattle, WA 98195; Depts. of Surg. Dent., and Cell. and Struct. Biol.[2], Univ. of Colo., Denver, CO 80262, and Facial Pain Ctr.[3], Univ. of Florida, Gainesville, FL 32610.

The distribution of dental afferents to the brain stem trigeminal nuclear complex (TNC) is being studied and compared in cat and monkey using intrapulpal application of the toxic lectin ricin (Ricinus communis agglutinins; RCA 60) and subsequent light microscopic methods for staining degeneration. We have previously shown that these preparations in cat probably demonstrate a more accurate and complete picture of the central distributions of dental afferents than is seen with transganglionic degeneration after dental lesions only or transganglionic transport of HRP from tooth pulps. Adult healthy Macaca fascicularis under deep anesthesia received unilateral intrapulpal injections of ricin (2-8 µl, 0.1% ricin-RCA 60) in all the teeth in both maxillary and mandibular quadrants. The dental pulps were exposed by an occlusal access and injections made immediately with minimal manipulation and only into the superficial part of the pulp. The animals were sacrificed after 8 days. The ganglia were prepared for cell stains and the brain stems frozen sectioned in the transverse plane; then serial (1 in 8) sections were stained by the Fink-Heimer method. The degeneration in the brain stem is striking, as it is distributed throughout all TNC subnuclei ipsilaterally. The distribution often appears as columns, with a medio-lateral orientation at dorsal, middle and ventral levels. In contrast to cat, also after ricin application, the monkey shows more ventral terminations in the main sensory nucleus and less of a ventral projection in the spinal subnuclei caudally. The monkey also has terminations in deeper laminae, often in isolated clusters, in subnucleus caudalis and at the C-1 level. The findings show clear differences, but with some similarities between primate and feline dental projection patterns.

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145.6 NEUROTRANSMITTER LOCALIZATION IN THE FELINE TRIGEMINAL MAIN SENSORY NUCLEUS. L.R. Johnson*[1], L.E. Westrum[2], and M.A. Henry[3] (Spon: B.R. Fink) [1] Depts. of Surgical Dentistry and Cellular and Structural Biology, Univ. of Colo., Denver, CO 80262, [2] Depts. of Neurological Surgery and Biological Structure, Univ. of Wash., Seattle, WA 98195, [3] Facial Pain Center, Univ. of Florida, Gainesville, FL 32610.

Previous studies have examined the localization of neurotransmitters within the caudal subnuclei of the brain stem trigeminal nuclear complex but apparently none have examined the main sensory nucleus (MSN). The purpose of the present study is to describe the immunocytochemical localization of neurotransmitters in the MSN.

Normal cats with no oral or facial pathosis were anesthetized with Ketamine and maintained on a respirator (O_2-Halothane) and then transcardially perfused with either 4% PLP in a 0.1 M PO_4 buffer or 0.1 M PO_4 buffered solution containing 3% paraformaldehyde and 0.1% picric acid followed by washes of 10 and 30% sucrose in the same buffer. Following the perfusions the brain stems were removed from the cranial vault and stored in 30% sucrose in 0.1 M PO_4 buffer for 48 hrs. Frozen sections (35um) were taken and processed using the indirect PAP method of Sternberger. Sections from the brain stems perfused with 4% PLP were incubated with primary antibodies to somatostatin, substance P (graciously provided by Dr. R.H. Ho), met-enkephalin or cholecystokinin. Sections perfused with the picric acid solution were incubated with antibodies to choline acetyltransferase.

Small and medium size somatostatin positive neuronal profiles are present in both the dorsomedial (DM/MSN) and ventrolateral (VL/MSN) MSN. Terminal arbors and boutons en passant positive for substance P are also present in both subdivisions of MSN. Met-enkephalin positive small and medium size cell bodies and terminals are only present in VL/MSN just rostral to pars oralis. A modest number of cholecystokinin positive terminals appear to be randomly distributed throughout the entire MSN. Large, medium and small neuronal cell bodies positive for choline acetytransferase are seen in VL/MSN, with the greatest numbers of positive profiles in VL/MSN just rostral to pars oralis, within DM/MSN a few medium and small choline acetytransferase containing neurons are present.

In summary this study indicates that systems using substance P and possibly cholecystokinin send projections to MSN, while projecting or intrinsic neurons in the MSN use somatostatin, met-enkephalin or acetylcholine as neurotransmitters. Supported by NIH grants DE04942 and NS20482. L.E.W. is an affiliate of the CDMRC Univ. of Washington.

145.7 FINE STRUCTURAL CHARACTERISTICS AND SYNAPTIC CONNECTIONS OF TRIGEMINOCEREBELLAR PROJECTION NEURONS IN RAT TRIGEMINAL NUCLEUS ORALIS. W.M. Falls, B.J. Moore*, and M.T. Schneider*. Dept. of Anatomy, Michigan State Univ., East Lansing, MI 48824.

Retrograde horseradish peroxidase (HRP) studies in our laboratory (Falls, W.M. and M.M. Alban, Somatosensory Res., 4:1-12, 1986) have shown that the dorsal one-half of the border zone (BZ) of rat trigeminal nucleus oralis includes among its neuronal population a morphologically distinct type of trigeminocerebellar projection neuron (TCPN) that innervates the orofacial portions of one or more of the four major tactile areas of the ipsilateral cerebellar cortex (i.e., crura I and II, the paramedian lobule, and the uvula). The presynaptic elements contacting BZ TCPN's were classified following electron microscopical examination of serial sections made from two of these cells which were retrogradely labeled with HRP following injections into ipsilateral crura I and II of the cerebellar hemispheres. In one experiment, an ipsilateral trigeminal sensory root rhizotomy was also performed in order to determine if identified BZ TCPN's receive direct primary trigeminal afferent input. Three types of axon terminals contacting proximal as well as higher order labeled dendrites have been recognized. By far the most numerous endings (Type I) are filled with clear, spherical to oval synaptic vesicles and form at least one asymmetrical axodendritic contact. Synapsing less frequently on dendritic shafts are boutons (Type II) containing pleomorphic synaptic vesicles and forming symmetrical to intermediate synaptic junctions. The least frequently encountered synaptic terminal (Type III) contains flattened synaptic vesicles and makes a single symmetrical synaptic contact with a dendritic shaft. The cytological heterogeneity of these three terminal types suggests that the activity, and therefore the efferent output of BZ TCPN's is influenced by axons from multiple neuronal origins. In addition, the density and placement of Type I endings along the entire dendritic tree would suggest that the cells of origin of this terminal type play a major role in controlling the activity of BZ TCPN's. The rhizotomy portion of this study indicates that a number of Type I endings are derived from primary trigeminal neurons. Type I endings, displaying dark degeneration after a two day survival period, are positioned centrally in simple glomeruli where each forms an axodendritic synapse on a single higher order dendrite of an identified BZ TCPN. No degenerating endings synapsed on proximal dendrites. It is at these synapses that the possible transfer of orofacial tactile inputs to BZ TCPN's are made by Type I endings for direct relay to crura I and II. Transmitter release at the axodendritic synapses may be modified presynaptically within the glomeruli by axoaxonic synapses between a terminal containing flattened synaptic vesicles and the Type I ending. (Supported by NIGH Grant DE06725)

145.8 PROGRESSIVE ACTIVATION OF THE PARATRIGEMINAL NUCLEUS DURING ENTRANCE TO HIBERNATION. T. S. Kilduff, C.D. Radeke*, F. R. Sharp and H. C. Heller*. Depts. of Psychiatry and Biological Sciences, Stanford Univ., Stanford, CA 94305 and Dept. of Neurology, Veterans Admin. Med. Cntr., San Francisco, CA 94121.

The paratrigeminal nucleus (PTN) is a brainstem structure whose function is unknown and whose anatomical connections are poorly understood. Originally described by Ramon y Cajal, the PTN was virtually ignored until the morphological and immunohistochemical studies of Chan-Palay in 1978. We have previously reported that, in the squirrel, the PTN is the only one of 85 brain structures examined to undergo a significant increase in its relative 2-deoxyglucose (2DG) uptake during deep hibernation in comparison to euthermia. We now report that, during the transition from euthermia to deep hibernation, there is a progressive activation of the PTN that is reversed during arousal.

Citellus lateralis, maintained at 5°C and under an LD 12:12 photoperiod, were prepared with chronic jugular catheters and subcutaneous re-entrant tubes. Animals were injected in darkness with [^{14}C]2-deoxyglucose (150 uCi/kg) during seven phases of the hibernation cycle: euthermia (n=16), three body temperature (T_b) intervals during entrance to hibernation (T_b=30°-25°C, n=4; T_b=25°-20°C, n=4; and T_b=20°-15°C, n=6), deep hibernation (T_b<8°C, n=6), and both early (T_b < 8°-35°C, n=4) and late (T_b=25°-35°C, n=5) in arousal from hibernation. The 2DG was allowed to incubate for 45 min in the euthermic animals. Because of the influence of T_b on metabolism, the incubation period was longer in the other experiments and was inversely proportional to the animal's metabolic rate. Animals were terminated by a sodium pentobarbital overdose, the brains were removed, frozen, sectioned at 20u and exposed to x-ray film for 7 days.

The PTN was readily apparent on the resultant autoradiographs of animals injected during the latter two phases of entrance and deep hibernation but not obvious during euthermia, arousal or the early phase of entrance to hibernation. Optical density (O.D.) measurements were made from the autoradiographs of the PTN and 94 other neural structures using a densitometer. O.D. ratios were calculated for all 95 structures as the ratio of the O.D. of the structure to the mean O.D. of all 95 brain structures. Analysis of variance revealed a highly significant increase in the O.D. ratio of the PTN as T_b decreases ($p < 0.001$; $F = 105.5$, $df = 4, 31$). As T_b increases during arousal from hibernation, the O.D. ratio progressively declines ($p < 0.001$; $F = 109.3$; $df = 3,27$). All 95 neural structures were also ranked in order of O.D. ratio, from highest to lowest, across the seven phases of the hibernation cycle. Whereas the PTN ranked #90 of 95 structures during euthermia, it rose during entrance from #55 in the T_b range 30°-25°C, to #36 in T_b=25°-20°C to #5 in T_b=20°-15°C to #1 during deep hibernation. These changes in ordinal rank indicate that the PTN is progressively increasing its 2DG uptake during entrance to hibernation relative to all brain structures.

These observations implicate the PTN as playing a role in the entrance and maintenance of hibernation and support the hypothesis that the PTN is a thermoafferent relay whose activity is enhanced by decreased T_b (Supported by NSF BNS-8216918 to HCH).

146.1 AREA CA_1 OF THE HIPPOCAMPUS OF THE RAT PROJECTS TO THE CONTRALATERAL HIPPOCAMPAL FORMATION. Th. van Groen* and J.M. Wyss (SPON: E.E. Geisert). Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

Extensive research into the commissural projections of the hippocampus in the rat has indicated that all commissural projections from the hippocampus proper originate in neurons within the CA_2, CA_3 and CA_4 regions. In contrast, the CA_1 pyramidal neurons were not considered the origin of any commissural projection but were thought to project only ipsilaterally to the subiculum, entorhinal cortex, lateral septal nuclei and perhaps to CA_1 itself. In order to further investigate the connections of area CA_1, anterograde and retrograde tracing studies were conducted. 5 - 20 nl injections of wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) or [^{3}H] amino acids were made ipsilaterally into area CA_1. Following all injections into the septal third of CA_1 anterograde label could be seen in the contralateral subiculum (pyramidal layer) and somewhat lighter labeling was present in area CA_1 (stratum oriens and stratum radiatum). Also, labeling was present in layers I, III and V of the contralateral postsubiculum. To confirm the CA_1 origin of the projection, injections of retrogradely transported fluorescent dye tracers were made into the subicular complex and/or area CA_1. Following all injections into the septal portion of the postsubiculum, subiculum, and CA_1 fields, neurons in the pyramidal layer of the contralateral area CA_1 were labeled. This labeling occurred over a rather discrete region of area CA_1 that was similar in septo-temporal extent to the injection site. The CA_1 projection appears to be confined to the septal third of the field. Together these results demonstrate that the small pyramidal cells of area CA_1 do project to the contralateral hippocampal formation.

146.2 BRAINSTEM DISTRIBUTION OF HORSERADISH PEROXIDASE AFTER INJECTION OF THE NODOSE GANGLION IN MACAQUES. A.P. Knox, N.L. Strominger and D.O. Carpenter. Dept. of Anatomy, Albany Medical College, Albany, NY 12208.

Horseradish peroxidase conjugated to wheat germ agglutinin [10 µl, 1%] was injected unilaterally into the nodose ganglion in four monkeys, 'Macaca fascicularis'. After 24-36 hours, animals were anesthetized and perfused transcardially with saline followed by 4-8% gluteraldehyde. Brainstems, blocked either transversely or in the horizontal plane, and cervical spinal cords were sectioned at 50 µm and reacted with tetramethyl benzidine. Alternate sections were stained with neutral red.

Retrograde labeling of perikarya occurred ipsilaterally in the following brainstem nuclei: dorsal motor of the vagus, ambiguus, retroambiguus, and occasionally in the various subnuclei of the solitary complex. Many neurons were labeled in the ipsilateral retrofacial nucleus as well as a few on the contralateral side. Afferent fibers and terminal label was present in the throughout the region of the solitary nuclei, in the area postrema and in the pre-area postrema. Within the cervical spinal cord, the dorsomedial nucleus and spinal nucleus of the accessory nerve were labeled ipsilaterally.

The nucleus ambiguus in all cases consisted of two populations of labeled neurons, a cluster of larger cells tending to be diffuse and located in dorsal and medial parts of the nucleus, and a cluster of smaller more compactly arranged neurons tending to be in ventral and lateral parts of the nucleus. The latter appeared similar in size and configuration to cells of the dorsal motor nucleus of the vagus.

146.3 IDENTIFICATION OF NUCLEI IN THE HUMAN BRAINSTEM: A CHEMOARCHITECTONIC APPROACH USING ACETYLCHOLINESTERASE REACTIVITY G. Paxinos and I. Tork. School of Psychology and School of Anatomy, University of New South Wales, Sydney, Australia 2033

The present study attempts to establish the human homologues to nuclei identified in other animals by considering the chemoarchitectonic profile of nuclei. We report that nuclei known to have intense acetylcholinesterase (AChE) reactivity in the rat are similarly reactive in the human. Thus, in the lateral tegmentum, the parabigeminal nucleus shows intense reactivity in both species. The most intense AChE reactivity in the tegmentum is associated with the pedunculopontine tegmental nucleus (PPTg). This nucleus straddles the lateral aspects of the superior cerebellar peduncle. The lateral dorsal tegmental nucleus is also reactive in both species. Cell bodies reactive for AChE were observed in the locus coeruleus, PPTg, and compact part of the substantia nigra. Cajal identified in the kitten the rhabdoid nucleus as a parvocellular zone girthing the posteroventral aspects of the decussation of the superior cerebellar peduncle and connecting the interpeduncular nucleus with the dorsal tegmental nucleus. The Rhabdoid nucleus is AChE reactive in all mammalian species studied including the human. We conclude that while species variations in the content of neuroactive compounds of different nuclei occurs, the chemoarchitectonic profile of homologous nuclei is remarkably stable.

146.4 THE STRUCTURE OF NEURONS THAT CONTRIBUTE TO LAYER I DENDRITIC BUNDLES IN THE RAT RETROSPLENIAL CORTEX. J.M. Wyss and Th. van Groen.* Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

Recently we have demonstrated that layer II neurons in retrosplenial cortex of the rat display a point to point commissural projection and that apical dendrites of these neurons form tight bundles in layer I. The region of the bundles receives thalamic input, while the area between the bundles receives cortical and little or no thalamic input. In the present study we employed the fixed slice preparation to identify the structure of the neurons that contribute dendrites to these dendritic bundles, and compared them to cells in layer III that do not appear to have dendrites in the bundles. In each of 10 rats, an initial injection (10-30 nl) of fluorogold (2%) was made into the right retrosplenial cortex and following a 4 day survival, the rats were perfused with 150 ml of 4% paraformaldehyde, the brain was removed from the skull, the retrosplenial cortex was blocked and 100-200 µm sections were cut on a vibratome and stored at 4^{0}C until used. Under fluorescent illumination, fluorogold labeled cells in layer II were impaled with a glass micro-pipette containing Lucifer Yellow (4%). When a layer II cell was penetrated, negative electrical current was passed through the pipette solution to force the dye into the cytoplasm. In other cases we have filled both commissural and noncommissural layer III cells. The results of these studies indicate that the layer II neurons of the retrosplenial granular cortex display long apical dendrites and only very short and thin basal dendrites. The apical dendrites invariably begin to arborize in layer Ib and reach their greatest extent of arborization in layer Ia. Many of the apical dendrites do not ascend perpendicular to the pial surface of the brain, but angle through layer Ic and then turn and ascend through layer Ib and Ia. Dendritic spines are primarily located on the distal portions of these dendrites. In contrast to layer II cells, layer III neurons display better developed basal dendrites, and apical dendrites that arborize extensively in layer Ib and Ic but have few branches in layer Ia. Further the layer III neurons have a larger and triangular shaped cell body while the somata of layer II neurons tend to be small and spindle shaped. These data clearly demonstrate that the layer II commissural neurons which form dendritic bundles in the retrosplenial cortex are morphologically different from layer III commissural and non-commissural neurons.

146.5 CORTICAL STRUCTURE IN FRONTAL REGIONS OF THE WEST INDIAN MANATEE (Trichechus manatus) R.L. Reep, J.I. Johnson, R.C. Switzer, W.I. Welker. Department of Neuroscience, University of Florida, Gainesville, FL 32610; Anatomy Department, Michigan State University, East Lansing, MI 48824; Department of Pathology, University of Tennessee, Knoxville, TN 37920; Department of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Cortical architecture was examined in Nissl, myelin, AChE and cytochrome oxidase material from four brains. In general, the cortex exhibits a high degree of organization, with well defined laminae, robust cell densities, and a markedly striated layer VI.

In the pregenual region, the medial wall and ventromedial cortex are characterized by a prominent layer II that is compact and continuous, broad layer III, no granular layer IV, and large celled layer V with clear layer Vb. Proceeding from the midline onto the dorsolateral surface, layer II becomes progressively less distinct; layer V is thinner, contains smaller cells which are more sparsely distributed, and a less distinct Vb. There appears to be a granular layer IV which merges with the small pyramidal cells of layer III.

Ventrolaterally, the medial wall structure continues until the junction of the olfactory peduncle with the basal cortex. Dorsal to the shallow rhinal fissure, layer II becomes more irregular and a lamina dissecans is seen between poorly defined layers III and V. There is no visible claustrum or extreme capsule. Instead, distinct cell clusters (150-500um dia) become visible in layer VI, and extend dorsally into in an otherwise typical dorsolateral cortex. These clusters are spaced at fairly regular 500-1000um intervals. Likewise, a deeply situated AChE-positive band extends dorsally from the rhinal fissure, becoming fragmented and coextensive with the cell clusters seen in layer VI. Proceeding caudally, layer VI clusters are distributed continuously into progressively more dorsal cortical areas, until a prominent fissure is reached.

Dexler (1913, Gegenbaus morphol. Jahrbuch 45:97) first noted these cell clusters in the brains of the other extant Sirenian genus, Dugong, and termed them Rindenkern, or cortical nuclei. They may represent a fragmented claustrum or specializations peculiar to somatic sensory cortex.

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146.6 **TELENCEPHALIC REGIONAL VOLUMETRIC ASYMMETRIES IN TARSIERS AND LORISES.** M-C de Lacoste, T. Adesanya*& D. J. Woodward. *Department of Cell Bio. & Anat. UTHSCD, Dallas, TX 75235.*

One of the goals in our laboratory is to detemine if cerebral asymmetry has been selected as a trait expressed during speciation within the primate order. In previous studies we have described telencephalic regional volumetric asymmetries in the family **Lemuridae** and in some species from superfamilies **Ceboidea, Cercopithecoidea & Hominoidea**. Outstanding prefrontal asymmetry appeared to be characteristic of ceboid cerebra, while most of the remaining primate brains exhibited striking retrocalcarine asymmetries [de Lacoste et al, 1986]. This study was undertaken to delineate hemispheric regional volumetric asymmetries in **Tarsiidae**[N=4], a family of Haplorhines, and in **Lorisidae** [N=12], a family of strepsirhines. There is a fairly wide range of brain morphology for the different species within family Lorisidae. The cerebrum of *Galago demidovii*, for example, is nearly lissencephalic, excepting a Sylvian fissure and calcarine complex. In contrast, the brain of *Nycticebus coucang* exhibits a number of sagitally oriented fissurets, some of which may serve as limiting sulci for cytoarchitectural zones [Sanides & Krishnamurti, 1966]. In family Tarsiidae, cerebral gyral and sulcal patterns are more uniform, albeit unique. The Sylvian or pseudosylvian fissure is actually a small groove, the corpus callosum is narrow and short, frontal cortex is relatively small, temporal cortex is poorly developed and cerebellar foliation is minimal. Striate and extrastriate areas, however, are very well developed and appear to comprise the largest part of the neocortex. We were, therefore, especially interested in determining if regional volumetric asymmetries in tarsiers are more pronounced in visual and visual-related areas.

Complete anterior to posterior series of coronal sections [cut at 10-20μ] were photographed and digitized. Sectional and regional volumes were integrated using the laboratory CARP system and SPSSx. Correction factors and a set of indices of asymmetry were calculated using algorithms developed for these purposes. Anatomical delimiters varied with the species but included the rectus, arcuate, central, intraparietal, lunate, para-, pre- and retro-calcarine sulci as well as the genu of the corpus callosum and lateral geniculate nucleus.

Results indicated that species within family Lorisidae manifest variant patterns of regional volumetric asymmetries. Some of the species [e.g., *Galago demidovii* and *Nycticebus coucang*] evidenced pronounced prefrontal but little striate and extrastriate regional volumetric asymmetries. Other species [e.g., *Galago senegalensis*] displayed significant retrocalcarine asymmetries similar to those described for other strepsirhines [de Lacoste et al, 1985]. In contrast, the tarsier cerebra consistently manifested a high percentage difference between left and right striate and extrastriate cortical volumes. We believe that the retrocalcarine asymmetry exhibited by the tarsiers demonstrates the significance of asymmetry in primate brain specialization.

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146.7 **ORGANIZATION OF MOTONEURONS INNERVATING EPAXIAL AND HYPAXIAL MUSCULATURE IN THE FROG, RAT, AND MONKEY.** M. Kramer, T. Deacon, A. Sokoloff, and A. Filler. Biological Anthropology, Harvard University, Cambridge, MA 02138.

Recent tracer studies have demonstrated a segregation of motoneurons innervating epaxial versus hypaxial muscles in snakes and rats but not in fish and mudpuppies, suggesting possible differences in the organization of motoneurons innervating axial muscles in amniotes and anamniotes (Fetcho, J., J. Comp. Neurol., 249:521, 1986). To establish a broad comparative base for investigating the organization of motoneurons that innervate axial musculature, we have used WGA-HRP to trace the innervation of individual epaxial and hypaxial muscles in two species of mammals, the rat and cynomolgus monkey, and an anuran species, the grass frog. Individual muscles were injected with from 0.5-2.5 microliters of tracer. After 24-72 hours animals were sacrificed and perfused, then muscles and spinal cords were removed, sectioned and processed for peroxidase using the tetramethyl benzidine histochemical method.

In the rat and the monkey a single unilateral injection of WGA-HRP into either an erector spinae or transversospinalis muscle labels a multisegmental column of motoneurons occupying the extreme ventral tip of the ipsilateral ventral horn. An injection into the rectus abdominis muscle in either mammalian species labels a column of motoneurons that is centrally placed in the ventral horn and extends from its medial to its lateral edge. Unilateral injections into the rectus muscle result in both ipsilateral and contralateral cell labeling. In the grass frog unilateral injections into epaxial musculature label a column of motoneurons located in the ventral tip of the ipsilateral ventral horn although a few labeled neurons are located more dorsally. Injections into the frog rectus muscle label motoneurons that are located centrally in the ventral horn although a few labeled cells are located near the ventral tip of the ventral horn. Unilateral injections into the frog rectus muscle result in both ipsilateral and contralateral cell labeling.

These results demonstrate a discrete dorsoventral segregation of motoneuron columns innervating epaxial and hypaxial muscles in the rat and monkey. A similar though less discrete pattern is also evident in an anamniote, the frog. A pattern of ipsilateral epaxial versus bilateral hypaxial muscle innervation is observed in both mammalian and anuran species. The multisegmental labeling observed following extremely small single muscle injections indicates that a relatively large number of neurons innervate individual axial muscles and suggests the possibility that many of these neurons project to multiple muscles.

146.8 SURFACE FEATURES OF THE FOREBRAIN OF THE BOWHEAD WHALE, BALAENA MYSTICETUS. D. W. Duffield* (SPON: W. Flory). Dept. of Veterinary Anatomy, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803.

Very little information is available on the brain of the bowhead whale, a large right whale that inhabits Arctic waters. Hunted almost to extinction in the last century by commercial whalers, it now may be threatened by oil and gas exploration and drilling within its range. It is now legally hunted only by Eskimos for their subsistence. Initial studies on the brain of this endangered species were done in conjunction with an environmental impact study.

Observations were made on 11 Eskimo-harvested bowhead whale brains that were removed via the extremely large foramen magnum. Tissues were fixed on site in 10% formalin. All were damaged in removal. Six had been sliced into 1 cm sections. The brain of the bowhead whale is similar to other cetacean brains and particularly to the brain of the Southern right whale. The hippocampus is relatively small, but does protrude into the lateral ventricle. An olfactory peduncle is present as in other baleen whales. An olfactory bulb has not been recovered, but is presumed to exist, because there are nerve fibers in the peduncle. The lateral part of the rostral rhinal sulcus is difficult to locate grossly. Semilunar and ambient gyri are visible on the rostromedial aspect of the temporal lobe. The olfactory tubercle is characteristically large, and the diagonal band is readily discernible. Clefts in the bowhead brain are similar to those described in other cetacean brains. The insula is modestly developed. The pineal body is present in the choroid plexus of the third ventricle ventral to the splenium corporis callosi. There is no interthalamic adhesion. The caudolateral thalamus is well developed, and the medial geniculate body is large. The lateral geniculate body is a definite raised structure on the caudolateral extremity of the thalamus.

The cooperation of the tissue collectors and whaling captains is gratefully acknowledged. Initial funding was by the University of Maryland from Bureau of Land Management funds and subsequent funding from the North Slope Borough, Barrow, AK.

146.9 DISTRIBUTION OF MONOAMINERGIC, CHOLINERGIC, GABAERGIC, AND PEPTIDERGIC MARKERS IN THE HUMAN CEREBRAL CORTEX. F. Javoy-Agid*, B. Scatton[a], P. Cervera*, M. Ruberg*, R. Raisman*, H. Taquet[b]*, J.J. Benoliel[b]*, A. Maubourgne[b]*, F. Cesselin[b]*, S. Jegou[c]*, H. Vaudry[c]*, Y. Agid* (SPON: A. Enjalbert). INSERM U 289 and b U 288 - CHU Pitié-Salpêtrière - 75013 Paris - a Synthélabo-LERS - 92220 Bagneux - c Groupe CNRS 650 - 76130 Rouen - FRANCE.

A detailed biochemical study of the distribution of various markers of neurotransmitter systems was investigated in the entirety of the cerebral cortex in human brain postmortem. The brains from two subjects with no known neurological or psychiatric disease were examined. The frozen hemispheres were cut in 9 mm frontal sections throughout the entire rostrocaudal extent. On each of the 23 sections -divided in 4 equal portions- the entire cortical gray matter (separated from the white matter) was dissected out. Biochemical assays were performed on an aliquot of the tissue of each different parts. The presence of classical neurotransmitters and neuropeptides in all cortical samples suggests a widespread distribution throughout the cerebral cortex. However, each biochemical marker distributed heterogeneously in a distinct pattern. The ascending monoaminergic and cholinergic systems project in a non uniform manner. The highest noradrenergic innervation was found in the fronto-parietal area, whereas the dopaminergic input seemed denser in the prefrontal cortex. The serotonin innervation predominated in the prefrontal and temporal regions. CAT activity, a marker for cholinergic systems, was high in prefrontal and temporal cortex. The distribution of GAD activity most likely corresponding to intrinsic GABAergic neurons appeared quite uniform. Low content in monoaminergic markers was found in the occipital region. In general, neuropeptides were concentrated in the prefrontal and rostral part of the temporal cortex (VIP, Substance-P, CCK-8), with an exception for opioid peptides (met and leu-enkephalin) which concentrated in the occipital region.

146.10 IMMUNOHISTOCHEMICAL MAPPING OF ADENOSINE DEAMINASE-CONTAINING NEURONS IN SEPTAL NUCLEI AND IN FIBER PROJECTIONS TO THE MEDIAL HABENULA. K.M. Dewar, T. Yamamoto, W.A. Staines, P.E. Daddona*, J.D. Geiger and J.I. Nagy. Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3E 0W3.

The localization of neurons containing adenosine deaminase (ADA) within various nuclei of the septum and the septal efferent projection to the medial habenula has been investigated. ADA-immunoreactive neurons were identified within the posterior septum, namely the septofimbrial and triangular septal nuclei, and within the dorsal and ventral regions of the lateral septal nuclei. Intense ADA-immunostaining was also seen in the densely packed neurons of the bed nucleus of the anterior commissure. Furthermore, numerous ADA-immunoreactive fibers were detected in the stria medullaris and these fibers appeared to give rise to an ADA-containing terminal field, which was of a fine punctate nature, in the medial habenula. The existence of this putative ADA-containing projection from the posterior septal nuclei to the medial habenula via fibers in the stria medullaris was further investigated by the use of retrograde tracing and lesion techniques. Microiontophoretic injection of Fluoro-gold into the medial habenula resulted in the retrograde labelling of a large number of ADA-positive neurons within the septofimbrial (95-97%) and triangular septal nuclei (95%), and in the majority of the neurons in the bed nucleus of the anterior commissure. In addition, unilateral transection of the stria medullaris effectively reduced immunostaining for ADA in the medial habenula posterior to the lesion with a concomitant reduction in ADA activity (40%) in the medial habenula ipsilateral to the side of the lesion.

In summary, the present study demonstrates ADA-immunostaining within subpopulations of neurons in septal nuclei. These findings are consistent with the highly restricted distribution of ADA-immunoreactivity elsewhere in the rat brain. Furthermore, a major projection of ADA-containing neurons from the posterior septal nuclei to the medial habenula has been documented. ADA may prove to be a useful marker to delineate septal subregions and to distinguish their anatomical relationships with other brain areas. Whether ADA-immunoreactivity in septal neurons and in neurons elsewhere in the rat CNS reflects their utilization of purines as transmitters and/or neuromodulators is still a matter of speculation.

146.11 EVOLUTION OF THE PRIMATE SUBICULUM. E. Armstrong and G. T. Frost*. Dept. of Anatomy, Louisiana State University Medical School, New Orleans, LA 70112.

Previous studies have shown that the sizes of structures in the limbic system are correlated both with brain size and phylogenetic position. A morphometric study on the subiculum, the major output zone of the hippocampal region in primates, allowed us to test several hypotheses regarding its size. The data were collected from 16 primate species, of which 6 were prosimians and 8 were nonhuman anthropoids. Data from human brains were collected as well. The subiculum was identified and the cell layer measured on Nissl stained serial sections. The border between the subiculum and CA1 of the hippocampus was identified by the loss of the stratum radiatum which is present in CA1. The lateral border of the subiculum, located deep to the presubicular granule cells, was identified by the presence of large pyramidal cells in the subiculum. The subiculum was measured by digitizing outlines of the subicular grey matter that had been magnified using flat-field optics, usually around 20X. Approximately 20 sections per brain were analyzed. Allometric or scaling studies on logarithmically transformed data were used to analyze the observed differences in size.

The results show that as the primate brain enlarges the subiculum does as well, but to a lesser degree than the total brain. This indicates that larger primate brains have disproportionately smaller subiculi. Prosimians and anthropoids, subordinal divisions of primates, differ in morphometric scaling: for a given brain weight prosimians have bigger subiculi than anthropoids. These results resemble the scaling seen in the connected limbic nuclei, the anterior thalamus (AP) and the medial mamillary body (MMB). Among prosimians the subiculum scales in a 1:1 fasion with AP and MMB, indicating that, among these species, size differences occur while maintaining the same proportions. On the other hand, the scaling among the nonhuman anthropoids, is somewhat less than 1, indicating that in anthropoids, the subiculum has expanded volumetrically more than AP or MMB. The human data do not fit the nonhuman anthropoid trend, however. The human subiculum is smaller than expected for an anthropoid on the basis of brain weight, AP or MB sizes, suggesting an altered morphometric relationship between the subiculum and the interconnected diencephalic nuclei occurred during human evolution.

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146.12 AN ANATOMICAL STUDY OF THE INSULAR AND CINGULATE CORTEX OF THE WALLABY, MACROPUS EUGENII. L. Mayner. Dept. of Behavioural Biology, R.S.B.S. Australian National University, Canberra, Australia.

The few cursorily anatomical studies on the cortical features of Australian marsupial have neglected the description of both insular and cingulate cortical regions. The following description of the cingulate and insular cortical regions, is part of an extensive study of the cortical cytoarchitectonic features of the wallaby, a diprotodontid Australian marsupial mammal. The method of identification of the two regions was from homologies of placement with eutherian mammals, as well as results from horseradish peroxidase (HRP) tracing studies for further confirmation. Thionin was used for the Nissl stain and the Mesulam TMB method was used for visualising the HRP product. The insular region contains two areas, an agranular area lying rostral to the granular area, placed lateroventrally behind the frontal cortex and in front of the parietal cortex. In the agranular area layer II appears to merge with layer III and layer IV is missing. Layer V contains deeply staining elongated cells which resemble cells found in layer II of other regions. Layer VI also contains elongated cells among the pseudopyramidal cells. A cell sparse layer appears to be present between layers V and VI. The granular insular region contains six layers. Within these are two prominent layers of sparsely packed cells, one between layers IV and V, the other between layers V and VI. HRP injections into the insular region result in both retrograde and orthograde label in the oral subdivision of the ventromedial nucleus of the thalamus. The cingulate region is found mostly in the medial cortical wall. Layer IV was not found within this region. Layer I is very wide however, layer II is not very prominent and at times blends with layer III cells. Layer III is a rather wide layer, at the point where the cingulate region encroaches onto the dorsolateral ridge of the cortex, a division in the form of a cell sparse layer, appears between layers III and V. This division is prominent in the dorsal convexity and extends about half way down the medial cortical area. Just above this, layer III cells appear to form a narrow band of densely packed, deeply staining cells. In layer V there are medium and some large pyramidal cells mostly moderately staining, and a few large pyramidal cells are deeply staining. Layer VI contains pseudopyramidal cells. After HRP injections into the anterior nuclei of the thalamus retrograde label was found in the cingulate cortex. These results show that the metatherian mammalian cortical plan for these two regions follows that of the eutherian mammal and that the organisation of the cortical plan of the wallaby has many similarities with eutherian mammals such as the rat.

146.13 MEDULLARY PROJECTIONS TO THE PONS AND BASAL FOREBRAIN: POSSIBLE SUBSTRATES INVOLVED IN DRINKING BEHAVIOR: G. L. Edwards, J. T. Cunningham*, T. G. Beltz*, and A. K. Johnson. Departments of Psychology and Pharmacology and The Cardiovascular Center, The University of Iowa, Iowa City, IA, 52242.

Numerous studies have implicated the basal forebrain in the control of drinking behavior. Notably, the tissue in the region of the anteroventral third ventricle (AV3V) has been implicated in these controls, particularly angiotensin II (ANG II)-induced drinking. Recent studies suggest the median preoptic nucleus (MnPO) may be the critical tissue in the AV3V region for the control of drinking (Neuroendo. 37: 73-77, 1983). Additionally, the lateral parabrachial nucleus (LPBN) in the pons is reported to be important in the control of ANG II-induced drinking (Am. J. Physiol. 251: R504-509, 1986).

Since the LPBN and MnPO both receive input from similar areas of the medulla it is of interest to determine if these inputs overlap or possibly neurons within the medulla project to both the MnPO and the LPBN. In order to investigate these possibilites, microinjections of either rhodamine labelled latex beads or Fluoro-Gold were made into the LPBN and MnPO. After a 4-5 day recovery period the animals were sacrificed and perfused. Serial sections of the medulla were mounted on slides and viewed with a fluorescence microscope. Neurons labeled from injections into the LPBN were observed primarily in the nucleus of the solitary tract (NTS), area postrema (AP), and the ventrolateral medulla in the region of the A1 catecholamine cell group. Neurons labeled by injections into the MnPO were observed primarily in the ventrolateral medulla in the region of the A1 cell group. Although retrograde label from injections into the MnPO was primarily observed in different cells than retrograde label from LPBN injections, there appear to be cells in the ventral medulla containing both Fluoro-Gold and rhodamine labeled beads. This observation suggests that a subpopulation of neurons in the medulla may project to both the LPBN and MnPO and subsequently affect information processed by these nuclei possibly relevant to drinking behavior. Ongoing studies are aimed at determining the chemical nature of these projections and their possible role in drinking behavior.

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146.14 THE TERMINAL NERVE OF DOLPHINS: GENERAL ANATOMY AND LHRH-IMMUNOCYTOCHEMISTRY.
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The gross morphology and general histology of the terminal nerve (TN) was studied in several specimens each of the common dolphin (Delphinus delphis) and the Pacific bottlenosed dolphin (Tursiops gilli). Brains for routine observation had been fixed in 10% formalin for some time. Nerve strands and ganglia were initially identified by treatment with 1% osmium. In Tursiops two nerves were fixed in Bouin's for LHRH immunocytochemistry (antisera to synthetic LHRH) and another one was immersed in 2-3% gluteraldehyde (in cacodylate buffer) for EM. In both cases the tissue was obtained within several hours after death. The latter was post-fixed in osmium. The TN is composed of many parallel strands runnning in the pia on the ventromedial surface of the frontal lobe. Its fibers enter the cranium from paired medial foramina located just above the frontal poles. TN bundles enter the brain in the anterior perforated substance or run along the optic chiasma or branches of the cerebral arteries. Ganglia are distributed along the TN with the largest situated at the point of cranial entry. The latter contains many (ca. 2,000-4,000) round cells (ca. 30 um) which have a capsule of satellite cells and are pear-shaped in some sections, suggesting a unipolar appearance. The nucleus is oval to irregular in shape with a prominent nucleolus. The cytoplasm contains round mitochondria, rough ER, lipofucsin granules and other inclusions. Synapses on the cells have not been observed; however, our observations are limited. A few scattered LHRH-immunoreactive (ir) cells, round or fusiform in shape with clear nuclei, are also present in the main ganglion. LHRH-ir fibers, single or in small fascicles, were also seen in the ganglion or along the bundle of the main nerve trunk. Occasionally LHRH fibers encircle or form a network around a single large non-ir cell. EM observations of LHRH-ir cells have not yet been made. Nerve strands contain many myelinated fibers (2-8 um), a feature seemingly unique to the TN of odontocetes. In at least Tursiops, some unmyelinated fibers are also present. Functional roles in sensory and/or autonomic control of nasal sacs and reproduction are suggested for the components of the dolphin TN.

146.15 THALAMIC PROJECTIONS TO VENTROLATERAL AND DORSOMEDIAL SUBSECTORS OF THE PREFRONTAL CORTEX IN THE RHESUS MONKEY. T. H. Henion* and H. Barbas. Dept. Health Sci. and Anatomy, Boston Univ. and Sch. of Med., Boston, MA 02215.

The thalamic input to ventrolateral (areas 11 and caudal 46) and dorsomedial (areas 32, 14 and 8a) subsectors of the prefrontal cortex was studied with the use of the retrograde transport of horseradish peroxidase (HRP) in rhesus monkeys. These areas constitute parts of a ventral and a dorsal trend of a gradual laminar differentiation observed within the prefrontal cortex (Barbas, H. and Pandya D.N., Neurosci. Abstr., 8:933, 1982). The orbitofrontal and medial prefrontal areas studied are architectonically less differentiated regions within each of the two trends than ventral area 46 and area 8a.

Most labeled thalamic neurons in each brain were found in the medial dorsal nucleus (MD). These neurons occupied predominantly the dorsal half of MD after HRP injection in dorsomedial prefrontal regions, and the central portion of MD after HRP injection in ventrolateral regions. In addition, dorsomedial prefrontal sites seemed to receive a greater proportion of their thalamic input from the intralaminar nuclei than did ventrolateral sites.

There were striking differences in the origin of thalamic projections to areas 8a and 46 when compared with the projections directed to the less architectonically differentiated areas 32, 14 and 11. Area 8a and ventral area 46 received most of their thalamic projections from the parvocellular (>60%), and a smaller proportion from the multiform (<10%) division of MD. Medial areas 32 and 14 and orbital area 11, however, received most of their MD projections from the magnocellular division. Moreover, a significant number of neurons from midline and anterior nuclei projected to medial prefrontal and orbitofrontal (>10% of the total number of labeled cells), but few, if any, projected to areas 8a or 46. The less architectonically differentiated areas 32, 14 and 11 had more widespread thalamic connections, whereas the more differentiated areas 8a and 46 had more focal thalamic connections.

The results suggest that the pattern of thalamic projections to the prefrontal cortex is correlated with the degree of architectonic differentiation of the prefrontal regions studied, and to some extent with the relative dorsomedial or ventrolateral location of each prefrontal target region.

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146.16 HIGH RESOLUTION BRAIN MAPPING. D.E. Hillman, M. Canaday*, G. Mahoney* and R. Crank*. Department of Physiol. and Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

A major difficulty in mapping immunolabels, receptor binding, functional labels and other details of the nervous system has been the restriction of low resolution frames of digital imaging techniques. Large digital images, however, contain so much data that they are difficult to analyze and virtually impossible to store. Solutions are found in applying combinations of technology in a comprehensive recording and analysis approach for digital mapping of the rat brain. High resolution mapping is done in two ways: (1) by interactive recording of structural boundaries using a moving stage, or (2) by digitizing 4000 x 4000 pixel frames. The single large frames (16 megabytes) are captured on a real-time, digital, video-disk of an image processor from a line scan camera. The approach allows automated image processing for edge enhancement, edge detection and selective processing of density defined structure through a cascade of operations generating images from which boundaries can be obtained as the structural maps. The advantage of multiple frame approach is that lenses having higher resolving power can be used and local visual analysis provides means to survey the entire section at the highest resolutions of the light microscope. The software, in both cases, is designed for mapping boundaries of structures within localized areas of the brain. Each object can be labeled and interactively amended as necessary to represent the original data. Digital brain atlases allow superimposition of structural boundaries over chemically defined structure at the cellular and the synaptic level for graphical display in the same coordinate system. Furthermore, three-dimensional representations by wireframe and rendered surfaces are useful for demonstrating relationships between objects in various perspectives. (NS13742 from NINCDS.]

146.17 DIGITAL RAT BRAIN: A COMPUTERIZED STEREOTAXIC ATLAS
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Mapping the central nervous system in accurate three space has historically taken the form of stereotaxic atlases. These maps of neuroanatomy not only help localize structures deep to the surface of the brain but also help us understand the relationships between those structures. Computerized digital imaging technologies (both 2D and 3D) have already proven extremely useful in the study of neurophysiology (Toga, et al., 1985,1986,1987; Hibbard & Hawkins, 1984; Smith, Schlusselberg & Culter, 1984). This paper describes an application of 3D neuroimaging to a stereotaxic rat brain atlas.

We have developed a system which maintains a 3 dimensional database of a neuroanatomic atlas. This database is derived from the drawings contained in a published atlas (with publishers permission). Software algorithms were developed which provided automatic contour extraction for the surface and outlines of certain neuroanatomic structures. The system automatically searches adjacent plates iteratively and extracts successive contours. Because the plates are separated by some distance (which can vary), we interpolated data between the contours. An interactive display package was developed whereby the user can synthesize images using the following utilities; wireframe modelling, solid modelling with surface rendering, shading, transparency, cut-away views, color indexing of neuroanatomic structures, perspective and rotation about all 3 axes.

The images which are produced can be labelled and functional data manually inserted by the user. Although the database is not comprehensive, it is constantly being expanded. Those structures most easily circumscribed have been completed. Examples include the corpus callosum, ventricular system, hippocampus, caudate and others.

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146.18 A COMPUTER METHOD FOR VOLUME DETERMINATION OF STRIATE AND EXTRASTRIATE CORTEX IN PRIMATE BRAIN.
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Previous work in our laboratory, using the **CARP** system (Computer Aided Reconstruction Package) for data compilation, has suggested that striate and extrastriate cortices are highly asymmetrical in primate brain [de Lacoste *et al*, 1986]. These studies, however, were limited in quantitative assessment in that telencephalic regions were defined using gross anatomical landmarks. This study was undertaken to develop methods for evaluating striate and extrastriate primate brain asymmetries using a video image analysis subsystem of **CARP** originally developed for 2-deoxyglucose autoradiography research. The aim was to develop more accurate methods for assessing volumes of cortical gray matter from histological preparations.

Formalin-fixed rhesus brains (N=4) obtained from Brooks Air Force Base were coronally sectioned (50 microns) with a freezing microtome and stained with Cresyl violet. Care was taken to assure uniformity of the staining procedure. The stained sections were mounted on glass slides and placed on a light box beneath a video camera. Video images were captured in a digital frame buffer and analyzed in an IKONAS color raster graphics system using the **CARP** software running on a Data General MV/8000 II host computer. Image processing routines were used to perform video averaging, to eliminate intensity gradients originating in the light source, and to correct geometrical anisotropy in the video digitizer.

The CSCAN utility of **CARP** was used first to isolate the cortex from the white matter in the sections and then to automatically detect and store the external and internal borders of the cortical gray matter in a computer database. The use of pseudocolor enhancement of the video digitized cortical cytoarchitecture was successful in determining the boundaries between striate and extrastriate cortex and between extrastriate cortex and surrounding areas. The line editing features of **CARP** allowed the construction of graphical line segments representing the cortical regions. Areas could be computed for these delineated regions of cortex on the left and right sides of each brain.

Areas in consecutive series can be reconstructed into a volume and quantitated using **CARP** utilities. These strategies provide a basic framework for determining regional volumes within cortical areas. Our long term goal is to include refined image analysis techniques to obtain quantitative information about details of cortical cytoarchitecture.

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146.19 **Whole-Brain Mapping: Strategies for the Construction of "Nested" Computerized Atlases.** C.A. Lemere*, C. Wurtz and R.B. Livingston. Brain Mapping Project, Dept. of Neurosciences S-001, UCSD, La Jolla, CA. 92093.

The anatomy of the brain has been studied at varying levels of magnification and resolution for centuries. However while micrographs, functional diagrams, and "wire frame" atlases have been produced, a complete brain map, corrected against preparation artifacts, has never been created. With the recent progress in digital image processing and computer databasing such an atlas is feasible for the first time. Ideally this atlas would contain a bit-map of all structures down to the limits of optical microscopy. In practice this creates an impossible amount of data...hundreds of terabytes to map at 20 μm resolution. Our strategy avoids this problem by retaining only the data needed to assign each cell soma an xyz address and a cytoarchitectural identifier (e.g. "pyramidal cell") These data are acquired by automated microscopy, by precessing a 10 μm thick section, stained for a specific cell type, under a CCD line camera. The CCD, along with the automated stage and host computer, assigns the address and size parameter and determines the cell type (based on the spectral response of the stained cell). Thus each cell requires only 10 bytes to define it for the map. Since we take one section every 100 μm, we are addressing approx. 10% of the cells in the brain, thus our human atlas will contain about 50 GBytes of neuronal data. To ensure 3-D accuracy and fidelity to the in vivo state we employ a sequential registration control process (hence the term "nested") utilizing the outer and ventricular surfaces and the pre-capillary vasculature as biological fiducials. We apply CT scans to correct for fixation, dissection, and freezing effects, and digital images of the blockface (acquired as the specimen is sectioned) to control for compression and shrinkage during histological processing. Finally we apply 2-D warping algorithms such that our map addresses accurately reflect the in vivo position of the given cell. We are currently acquiring data from the rat, the cynomologous monkey, and the human, for which we will construct complete maps. These atlases are ultimately more than a collection of precise spatial coordinates for the neuronal somata, glial cells and fiber tracts. They will be the framework for a computer-manipulatable neurobiological database, capable of containing physiological, neurochemical, and functional information.
Supported by: DAMD17-86-C-6093

146.20 CAPSAICIN-INDUCED DAMAGE TO SUBDIAPHRAGMATIC VAGAL FIBERS AND CENTRAL AFFERENT TERMINALS FROM THE STOMACH. S.S. Bernier*, E.H. South, and R.C. Ritter. (SPON: D. Fragaszy). Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520 and WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID 83843

The neurotoxin capsaicin destroys small unmyelinated primary sensory neurons in a variety of peripheral nerves. Recently, we reported behavioral data which indicate that capsaicin pretreatment attenuates suppression of food intake by stimuli thought to act via vagal afferents. To assess capsaicin-induced vagal damage, we performed transmission electron microscopy on the subdiaphragmatic vagi of adult rats treated with 175 mg/kg capsaicin (dosage series: 25, 50, 50, and 50 mg/kg). We also examined labeling of cell bodies and terminals in the dorsal hindbrain following injection of wheat germ agglutinin conjugated horseradish peroxidase (WGA/HRP) into the stomach wall of intact and capsaicin-treated rats. Twenty-four hours after capsaicin treatment, unmyelinated fibers in the vagi exhibited marked vacuolation and swollen mitochondria. By 72 h post-capsaicin a profound disruption of the integrity of unmyelinated fibers was observed. One week after capsaicin injection the disruption was less evident and by 23 days post-capsaicin the vagal fiber structure was overtly no different from controls. No damage to myelinated vagal fibers was observed at any time. Light microscopic evaluation of dorsal hindbrain sections of rats receiving stomach wall injections of WGA/HRP revealed terminal labeling in the subnuclei of the nucleus of the solitary tract (NST) and the dorsal motor nucleus (DVM) associated with gastric projections of the vagus (Shapiro and Miselis, 1985). The DVM also exhibited marked retrograde filling of the motor neuron cell bodies. In contrast to controls, capsaicin-treated rats exhibited dramatic reductions of terminal labeling in the NST. Loss of labeling was apparent at 24 h post-capsaicin onward and was still evident at least 23 d post-capsaicin. There was no apparent loss of filling of the DVM cell bodies.

Capsaicin-induced vagal damage results in reduced labeling of sensory projections of the gastric vagus in the dorsal hindbrain. These findings are consistent with the silver impregnation studies of Dinh and S. Ritter (1985) as well as our behavioral and electrophysiological findings (Ritter and Ladenheim, 1985; South and Ritter, 1986; Yox and Ritter, 1986; Ritter et al., 1987) indicating that capsaicin destroys a subpopulation of vagal afferent fibers which carry information from the gastrointestinal tract. Supported by NIH grants R01 NS20561 and R01 NS21805.

147.1 FOUR POLYPEPTIDES PURIFIED FROM GREEN MAMBA VENOM, WHICH BLOCK K CHANNELS, LABEL SPECIFIC RECEPTORS ON RAT BRAIN SYNAPTIC MEMBRANES. R.G. Sorensen and M.P. Blaustein. Dept. of Physiology, Univ. of Maryland Med. School, Baltimore, MD 21201.

Four polypeptides, (designated α-, β-, γ- and δ-DaTx), purified from the venom of the green mamba, Dendroaspis angusticeps, have been recently shown to block ^{86}Rb efflux from rat brain synaptosomes, [Benishin et. al., Fed. Proc. 46:504, 1987.] Two of the polypeptides, α- and δ-DaTx, preferentially block a component of ^{86}Rb efflux corresponding to a voltage-gated, rapidly-inactivating K channel. The other two, β- and γ-DaTx, preferentially block a component of ^{86}Rb efflux corresponding to a voltage-gated, non-inactivating K channel. Here, we characterize the binding of these four venom polypeptides to synaptic membranes prepared from rat brain.

The four polypeptides were radioiodinated. Their binding was determined from competitive displacement assays in which the ability of increasing amounts of an unlabelled polypeptide to displace the binding of a radiolabelled polypeptide was measured. Each polypeptide labelled a specific receptor on rat brain synaptic membranes. Maximal binding was observed in physiological salt solutions. Preliminary data indicate that [^{125}I] α-DaTx and [^{125}I] δ-DaTx both bind with a $K_D \simeq 1$ nM and $B_{max} \simeq 1.5$ pmol ligand bound / mg protein. [^{125}] β-DaTx and [^{125}I] γ-DaTx have about 10-fold lower affinity, $K_D \simeq 10$-15 nM, but a similar number of binding sites, $B_{max} \simeq 2.5$ pmol / mg protein. Radioiodinated α and γDaTx were crosslinked to synaptic membranes to identify the receptor polypeptides. The radioiodinated toxin was equilibrated with synaptic membranes for 30 min at 37°. Crosslinking of the toxins to membrane polypeptides was initiated by the addition of 0.05 to 1 mg/ml dimethyl suberimidate for 2-4 hr. The membrane polypeptides were subsequently separated by SDS-polyacrylamide gel electrophoresis and autoradiograms were prepared to determine attachment of the labelled ligand. Preliminary results indicate that α-DaTx and γ-DaTx bind to different receptors, as each polypeptide appears to be covalently crosslinked to a distinct polypeptide. [^{125}I] α-DaTx specifically labels a single polypeptide with apparent M_R = 80 kDa. [^{125}I] γ-DaTx specifically labels two polypeptides with M_R = 35 and 80 kDa. The different labelling patterns for the two toxins seems consistent with their observed abilities to block different K channels in rat brain. These venom components may be useful as potential ligands for the isolation and purification of mammalian voltage-gated K channels. [Supported by NIH grant NS-16106].

147.2 SINGLE-CHANNEL POTASSIUM CURRENTS IN MEMBRANE VESICLES DERIVED FROM NORMAL AND MUTANT *DROSOPHILA* LARVAL MUSCLES. A. Komatsu and C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Single-channel analysis of *Drosophila* mutants with altered membrane currents may provide insights into the molecular mechanisms of ion channel function. We have developed a sarcolemmal vesicle preparation suitable for patch-clamp recordings. Single-channel recordings using this preparation can be compared with macroscopic current data previously obtained from conventional voltage-clamp experiments in both larval and adult muscles.

Spherical membrane vesicles were prepared by exposing 3rd instar larval musculature to a high KCl solution containing collagenase (1 mg/ml). The experiments were conducted in the "cell"-attached configuration using vesicles bathed in 130 mM K^+ solution to maintain a resting potential near zero. One-third of the patches in which a gigaohm seal was formed contained complex channel activity which may consist of multiple channels. These patches exhibited only outward currents when pipette contained 2.5 mM K^+. When pipettes contained 130 mM K^+, these currents were reversed around 0 mV and became inward at hyperpolarizing potentials.

Other patches showed distinct single-channel activities that can be classified into at least two types: a voltage-dependent channel (type I) and a non-voltage-dependent channel with sustained activity (type II). Type I channels had a unit conductance of 10 pS and an extrapolated reversal potential of -100 mV when pipette contained 2.5 mM K^+. When pipette contained 130 mM K^+, this type of channel had a unit conductance of 40 pS and a reversal potential of 0 mV. The probability of channel opening peaked at 20-30 msec following steps of membrane depolarization and slowly declined over hundreds of msec.

Type II channels had a unit conductance of 50 pS at 0 mV and a reversal potential lower than -60 mV when pipette contained 2.5 mM K^+. When pipette contained 130 mM K^+, this type of channel had a unit conductance of 90 pS and a reversal potential of 0 mV. The I-V relations for this type of channel can be described by the Goldman-Hodgkin-Katz constant field equation and the kinetics resemble those of Ca^{++}-dependent K^+ channels in other preparations. Preliminary experiments with inside-out patches suggest that this channel is Ca^{++}-dependent and that 10 mM TEA causes a 20% reduction in unit conductance.

Comparisons of single-channel currents were made between normal and *slowpoke (slo)* mutant larvae, which lack a macroscopic Ca^{++}-dependent K^+ current (Elkins *et al, PNAS*, 83:8415, 1986). In normal larvae, 23% of the active patches (n=57) contained type II channel activity. This is in contrast to *slo* mutants in which no patches (in a total of n=58) contained type II channel activity. However, the proportion of patches showing complex channel activity were similar: 35.1% in normal and 43.1 % in *slo* mutant larvae. These results suggest that the *slo* mutant lacks a functional Ca^{++}-dependent K^+ channel. Further pharmacological experiments in inside-out patches are under way to determine whether the defect resides in the channel itself or in its activation mechanism.

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147.3 CONCOMITANT ALTERATION OF POTASSIUM CHANNEL GATING AND PHARMACOLOGY IN A SHAKER MUTANT OF DROSOPHILA. F.N. Haugland and C.-F. Wu, Dept. of Biology and Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA. 52242.

Electrophysiological analysis of mutations affecting ion channels allows the various functional properties of channels to be correlated with specific structural domains altered by individual mutations. In Drosophila, mutations of the Shaker (Sh) locus affect a transient potassium current, I_A, in larval muscle fibers (Wu and Haugland, J. Neurosci. 5:2626, 1985). Analysis of defective I_A in Sh mutants can reveal the functional roles of the affected structural domains within the I_A channel as determined by molecular genetic techniques.

Membrane currents in larval muscle fibers of the Sh^5 mutant were compared with normal using two-microelectrode voltage clamp. The current-voltage (I-V) relation was shifted to more positive potentials and steepened. Step depolarizations from -80 mV to potentials between -20 and +20 mV elicited an I_A whose amplitude was markedly reduced while steps to more positive potentials induced an I_A of nearly normal amplitude. The time course of I_A in the mutant was only slightly slower than normal. In a paradoxical fashion, I_A tail currents following repolarizations from +40 to 0 mV or more negative potentials had a normal amplitude but with abnormally rapid kinetics of decay. A mathematical model demonstrated that this seeming contradiction can result from a simple alteration in a kinetic scheme of I_A channel gating. Further voltage-clamp experiments revealed altered pharmacological properties. Sh^5 channels were much more sensitive to blockade by 4-aminopyridine (4-AP). At a concentration of 5 μM 4-AP, Sh^5 I_A was blocked by more than 90% while normal I_A was blocked by less than 65%. Since the Sh^5 mutation was induced by ethyl-methane-sulfonate, a mutagen which often results in single amino acid replacements, our data raise the possibility that the gating and 4-AP binding functions reside within the same structural domain of the I_A channel. Alternatively, these two functions may be governed by separate regions that may be influenced by the Sh^5 mutation site. This work was supported by NIH grants NS00675 and NS18500 to CFW.

147.4 PROPERTIES OF THE A-CURRENT IN MATURE, DISSOCIATED AMPHIBIAN SPINAL NEURONS IN CULTURE. A. Hernandez-Cruz.* (SPON: Paul R. Adams) Lab. of Neurobiology, The Rockefeller University, 1230 York Ave, New York, N.Y. 10021

Acutely dissociated neurons from the adult salamander spinal cord exhibit fast Na-dependent action potentials which repolarize very rapidly by the activation of K^+ currents. In a previous communication, evidence was presented for the presence of two sustained K^+ currents: The Ca-dependent K^+ current and the delayed rectifier (Hernandez-Cruz & MacLeish, Soc. Neurosci. Abst. Part 2 , pp 1348, 1986). In this report, the properties of a third component, similar to the A-current (I_A), are examined. Enzymatically dissociated cells were plated on plastic petri dishes and recorded (after 1 to 5 days in culture) with patch electrodes containing (in mM) 90 KAsp, 20 Sucrose, 10 NaH_2PO_4 , 4 $MgCl_2$, 1 ATP and 0.5 K_2EGTA (pH 7.3). Step depolarizations elicited a transient outward current when cells were held at rest (-70 mV) and bathed in a solution containing (in mM): 20 TEA, 0 NaCl, 0.2 $CaCl_2$, and 10 $MgCl_2$. This current peaked within 8-10 ms and inactivated exponentially with a time constant between 30 and 45 ms (19-22 °C). It was eliminated by 1.5 mM 4-AP or by holding potentials positive to about -30 mV. Half-activation of this current was observed at about -10 mV and half-inactivation at about -40 mV, with a small overlap near -30 mV. Both size and steady-state inactivation of I_A varied widely among different neurons from the same cultures, being absent in some of them. In general, inactivation curves were displaced 10 -15 mV in the positive direction, as compared to most neuronal systems, suggesting that a significant fraction of A channels could be available for activation during repetitive firing and not only at potentials near rest. Cadmium (0.5 mM) increased the I_A amplitude and shifted the inactivation curve even more into the positive region without affecting the inactivation time constant. This effect could not be attributed to screening effects or to blockade of Ca^{2+} channels. In current clamp experiments, Cd^{2+} and 4-AP were used to examine the possible role of I_A in the cell electrical behavior. When 4-AP was added to normal Ringer solution, the spike elicited by short pulses increased in size and broadened. On the other hand, Cd^{2+} reduced spike amplitude and shortened its duration slightly. In the last case, an effect due to Ca-current blockade by Cd^{2+} could not be completely ruled out, although similar changes in spike morphology were not observed when $[Ca^{2+}]_o$ was reduced from 2 to 0.2 mM. A decrease in firing frequency and a faster adaptation was observed in the presence of 4-AP. This was interpreted as a result of a more extensive activation of the delayed rectifier and Ca-activated K^+ currents secondary to spike broadening.

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147.5 CHARACTERIZATION OF A TRANSIENT K^+-CURRENT IN TRIGEMINAL ROOT GANGLION SENSORY NEURONS. I. Spigelman* and E. Puil (SPON: D. M. J. Quastel). Department of Pharmacology Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

Sensory neurons in the trigeminal root ganglion (TRG) of the guinea-pig share certain properties with other craniospinal neurons of mammals. For example, they both exhibit action potentials that are followed by brief afterhyperpolarizations (AHPs) lasting 1-20 ms. The AHPs are sensitive to blockade by external application of tetraethylammonium (TEA; 10 mM), which also prolongs the spike duration and decreases repetitive spike discharge (Puil and Spigelman, Neuroscience, in press). In preliminary studies using conventional intracellular recording techniques, we have found that bath administration of 4-aminopyridine (4-AP; 1 mM) also prolonged spike duration but, unlike TEA, 4-AP did not appreciably reduce the amplitude of AHPs and enhanced repetitive firing.

To investigate the effects of K^+-channel blockers more extensively, and to characterize an ionic current which may give rise to the AHP, we have performed pharmacological analyses of neuronal properties in slices of the TRG using single electrode voltage clamp techniques. In TRG neurons which were clamped at holding potentials of ~-40 mV, a transient outward current was evident at termination of hyperpolarizing voltage command steps (5-40 mV). This fast current had an onset of <5 ms and its deactivation was complete by ~40 ms. Similar currents could be evoked during application of tetrodotoxin (1 μM) using depolarizing voltage commands when the holding potential was near -65 mV. In addition, the transient outward current (holding potential -40 mV) could be reduced by increasing the extracellular K^+-concentration from 5 mM to 20-40 mM. These currents were blocked by bath application of TEA (1-10 mM). Similar applications of 4-AP (1-5 mM) did not significantly reduce the transient outward current. However, administration of either K^+-channel blocker elicited a decrease in leak conductance. These results suggest that the transient outward current in TRG neurons is mediated by K^+ ions. The characteristics of this current indicate its participation in the genesis of the short-lasting AHPs following spikes and presumably would be important in the regulation of repetitive spike discharge in TRG sensory neurons.

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147.6 MEMBRANE RESONANCE IN TRIGEMINAL ROOT GANGLION NEURONS: INVOLVEMENT OF K^+-CONDUCTANCES. E. Puil, B. Gimbarzevsky* and I. Spigelman*. Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

An outstanding feature of the plasma membrane in the trigeminal root ganglion (TRG) neuron and in other excitable cells is that during a small perturbation by a stimulus, there is a natural frequency of oscillation or resonance. The impedance of such a system has a maximal value at the resonant frequency, and like a low bandpass filter, falls rapidly at frequencies on either side of the peak resonant frequency. The characteristics of this TRG neuronal filter are based on the values of capacitive, as well as on the resistive and inductive components in the membrane. Since the resistive and inductive components are attributable to various time- and voltage-dependent conductances, we have investigated the effects of ionic channel blockers on the genesis of membrane resonance. The complex impedances and impedance magnitude functions of neurons were determined in in vitro slices of the TRG of guinea pigs using frequency-domain analyses of intracellularly recorded voltage responses to specified oscillatory input currents. At their initial resting membrane potentials, all neurons exhibited resonance in the range of 30 to 200 Hz in their impedance magnitude functions. Bath applications of the selective Na^+-channel blocker, tetrodotoxin (10^{-6} M) produced only minor changes, whereas tetraethylammonium (TEA: 10^{-2}) markedly increased the impedance magnitude and also suppressed the resonant behavior in a reversible manner. Such applications of 4-aminopyridine (4-AP; 10^{-3} M) decreased the peak resonant frequency in a manner similar to TEA application. Unlike TEA, however, 4-AP usually increased the sharpness of the resonant hump, in addition to the impedance magnitude in many neurons. For quantification, the complex impedance data were fitted with a neuronal model derived from linearized Hodgkin-Huxley-like equations. This yielded estimates for membrane electrical properties. Although input capacitance was unchanged by drug administration, both K^+-channel blockers decreased the time-invariant resting conductance that includes a lumped leak conductance component. The time- and voltage-dependent conductance was decreased by TEA, and increased by 4-AP. The time constant which is associated with this conductance as well as with the inductive property of the membrane also was decreased by TEA, and increased by 4-AP. These data suggest a primary involvement of two K^+-conductance systems in membrane resonance, or separate sites of action of TEA and 4-AP in the blockade of the K^+-channels.

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147.7 DIFFERENTIATION OF NEUROBLASTOMA CELLS INDUCES EXPRESSION OF K CHANNELS. C.J. Smith-Maxwell*, R.A. Eatock and T. Begenisich*. Dept. Physiol., University of Rochester, Rochester, NY 14642.

We have examined whole-cell currents in two closely related mouse neuroblastoma cell lines: Neuro-2a and Neuro-2a-AB-1 ("AB-1"). The AB-1 line was derived from the Neuro-2a line in J. Olmsted's laboratory. Depolarizing voltage steps elicit both Na^+ and K^+ currents in Neuro-2a cells but only Na^+ currents in AB-1 cells raised in standard culture conditions. We were interested in whether the AB-1's had lost K-channel genes or simply the ability to express those genes in standard conditions. Our approach was to see whether the cells would express K^+ currents if induced to differentiate. We applied agents that have been shown to differentiate AB-1's (Notter and Leary, Dev. Brain Res. 26:59-68, 1985) or other neuroblastoma cells, as assayed by changes in cell division and morphological changes (extension of neurites, hypertrophy, expression of cell surface markers).

AB-1 cells were grown at 37°C in humid 5% CO_2/95% air in one of the following media: *a) control medium:* Ham's F12 plus 10% fetal calf serum (FCS); *b) DMSO:* 7-13 days in F12 containing 1.3% dimethylsulfoxide and 0.3% FCS (Smith, J. Physiol. 382:191P, 1987); *c) mitomycin/Brdu:* 5 days in F12 containing 10% FCS, 500 ng/ml mitomycin C and 10 μM bromodeoxyuridine (the Brdu was added 6 hrs. after the mitomycin). All media contained 50 μg/ml gentaminicin and 2.5 μg/ml fungizone. In both treatments (b-c), cells were larger than controls and cell division was slowed or stopped. DMSO-treated cells were round with no processes or with thick bud-like processes. Mitomycin/Brdu promoted neurite extension.

Membrane currents were studied using the whole-cell gigohm-seal technique to clamp membrane voltage at depolarized levels. The bath contained a high-Na^+ solution while the pipette contained KCl or K-aspartate, 11 mM EGTA, and 3 mM K_2-ATP. Both DMSO and mitomycin/Brdu induced K^+ currents; Na^+ currents, present in both control and treated cells, appeared relatively unaffected. The induced K^+ currents were smaller than K^+ currents in the Neuro-2a cells. They were largely blocked by adding 10 mM tetraethylammonium to the bath. Given the high EGTA in the pipette and the low bath concentration of Ca^{2+} (2 mM), it seems likely that the induced K^+ currents were not primarily Ca^{2+}-activated.

We conclude that AB-1 cells possess K-channel genes whose expression is negligible in standard conditions and greatly enhanced by differentiating agents.

147.8 POTASSIUM CHANNELS IN ENCEPHALITOGENIC RAT LYMPHOCYTES: CHARACTERISTICS OF INACTIVATION AND ROLE IN CELL ACTIVATION S.I.V. Judge*, J.Z. Yeh*, M.D. Mannie* and P.Y. Paterson*(SPON: L.D. BROWN). Dept. of Pharmacol. and Dept. of Microbiol.-Immunol., Northwestern Univ. Med. Sch., Chicago, IL. 60611.

Myelin basic protein (MBP)-reactive, Lewis (Le) rat T lymphocytes mediate the transfer of experimental allergic encephalomyelitis (EAE) into normal Le rats following MBP stimulation of cell activation and proliferation. Using the gigohm-seal whole-cell variation of the patch clamp, we studied voltage-gated potassium (K) channels in both MBP-sensitized lymph node cells (LNC) and the MBP-reactive GP1 continuous cell line at rest and during MBP stimulated proliferation-activation. Characterization of K channel inactivation under conditions of prolonged depolarization revealed a shift in the voltage-dependence of steady-state (SS) inactivation, which correlated in a time-dependent manner with the extent of cell activation and proliferation. Over the 9-10 day cell cycle, SS inactivation was half-maximal (V1/2) at progressively more depolarized potentials during the first 6-7 days. The subsequent 2-3 days evidenced a rapid shift of V1/2 back to resting levels. V1/2 occurred around -50 mV (slope factor: SF=4.4) at rest, within 1-2 mV of the current activating potential -40 mV (SF between 4.8 and 5.2) during peak cellular activation, at progressively more depolarized potentials up to -28 mV (SF=4.0) during proliferation, and reversed over the course of one day to -40 mV (SF=4.0) as cells began returning to rest. For all T cells studied, the decay of outward current was fit by a single exponential. The time constants of inactivation showed a nonsystematic variability of 300-500 ms at all potential steps, which did not correlate with the resting or activated states of these cells. LNC showed an almost complete inactivation of current at all potentials during the 1.8 s depolarizing pulses, in contrast to the GP1 cells which showed a residual non-inactivating current that increased as the cells progressed through their activation phase.

The capacity of these cells to transfer EAE constitutes a measure of cell activation. Therefore, cellular activation assays were performed on MBP-sensitized LNC to simultaneously assess the involvement of K channels in cell activation and subsequent EAE induction. Cells were incubated with tetraethylammonium (TEA; 100mM), methoxyverapamil (D600; 200uM), 4-aminopyridine (4-AP; 10mM) or nifedipine (200uM) during activation cultures with MBP. These blockers did not inhibit or modify the induction of EAE into Le rats. Concurrent proliferation assays showed that, at doses above 100uM, both D600 and nifedipine inhibited proliferation as measured by [^{3}H] thymidine uptake, while TEA and 4-AP had no effect. SITS (4-acetamido-4'-isothiocyanostibene2,2'disulfonic acid), a modulator of neuronal K channels, strongly stimulated proliferation.

In summary, our results demonstrate a depolarizing-hyperpolarizing pattern of shifts in the voltage-sensitivity of K channel SS inactivation after MBP stimulation. The amount of non-inactivating current correlates with the level of cellular activation for the GP1 cells but not for LNC. We also show that K channel blockers do not prevent the activation of T cells and their ability to mediate the transfer of EAE.

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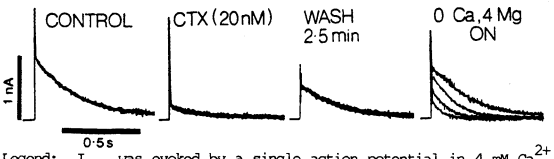
147.9 **EFFECTS OF CHARYBDOTOXIN AND EXTRACELLULAR CALCIUM ON THE TIME COURSE OF THE AFTERHYPERPOLARIZATION CURRENT (I_{AHP}) OF BULLFROG GANGLION NEURONS.**
P.S. Pennefather and J.W. Goh. Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 1A1, Canada.

Charybdotoxin (CTX) is a basic protein isolated from the venom of the Israeli scorpion, Leirus quinquestriatus which has been shown to block the large voltage and calcium activated potassium channel found in many types of cells (Smith et al., J. Biol. Chem., 261:14607). We find that low concentrations of CTX (4-20 nM) reversibly prolongs the action potential recorded in neurons of the Bullfrog sympathetic ganglion. This result is consistent with the reported action of the toxin, since a large, voltage and calcium activated potassium channel (I_C) is known to be responsible for spike repolarization in these cells. However, we find that another calcium-activated potassium channel called I_{AHP}, is also blocked by similar concentrations of CTX. Superficially, the action on both currents resembled that produced by blocking calcium influx but, a more detailed analysis of the effect ruled out this possible mechanism. The exponential decay of I_{AHP} evoked by either a single action potential or a train of spikes is always longer following the train. Lowering extracellular calcium causes the decay rate of I_{AHP} to increase (see last panel of Figure). Between 4 and 1 mM calcium, the half decay time is reduced 1.4-fold per halving of calcium levels. Thus, the time course of I_{AHP} is a measure of the calcium load produced by action potentials. CTX reduced I_{AHP} amplitude without affecting time course (see Figure). It is thus, unlikely that CTX reduces calcium influx and we conclude that two channels with very different calcium, voltage and time dependencies share a common type of receptor for CTX.



Legend: I_{AHP} was evoked by a single action potential in 4 mM Ca^{2+} and was measured by switching to voltage clamp mode during the repolarization phase of the spike. 20 nM CTX caused an almost complete inhibition of I_{AHP}. Recovery had a half time of about 3 minutes. The last panel shows superimposed records taken at successive 30 second intervals after changing the bathing solution to one that contained 4 mM Mg^{2+} and no calcium.

Supported by a grant from the Canadian MRC. PSP is a career scientist of the Ontario Ministry of Health. JWG is the recipient of a Postdoctoral Fellowship from MRC of Canada.

147.10 CALCIUM-ACTIVATED POTASSIUM CHANNELS IN RAT CORTICAL NEURONS IN CELL CULTURE. C.Zona, G.Pirrone*, M.Deodati*, A.Brancati*. Chair of Human Physiology, Department of Experimental Medicine, University of Rome, Tor Vergata, Rome, Italy.

Ca-activated K channels from rat cortical neurons in cell culture were studied in detached inside-out membrane patches (Hamill et al., Pflügers Arch. 391: 85, 1981).The neurons were derived from 13-15 days rat embryos cortices and were dissociated with trypsin and plated on collagen-polylysine coated coverslips (Dichter, Brain Res. 149 : 279, 1978). In a physiological K gradient ($[K]_o$ = 3 mM and $[K]_i$ = 120 mM where $[K]_o$ is the K concentration in the pipette and $[K]_i$ is the concentration in the bathing solution), when the patches were depolarized, outward currents were recorded. The Ca-activated K channel was identified by its Ca and voltage sensitivity, and this was the channel most commonly observed under the conditions of these experiments. Other channels were, however, occasionally observed. The increased current noise when the channel was open was apparent and was useful for distinguishing between open and closed states. The channel was observed in 90% of all patches and even with small pipettes, multiple channels were obserbed. The channel was voltage gated. Both the frequency of channel opening and the duration of the open state increased as the patch was depolarized from 0 to 50 mV at a constant Ca concentration. The conductance of the channel in physiological K gradient over this range was of about 120 pS. The activity of this channel was regulated also by the Ca concentration on the cytoplasmic surface of the membrane. As the internal Ca concentration decreased both the probability of channel opening and the duration of the open state decreased. The effect of small ions on permeation through the channel and the mechanisms by which this current interact to determine cortical neuronal excitability will be discussed.

147.11 DOPAMINE INCREASES A K^+ CONDUCTANCE AND INHIBITS SPONTANEOUS ACTION POTENTIALS RECORDED FROM MELANOTROPIC CELLS OF RAT PITUITARY. J.Stack* A. Surprenant & R.G. Allen. (SPON:L. Bazier) Vollum Institute, Oregon Health Sciences University, Portland, OR. 97201.

Basal secretion of α-melanotropin (α-MSH) and other proopiomelanocortin (POMC)-derived peptides in the rat intermediate pituitary is calcium-dependent and this basal secretory activity is inhibited by activation of dopamine (D_2) receptors; however ionic mechanisms which may underlie these actions are not known. Therefore we have made intracellular recordings of membrane voltages and membrane currents (using single-electrode voltage clamp) from rat intermediate pituitary cells.

Neurointermediate lobes were enzymatically and mechanically dispersed in a collagenase Hanks Hepes solution at 37°C; cells were plated onto 12 mm coverslips that had been treated with poly-L-lysine. Intracellular recordings were made 4 hr - 3 days after dispersion, temperature was 35-37°C. Parallel release studies, using β-endorphin immunoassays, showed that basal secretion was approx. 0.1 pmoles/min/lobe equivalent; 1 μM bromocryptine abolished basal release and this inhibition was blocked by 1 μM haloperidol.

Spontaneous electrical activity was recorded from all cells whose input resistances were greater than 600 MΩ. Spontaneous TTX-sensitive action potentials (APs; 1-2 ms duration, 50 mV amplitude), occurred at 0.2 - 2 Hz in about 50% of spontaneously active cells; TTX-insensitive/cobalt-sensitive membrane oscillations (10 ms duration, 2-10 mV amplitude) occurred in all cells and spontaneous hyperpolarizations (50 ms duration, 5-20 mV amplitude) occurred in cells in which both sodium and calcium APs were present. The hyperpolarization was associated with an increased membrane conductance, was dependent on extracellular K^+ concentration and was blocked by cobalt. Bromocryptine (1 μM) and dopamine (5 μM) hyperpolarized the membrane, increased the membrane conductance and abolished spontaneous APs. The amplitude of the dopamine hyperpolarization decreased when the membrane was hyperpolarized and reversed polarity at the K^+ equilibrium potential.

These results indicate that activation of dopamine receptors on melanotropes increases a K^+ conductance in the membrane. The occurrence of spontaneous calcium-dependent depolarizations in cells shown to be functionally active (by immunoassay) provides a mechanism by which basal hormone secretion may be directly coupled to calcium influx.

147.12 POSSIBLE INVOLVEMENT OF GTP-BINDING PROTEINS IN COUPLING OF MUSCARINIC RECEPTORS TO M-CURRENT IN BULLFROG GANGLION CELLS. H.S. Lopez*, D. Brown and P.R. Adams. (SPON: P.K. Coyle). Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

In bullfrog ganglion "B" cells application of muscarine or neuropeptides reduces a voltage-dependent K-current (Im) as well as producing some increase in leakage. We have investigated the possible involvement of G proteins in receptor/conductance coupling. Firstly, the effect of brief applications (30-40 sec) of 1 uM muscarine on control responses elicited by hyperpolarizing 20 mV voltage steps from -30 or -40 holding potentials were measured. This concentration of muscarine on average inhibited 55% of Im, and after 30-120 sec washing almost complete recovery was seen. However, the extent of inhibition in individual cells was quite variable (30-80%). A second pipette, containing GTP-gamma-S, was then inserted into the cell and negative current (-2 to -4 nA) applied for 2 minutes, and this electrode then removed. The effect of 1 uM muscarine was then retested. Inhibitions of Im ranging from 0 to 100% were observed. However, most of the cells did not recover following washout. In all cells showing greater than 30% inhibition of Im by muscarine after GTP-gamma-S injection, no recovery was seen. Several GTP-gamma-S injected cells showed no response to muscarine, although they did before GTP-gamma-S injection. The injected GTP-gamma-S appeared to reduce Im by itself although this effect might be due to the trauma of double impalement and iontophoretic injection. GTP-gamma-S injected cells also responded to muscarine with a larger inward current instantaneously appearing at -50 or -60 mV potentials than control cells, and this inward current (presumably reflecting increased leakage) also failed to recover. These data suggest that muscarinic receptors activation in "B" cells reduces M conductance via activation of a GTP-binding protein.

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147.13 GTP-γ-S INHIBITS THE M-CURRENT OF DISSOCIATED BULLFROG SYMPATHETIC NEURONS. Stephen W. Jones. Dept. Physiology and Biophysics, Case Western Reserve University, Cleveland OH 44106.

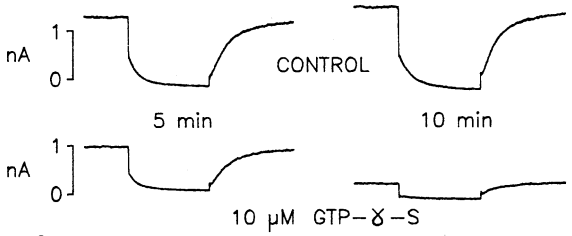
The M-current of bullfrog sympathetic neurons is a voltage-dependent potassium current that is inhibited by muscarinic agonists and certain neuropeptides. It has been supposed that the coupling between the receptors and the M-channel is indirect, but a second messenger has not been conclusively identified. Whole-cell patch clamp recording has been used to test the action of GTP-γ-S, a metabolically stable analog of GTP that produces prolonged activation of GTP-binding proteins.

Sympathetic ganglia were dissociated, and isolated neurons were clamped with 1-1.5 MΩ electrodes [filled with (mM) 100 KCl, 1 K_2EGTA, 5 Na_2ATP, 4 $MgCl_2$, and 2.5 NaHEPES, titrated to pH 7.2 with KOH, adding ~8 mM K]. The extracellular medium contained (mM) 115 NaCl, 1.5 KCl, 2 $CaCl_2$, and 2.5 NaHEPES, pH 7.2.

Without GTP-γ-S, the M-current increased in the first few minutes of whole-cell recording, but was relatively stable thereafter for approximately 1 hour. 10-100 μM GTP-γ-S in the pipet either prevented the initial increase in M-current, or decreased the M-current over a period of minutes (see Figure). The leakage conductance often increased with GTP-γ-S. The M-current (and/or leak increase) sometimes recovered partially over tens of minutes following the maximal effect.

This suggests that a GTP-binding protein might be involved in coupling of receptors to M-channels. Further experiments are necessary to test that hypothesis, and to establish whether the GTP-binding protein acts directly or via generation of cytoplasmic second messengers.

Supported by NIH grant NS 24471.



Currents are in response to 0.6 sec steps to -60 mV from the holding potential of -30 mV, in a cell with 10 μM GTP-γ-S in the pipet (below), or in a control cell (above).

147.14 EXCITATORY ACTIONS OF TETRAHYDRO-9-AMINO-ACRIDINE ON HIPPOCAMPAL PYRAMIDAL NEURONS. D. R. Stevens and C. W. Cotman . Dept. of Psychobiology, University of California, Irvine, Ca. 92717

We have examined the actions of tetrahydro-9-aminoacridine (THA) on guinea pig hippocampal pyramidal neurons in an *in vitro* brain slice preparation using intracellular recording techniques. THA (10-1000μM) depolarized CA_1 pyramidal neurons. This action of THA was accompanied by an increase in action potential frequency and a decrease in membrane conductance. Since THA is a potent anti-cholinesterase, we examined it's action in the presence of atropine. Neither atropine nor tetrodotoxin prevented THA's excitatory action. These results indicate that the excitatory action of THA is independent of the release of neurotransmitter.

Repolarization of membrane potential did not reverse the conductance decrease associated with the action of THA, demonstrating that this change is a direct action of THA. In twelve neurons the extrapolated reversal potential was -88 ± 5 mV, near the reversal potential of potassium ions in this preparation.

THA also excited CA_3 pyramidal neurons. In these neurons, THA caused increased action potential frequency and the appearance of bursts of action potentials followed by large afterhyperpolarizations. When the membrane potential was hyperpolarized to prevent bursts a decrease in conductance was observed. This result suggests that the mechanism of THA's excitatory action on CA_3 pyramidal neurons was a decrease in potassium conductance similar to that observed in CA_1 neurons. In addition, a broadening of the action potential was observed which could enhance the bursting behavior.

These experiments demonstrate that THA has excitatory actions on hippocampal pyramidal neurons which result from blockade of potassium conductance(s) and are independent of THA's anti-cholinesterase activity. The potassium blocking action of THA resembles the action of acetylcholine on "m" currents and appears to account for the excitatory action of THA in both CA_1 and CA_3 pyramidal neurons. The qualitative differences in the effects of THA on CA_1 and CA_3 pyramidal neurons may result from intrinsic properties of these two populations of neurons.

This potassium current blockade may contribute to the reported efficacy of THA in improving cognitive function of patients suffering from Alzheimer's disease.

This research was supported by an NRSA fellowship to DRS and NIA grant AG 00096.

147.15 CARBACHOL BLOCKS A K^+ LEAK CHANNEL IN HIPPOCAMPAL NEURONS. R.D. Blitzer, D.M. Benson, and E.M. Landau. Depts. of Psychiatry, Mt. Sinai School of Medicine and Bronx V.A. Medical Center, Bronx, NY 10468.

Carbachol (CCh) depolarizes hippocampal pyramidal cells while increasing their membrane resistance. It is known that CCh blocks two voltage-dependent K^+ channels, I_{AHP} and I_m. In addition, we have previously shown (Landau *et al*, *Soc. Neurosci. Abstr.*, 11:572, 1986) that CCh blocks an additional, voltage-insensitive K^+ current (a "leak" channel). We now provide further evidence for the K^+- dependence of the CCh effect, and investigate the pharmacology of the "leak" channel.

Slices of guinea pig hippocampus were maintained in a submerged tissue chamber at room temperature, and CA1 neurons were impaled with 3M KCl-filled electrodes. For voltage-clamp experiments, a single-electrode clamping amplifier was used (Axoclamp-2, switching freq. = 2-3 kHz). CCh effects on the I-V relationship of voltage-clamped cells were determined by measuring the instantaneous membrane currents resulting from 3-sec voltage steps. Changing the K^+ concentration in the bath predictably altered the reversal potential of the CCh effect. In 5 mM K^+, 10 μM CCh decreased membrane conductance, resulting in a crossover at -91 ± 9.4 mV (n=12). In 25 mM K^+, the crossover shifted to -49 ± 3.4 mV (n=6). These values were similar to those seen with 10 μM serotonin (-84 ± 8.4 mV (n=10), and -46 ± 2.7 mV (n=6), respectively). However, in some cells (n=3), CCh induced an inward current without an I-V crossover.

We studied the effects of various K^+ channel blockers on the depolarization or inward current produced by 10 μM CCh (ΔV = 9.7 ± 4.6 mV, n=18; ΔI = 90 ± 60 pA, n=12). 2M tetraethylammonium, in the electrode, blocked the depolarizing effect of CCh (ΔV = -2 ± 1.9 mV, n=9). Prolonged exposure to 4 mM Ba^{+2} (applied in the bath) also blocked the CCh effect (ΔV = 0 mV, n=1; ΔI = 2.5 ± 5 pA, n=4). Neither 4-aminopyridine (in the bath) nor cesium (in the electrode) had any effect on the depolarization induced by CCh. We conclude that CCh exerts its effects by blocking 3 different K^+ channels, including a voltage-insensitive "leak" channel (I_L) which is blocked by TEA and Ba^{+2}.

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147.16 TIME-DEPENDENT INWARD RECTIFICATION IN RAT SUBSTANTIA NIGRA COMPACTA NEURONS: VOLTAGE-CLAMP ANALYSIS. M.G. Lacey,* and R.A. North. (SPON: R.A. Baird) Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from neurons in the substantia nigra zona compacta in a rat brain slice maintained at 36°C. Cells fired spontaneous action potentials at rates of 1-5 Hz and were hyperpolarized by dopamine. Membrane currents were measured under voltage clamp at holding potentials of -50 to -60 mV during hyperpolarizing steps to between -60 and -120 mV.

Hyperpolarizing steps evoked a slowly developing inward current; its amplitude was larger for greater hyperpolarizations, reaching five times the value of the instantaneous or 'leak' current. This current took between 0.5 and 20 s to reach a steady-state value; the time course was faster at more negative potentials. Superfusion with Cs^{++} (1-10 mM) markedly reduced this current, rendering the steady-state membrane current similar to the instantaneous current. This current was unaffected by addition of Mg^{++} (10 mM), Co^{++} (1 mM), Cd^{++} (0.3 mM), Ni^{++} (0.3 mM), 4-aminopyridine (1 mM) or TTX (1 μM) to the superfusate.

A further membrane current was observed at the termination of hyperpolarizing commands to potentials more negative than -65 mV. This was an outward tail current which declined within about 20 s and was unaffected by extracellular Cs. This was probably a potassium current as it was reduced by TEA (30 mM) and was negligible during recordings with Cs-filled electrodes. This novel current was also insensitive to Mg^{++} (10 mM), Co^{++} (1 mM), Cd^{++} (0.3 mM), Ni^{++} (0.3 mM), 4-aminopyridine (1 mM) or TTX (1 μM).

The Cs-sensitive inward current activated by hyperpolarization from resting levels is similar to that variously called I_h, I_f/I_{Kz} or I_Q in other cells. An additional smaller outward current, probably carried by potassium ions, is inactivated by hyperpolarization and rapidly activated by repolarization, complicating the study of the Cs-sensitive inward current. The activation of the potassium current at the termination of a hyperpolarization produces a slowing of action potential discharge lasting several seconds.

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147.17 ENHANCEMENT OF ANOMALOUS RECTIFICATION IN RAT LOCUS COERULEUS NEURONS BY ETHANOL. S. A. Shefner and S. S. Osmanovic. Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

Intracellular recordings were made from rat locus coeruleus (LC) neurons in a totally submerged brain slice preparation. Voltage-current (V/I) curves were constructed by plotting the amplitude of electrotonic potentials elicited by hyperpolarizing current pulses as a function of current amplitude. V/I curves determined in control artificial cerebrospinal fluid show anomalous rectification (AR) as indicated by a progressive decrease in slope resistance with membrane hyperpolarization. The V/I relation becomes linear at membrane potentials more negative than -105 to -115 mV (transitional zone), with a slope resistance much lower than that observed near the resting membrane potential. An anomalous rectification ratio (AR ratio) was obtained by dividing the slope resistance immediately below the resting potential by the slope resistance from the linear part of the V/I curve below the transitional zone. The AR ratio ranged from 1.5 to 6.6 with a mean of 3.2 $\pm$ 0.2 (S.E.M., n = 30 cells). Raising the external K^+ concentration shifted the V/I curves and transitional zone in the depolarizing direction by 58.5 $\pm$ 2 mV per ten-fold change in K^+ concentration (n = 6) as predicted by the Nernst equation. Both external Ba^{2+} (0.5 - 2 mM) and Cs^+ (0.5 - 3 mM) depressed AR making V/I relations almost linear throughout the whole potential range and increasing slope resistance. When K-acetate recording electrodes were used, AR was present immediately after impalement but completely disappeared by about 1 hour of recording (n = 25).

V/I curves were determined before, during and after washout of ethanol (30-100 mM) applied by superfusion in the bath. In 8 of 12 LC neurons tested, ethanol inhibited spontaneous firing and increased the AR ratio by 41 $\pm$ 9% (mean $\pm$ S.E.M.). These effects were dose-dependent and reversed with washout of ethanol. Percent inhibition of firing in response to ethanol was found to be correlated with the change in AR ratio (correlation coefficient = 0.84, df = 10, significant, $p < .01$).

On the basis of sensitivity to external Ba^{2+} and Cs^+ and its dependence on external K^+, anomalous rectification in LC neurons resembles the classical K^+-specific anomalous rectifier described in marine egg cells and skeletal muscle. Whether ethanol enhancement of AR in LC neurons is functionally related to other inhibitory effects of ethanol on these neurons remains to be determined.

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147.18 LOCALIZATION OF POTASSIUM CHANNELS ON MYELINATED MAMMALIAN AXONS. T.R. Gordon, D.L. Eng*, J.D. Kocsis & S.G. Waxman. Depts. of Neurology, Yale Medical School and V.A. Medical Center, Palo Alto CA 94304.

Recent studies have indicated the presence of several pharmacologically distinct types of potassium conductances on mammalian myelinated axons of both the central and peripheral nervous systems. This study uses intracellular and sucrose gap recording techniques to examine the effects of the potassium channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) on rat sciatic nerve. The resultsyy indicate that 4-AP- and TEA-sensitive channels are localized differently from each other on the axon.

Sciatic nerves from Wistar rats, aged 11 days to 23 weeks, were stimulated with a single supramaximal stimulus in a sucrose gap chamber. Young nerves demonstrated compound action potential broadening followed by a TEA-sensitive postspike positivity (PP) several hundred milliseconds in duration in the presence of 4-AP. These 4-AP effects attenuated markedly with age: there was minimal broadening and no measurable PP from mature nerves. However, a 200 Hz 100 ms duration stimulus train evoked a TEA-sensitive PP in Krebs' solution (NS) in all ages which was of similar waveform and relative amplitude to that of young nerves in the presence of 4-AP. TEA alone had no appreciable effect on action potential waveform after a single shock. Intracellular recordings in the presence of 4-AP showed a prolonged depolarization, from which bursts of action potentials could arise, followed by an afterhyperpolarization (AHP) with a time course similar to that of the PP. All fibers studied in NS alone exhibited the AHP following a stimulus train. In TEA alone a stimulus train lead to a brief AHP but not to the prolonged AHP. The brief AHP was sensitive to 4-AP.

These data confirm the presence of two pharmacologically distinct types of potassium channels on rat sciatic nerve, one sensitive to 4-AP and the other to TEA. The response to 4-AP greatly attenuates with maturation (myelination) suggesting that 4-AP-sensitive channels are primarily represented at internodal regions. The TEA-sensitive channels require a prolonged depolarization for activation and are blocked by TEA in both immature and mature nerves, suggesting that these channels have an appreciable nodal representation. Supported by the VA and the NIH.

147.19 4-AMINOPYRIDINE-SENSITIVE POTASSIUM CHANNELS IN THE NONMYELINATED AXONS OF THE TURTLE OLFACTORY NERVE. D.L. Eng* and J.D. Kocsis. Depts. of Neurology, Stanford and Yale Med. Sch.; and V.A. Med. Ctr. Palo Alto, CA 94303

Recent studies on amphibian and mammalian myelinated axons indicate that 4-aminopyridine-sensitive potassium channels may be important in action potential repolarization, and a tetraethylammonium-sensitive potassium channel, which has a longer time course, may be important in the regulation of repetitive firing. The purpose of this study is to determine the 4-AP and TEA-sensitivity of a different functional class of axons: the nonmyelinated axons of the turtle olfactory nerve (ON). The ON is an ideal experimental preparation for the study of nonmyelinated axons. Pseudemys scripta (carapace 7"-8") were decapitated, and the olfactory nerve dissected into several bundles. A sucrose gap recording method was used to examine the compound action potential and changes in membrane pot-ential of the fine calibre (0.1-0.2 µm) ON axons. Recordings were conducted at room temperature (24°C). A normal compound action potential recorded in the sucrose gap consisted of a monophasic negative wave followed by a negative tail having a duration up to several seconds. 4-AP broadened the compound action potential in a dose-dependent manner (25 µM-100 µM) Higher concentrations of 4-AP lead to conduction block. 4-AP did not lead to a change in membrane potential. TEA (1 mM-10 mM) did not change the compound action potential, the delayed negativity, or membrane potential. CsCl (25 µM to 5mM) also had no effect on the compound action potential or membrane potential. Capsaicin (100 µM) broadened the compound action potential and caused a membrane depolarization. 100 µM 4-AP and 5 mM TEA applied simultaneously, slightly broadened the compound action potential compared to 4-AP applied alone; repetitive stimulation (5-40 Hz for 1 sec.) elicited either a modest or no post-train posit-ivity. When 100 µM 4-AP, 5 mM TEA, and 5 mM CsCl were applied simultaneously, 5-20 Hz stimulation elicited a strophanthidin-sensitive post-train positivity.

These results indicate that 4-AP-sensitive potassium channels are important for action potential repolarization in the turtle olfactory nerve. The prominent TEA-sensitive afterhyperpolarization observed in mammalian myelinated axons is not observed in the turtle ON suggesting a difference in the organization of potassium channels in these axon types.

147.20 GABA-INDUCED DEPOLARIZATION OF MYELINATED RAT DORSAL ROOT AXONS; PHARMACOLOGY AND IONIC MECHANISMS. R.D. Fields, R.B. Bhisitkul* and J.D. Kocsis. Dept. of Neurology, Yale Med. Sch., and VA Med. Center, Palo Alto, CA 94304.

Many axon types in the peripheral nervous system of amphibians and mammals exhibit a depolarization in response to gamma-aminobutyric acid (GABA). Moreover, this response is seen in in vitro preparations of rat spinal roots where fibers are isolated from the cell body and synaptic terminals, thus suggesting the presence of receptors for GABA on the axonal membrane at asynaptic regions. Axonal GABA receptors are selectively localized on sensory fibers, as opposed to motor fibers, in rat spinal roots. In an effort to characterize this axonal GABA receptor and the ionic mechanisms of the depolarization, we have studied the effects of GABA agonists on rat dorsal roots in isolated preparations using a variety of extracellular recording methods including the sucrose gap technique, and intra-axonal recording and stimulation. Our results show a rapid depolarization of approximately 8 mV upon application of 400 µM GABA or 200 µM muscimol, which is accompanied by a reduction in input resistance of about 15%. Changes in membrane conductance were measured by intracellular current injection through the recording microelectrode equipped with a bridge circuit, and by extracellular measurements of rheobase and propagation of electrotonic potentials. The GABA-induced depolarization could be blocked by the $GABA_A$ receptor antagonist bicuculline methiodide (200 µM), but was not affected by the $GABA_B$ receptor agonist baclofen (200 µM). In addition, the GABA effects were potentiated by 200 µM diazepam, further suggesting that these peripheral axonal receptors resemble the $GABA_A$ receptor described in the central nervous system. Furosemide, a Cl^- pump inhibitor, or Zn^{2+}, which blocks Cl^- channels in some systems, both prevent the GABA-induced depolarization of dorsal roots, suggesting that the increased membrane conductance may result from the activation of Cl^- selective channels. This interpretation is supported by measurements showing a relationship to the chloride equilibrium potential and ionic substitution experiments which are being pursued currently.

These results suggest that $GABA_A$-like receptors are present on at least some mammalian myelinated sensory axons. The activation of these receptors leads to increased Cl^- conductance with an attendant depolarization if an outwardly directed Cl^- gradient is maintained by a furosemide-sensitive Cl^- pump.
Supported in part by the Medical Research Service of the VA and the NIH.

148.1 SUBTYPES OF MAMMALIAN DORSAL ROOT GANGLION NEURONS IN CELL CULTURE: ELECTROPHYSIOLOGICAL STUDIES USING INTRACELLULAR AND PATCH CLAMP TECHNIQUES. M.J. McLean and P.B. Bennett*, Dept. of Neurology and Cardiovasc. Res. Program, Vanderbilt Univ. Med. Ctr., Nashville,TN 37212

Subtypes of dorsal root ganglion neurons (DRGn) are identifiable in intact ganglia in situ by conduction velocity and action potential (AP) waveform. In this study, adult mouse and human ganglia were dissociated to single cells after incubation in enzyme containing solutions. Conventional intracellular electrophysiological recordings were made in phosphate buffer from DRGn plated in collagen-coated dishes and maintained in vitro for up to 5 months prior to experiments. Patch clamp experiments were performed on freshly dissociated neurons. As in intact ganglia, "A-like" neurons (ALN) in vitro fired brief (<1.5 msec duration) APs which were reversibly abolished by tetrodotoxin (TTX, 10^{-8}M) and fired only a few times during long intracellularly-applied current pulses. Whole-cell inward currents (K^+ replaced by Cs^+ in pipette solution) included a large rapidly-inactivating early component abolished by TTX and a small slowly-inactivating component resistant to TTX. Both currents varied with $[Na^+]_o$, but not $[Ca^{++}]_o$. "C-like" neurons had long-duration APs (2-8 msec) which fired in sustained repetitive fashion during long depolarizations and persisted in 30 μM TTX. I_{Na} consisted of a small TTX-sensitive early component and a large slowly-inactivating TTX-resistant component. Both ALN and CLN were quiescent at rest. CLN, but not ALN, depolarized and fired bursts of APs in response to capsaicin, histamine and bradykinin applied to somata by pressure ejection from blunt micropipettes positioned nearby. Similar findings were observed in both mouse and human DRGn. Thus, DRGn in monolayer dissociated cell culture maintain properties of subtypes of DRGn in intact ganglia and can be classified further in vitro by the proportion of two inward Na^+ currents generating the upstrokes of the APs. In addition, CLN in cell culture are sensitive to pain-producing substances which activate nociceptive DRGn in situ. Thus, the DRGn in cell culture provide a useful model for studying properties and effects of chemical substances at the cellular level for comparison with those on subtypes of neurons in animals and man.

148.2 VOLTAGE-DEPENDENT CURRENTS OF MYENTERIC NEURONS IN CELL CULTURE. J.L. FRANKLIN* & A.L. WILLARD. Dept. of Physiology 206H, University of North Carolina, Chapel Hill, NC 27514

Myenteric neurons have diverse electrophysiological properties and are very heterogeneous with respect to their neurotransmitter content and the types of synaptic potentials they cause, both in vivo (North, Neuroscience 7:315; Gershon, A. Rev. Neurosci. 4: 227) and in culture (Nishi & Willard, Neuroscience 16:187; Willard & Nishi, Neuroscience 16:201, 213). We are interested in how functions of enteric neurons are related to their electrophysiological properties and in how those properties can be altered by synaptic activity. As a first step toward that end, we have determined the voltage-dependent currents found in myenteric neurons.

Whole cell patch clamp recordings were made at 20 - 22° C from rat myenteric neurons grown in cell culture for 4-22 days. At least 5 different voltage-dependent currents were detected in all cells tested: 1. A fast TTX-sensitive Na current. 2. A fast "A" type K current. This current rapidly activates (t_{peak}<10 ms) and inactivates ($t_{0.5}$ 10 ms). Like other A currents, it is fully inactivated at holding potentials positive to -55 mV, blocked selectively by millimolar 4-aminopyridine and relatively insensitive to tetraethylammonium (TEA) up to 25 mM. In contrast to many A currents, it is blocked when Ba replaces Ca or when Ca currents are suppressed by Cd or Co. 3. A "delayed rectifier" K current. This current does not inactivate during 175 msec pulses, is sensitive to TEA (IC_{50} 5 mM) and shows no Ca-dependence. 4. A rapidly activating (5 msec) and inactivating ($t_{0.5}$ 12 msec) Ca current. 5. A slower (t_{peak} 15-20 ms) non-inactivating Ca current.

Although all 5 currents appear to be present in all cells, their relative amplitudes vary significantly among different cells. At present, we have no evidence that there are qualitative differences in the currents found in subsets of neurons. Experiments are in progress to determine how the relative amplitudes of the different currents can be altered by synaptic and electrical activity in these neurons. Supported by NIH grant NS24362 and by an Alfred P. Sloan Fellowship to ALW.

148.3 PUMILIOTOXIN-B INCREASES EXCITABILITY AND INDUCES REPETITIVE ACTION POTENTIAL DISCHARGES IN BULLFROG SYMPATHETIC NEURONS. Geoffrey G. Schofield Forrest F. Weight, and Stephen R. Ikeda. Section of Electrophysiology, Lab. of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The alkaloid pumiliotoxin-B (PTX-B), isolated from the skin of the Panamanian frog *Dendrobates pumilio*, has been shown to prolong and potentiate muscle twitches, cause repetitive EPP discharges in response to a single nerve shock, and cause repetitive action potential discharge superimposed on a depolarizing after-potential in response to a single brief depolarization (Rao, K.S. *et al*, Mol. Pharmacol. in press). To characterize the effects of PTX-B on nervous tissue we have investigated the effects of the toxin on bullfrog postganglionic sympathetic neurons using conventional intracellular microelectrode techniques.

Under control conditions, orthodromic or antidromic stimulation, or brief (10 msec) depolarizing current pulses injected into the soma via the recording pipette induced a single large overshooting somal action potential followed by an after-hyperpolarization (AHP). After superfusion with 2 μM PTX-B these stimuli induced either self-sustaining repetitive action potential discharges or bursting pacemaker activity. Prior to the initiation of spontaneous activity a post-AHP depolarization was observed. The amplitude of the post-AHP depolarization was dependent on the stimulation frequency, and appeared to be responsible for the spontaneous action potential discharge. When repetitive activity was induced by orthodromic stimulation it was often associated with bursts of EPSPs whereas antidromic stimulation or intracellular current injection resulted in repetitive activity free of these EPSP bursts.

PTX-B-induced repetitive action potential discharges were not dependent on external Ca^{2+} as they persisted in Ca^{2+}-free or Cd^{2+} (0.5 mM) containing solutions. However, superfusion with Na^+-free or TTX (3 μM) containing solutions resulted in a cessation of repetitive activity which returned on reapplication of Na^+ or washout of TTX.

We conclude that PTX-B induces a use-dependent increase in neuronal excitability. Voltage-clamp experiments are being conducted to elucidate the mechanism of action of PTX-B on sympathetic ganglion neurons.

We would like to thank Dr. E. X. Albuquerque for providing the toxin and for helpful discussions during the course of these experiments.

148.4 ELECTROPHYSIOLOGY OF ISOLATED NEURONS FROM BULLFROG INTRACARDIAC GANGLION. A. Tse*, R.B. Clark* and W. Giles*, (SPON: J. Remmers). Dept. of Physiology, Faculty of Medicine, University of Calgary, Calgary, Canada T2N 4N1.

Neuronal cell bodies from the parasympathetic ganglia of the interatrial septum of bullfrog (Rana catesbeiana) heart were isolated using an enzymatic digestion procedure. The vagus nerve trunk was separated from the septum, cut into 1 - 2 mm lengths, and incubated in Ca^{2+}-Ringer containing 0.2% Collagenase and 0.1% Elastase for 0.5 hr at 30°C. Cell bodies, released by triturating the segments of nerve trunk through a fire-polished Pasteur pipette, were maintained in tissue culture medium (50% L - 15) for 24 hrs before use.

Isolated cell bodies were spherical or slightly ellipsoidal, with diameters of 20 - 25 μm. Electrophysiological recordings were made at 20° C using the "gigaseal" method. Resting membrane potential (r.p.) was -49.5 mV (S.D., 4.9; n = 6). Injection of current pulses (1 ms, 2 - 3 nA) evoked action potentials with the following characteristics: threshold, -31.5 ± 3.5 mV; maximum overshoot, +40.6 ± 6.5 mV; maximum after-hyperpolarization, -74.1 ± 3.2 mV; duration (at 0 mV), 2.6 ± 0.3 ms; maximum rate-of-rise, 85 ± 26 V/sec. The after-hyperpolarization decayed with a time constant of 100 - 150 ms; this slow decay arose from the large input resistance (0.5 - 2 Gohms) of the neurons negative to r.p. Injection of hyperpolarizing current pulses (1 ms, 1 nA) produced electrotonic decays with a similar time course. Cell capacitance was 50 - 65 pFd.

Preliminary voltage clamp experiments identified a rapid TTX-sensitive inward current, a slower Cd^{2+}-sensitive inward current, and at least two components of outward current. The reversal potential of the outward currents, estimated from current tails was -75.0 ± 1.2 mV (n = 3) in 2.5 mM K^+ Ringers, not significantly different from the maximum after-hyperpolarization of the action potential. Outward current tails decayed rapidly in the membrane potential range -50 to -70 mV: in 3 cells the time constant was 5.6 ± 2.0 ms at -50 mV, 4.5 ± 1.7 ms at -60 mV, and 3.6 ± 2.2 ms at -70 mV. Cd^{2+}-containing (0.3 mM) Ringers abolished 50 - 90% of the outward current, implying that most of the outward current in these isolated neurons is generated by a Ca^{2+}-dependent K^+-conductance.

Supported by an A.H.F.M.R. grant to W. Giles.

148.5 DIRECT EFFECTS OF THE LINCOSAMIDE ANTIBIOTIC, CLINDAMYCIN, ON SYMPATHETIC GANGLION CELLS. L.M. Konopka* and R.L. Parsons. (SPON: S. Freedman). Department of Anatomy and Neurobiology, College of Medicine, The University of Vermont, Burlington, Vermont 05405.

The lincosamides are one of the most commonly used types of antibiotics. One side effect of these compounds is interference with cholinergic neurotransmission, especially well documented at the neuromuscular junction but also occurring at autonomic ganglia. However, only limited information is available concerning possible direct effects of these agents on the general properties of neurons. Therefore, the present study was undertaken to identify possible direct actions of one lincosamide, clindamycin, on the properties of sympathetic ganglion cells and to compare the concentration dependence of direct effects on ganglion cell properties to the development of ganglionic blockade. All experiments were done in vitro using sympathetic ganglion B cells of the bullfrog, Rana catesbeiana, maintained in a HEPES buffered solution at 20-23°C.

We found that clindamycin produced a number of concentration and time dependent alterations in the properties of individual ganglion cells. Many ganglion cells were depolarized during either bath application (1×10^{-5} - 5×10^{-4} M) or local "puffer" application of clindamycin. The depolarization could be induced after pretreatment with either atropine or curare indicating that it was not mediated by activation of either nicotinic or muscarinic receptors. Clindamycin also produced a decrease in the rate of rise and fall and a decrease of the overshoot of directly stimulated action potentials. Further, in the presence of clindamycin the hyperpolarizing afterpotential (HAP) of the action potential was dramatically shortened. The change in the HAP duration by clindamycin appeared to be due to a selective decrease of the slow component of the HAP. In addition, the alteration in the HAP occurred with concentrations lower than those required either to alter the other action potential parameters or interfere with ganglionic transmission.
Supported by PHS RO1-23978.

148.6 4-AMINOPYRIDINE EFFECTS ON FAST CONDUCTING AND MIDDLE CONDUCTING MYELINATED OPTIC TRACT AXONS: IN VIVO STUDY. D.-Y. Ruan*, D. Impelman and D. A. Fox. College of Optometry, University of Houston, Houston, TX 77004.

Recent in vitro studies in rat optic nerve demonstrate that the 4-aminopyridine (4-AP) sensitive potassium channel plays a primary role in action potential repolarization. 4-AP, but not tetraethylammonium (TEA), causes a marked broadening of the compound action potential (CAP) response followed by a postspike positivity (Kocsis et al., Brain Res 383: 357, 1986). Due to the short conduction distance, however, only a single monophasic negative component of the response is present in the recordings. In order to examine the possible differential effects of 4-AP on large diameter, fast conducting t1 optic tract (OT) axons and medium diameter, middle conducting t2 OT axons we utilized an in vivo preparation in 90 day old female Long-Evans hooded rats. CAP recordings were made from distal OT while stimulating the optic chiasm. Time course studies (0-60 min) of response amplitudes, peak latencies, rise times, and CAP duration were determined from single-shock experiments before and after injection of 5 µL of 1 mM 4-AP into distal OT. Rheobase, chronaxie, frequency following, relative refractory periods (RRPs), and percent amplitude recovery were determined from single-shock, paired-shock and train stimulation experiments before and after stabilization (1-2 hours) of the 4-AP response. Time course studies reveal that initially (1-5 min) most t1 and t2 measures are similarly affected by 4-AP. Response amplitude is decreased, peak latency and rise time are increased while t1 CAP duration is markedly increased compared to t2. 20-60 min following 4-AP similar results are observed for t1 and t2 except that during this time period t1 amplitude is partially recovered resulting in a larger amplitude decrease in t2 than t1. Additional studies show that 4-AP markedly increases t1 and t2 RRP, rheobase and chronaxie with the latter exhibiting greater effects in t2 than t1. No effect on absolute percent amplitude recovery is observed for t1 or t2. Furthermore, after 4-AP frequency following is equally decreased in t1 and t2 up to 300 Hz while from 300-700 Hz t2 exhibits a slightly greater decrease. These results suggest that the amplitude decrease and waveform broadening observed by Kocsis et al. primarily result from effects on t2 and t1, respectively. In addition, these data demonstrate that 4-AP produces differential effects on the firing characteristics of t1 and t2 OT axons as assessed by paired-pulse and train stimulation. The single pulse effects on amplitude, duration, rheobase and chronaxie are probably due to prolonged membrane depolarization while the paired-pulse recovery function and frequency following effects are most likely associated with the postspike positivity (i.e., intracellular hyperpolarization).
Supported by NIEHS Grant ES 03183 (DAF).

148.7 **IONIC CURRENTS IN ACUTELY DISPERSED CELLS FROM THE PINEAL GLAND OF THE ADULT RAT.** Luis G. Aguayo* and Forrest F. Weight. Section of Electrophysiology, Laboratory of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md. 20852.

Ionic currents of pineal cells were studied using the whole-cell variation of the patch-clamp recording technique. Pineal glands were removed approximately 2 1/2 hrs after light onset from male Sprague-Dawley rats (200-300 g) kept under LD 12:12 with lights on from 6 A.M. to 6 P.M. Single cells were acutely separated with enzymatic and mechanical dispersion techniques. The macroscopic ionic current observed in control external solution (mM: 150 NaCl; 5.4 KCl; 2 $CaCl_2$. 1 $MgCl_2$; and 10 HEPES; pH 7.4) and internal solution (mM: 130 KCl; 1 $CaCl_2$; 2 $MgCl_2$; 10 HEPES; and 11 EGTA; pH 7.35) was dominated by an outward current with little or no apparent inward current. Membrane currents evoked from holding potentials of -50 and -80 mV showed distinctive features with the one from -80 mV having the fastest rising phase and the largest amplitude. Currents obtained in the presence of 1 µM TTX were similar to those obtained in control solutions.

Study of the outward current in external solution without added Ca^{2+} revealed the existence of two distinct outward currents. Depolarizations from a holding potential of -100 mV activated a fast current which reached a peak within 15 ms (at command potentials positive to -20 mV) and decayed completely in about 150 ms. The time to peak of this transient current decreased linearly with the command potential, being 25 and 10 ms at -40 and -5 mV, respectively. This current was activated at potentials more positive than -50 mV and displayed steady-state inactivation at depolarizing voltages with half-inactivation near -80 mV. The second outward current component isolated from a holding potential of -50 mV was activated at potentials positive to -20 mV, reached a steady-state current amplitude within 50 ms and was sustained up to 400 ms. In the presence of 2 mM external Ca^{2+} the I-V relationship for the outward current obtained from -50 mV did not display a region of negative slope conductance (N-shape) and this may suggest that a Ca^{2+}-activated K^+ current did not contribute significantly to the outward current.

We conclude that acutely dissociated pineal cells dispersed at the begining of the light period display two distinct K^+ currents: (i) a transient current similar to the A current (I_A); and (ii) a slowly activating, sustained current similar to the delayed rectifier current (I_K). Under the conditions of our experiments, Ca^{2+}-dependent currents appear to contribute only a small fraction of the total membrane current.

148.8 ELECTROPHYSIOLOGY OF GUINEA PIG MAMMILLARY BODIES *IN VITRO*. A. Alonso* and R. Llinás (SPON: V. Decrescito). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Intracellular recordings were obtained from both medial and lateral mammillary neurons in guinea pig brain slices. The cells were grouped into two categories according to electrophysiological criteria. Those in the first category were characterized by a high input resistance ranging from 100 to 200 megohms, by the presence of a powerful A-conductance, and by dendritic calcium spiking following blockage of potassium conductance by tetraethylammonium (10 mM). These cells also demonstrated a clear calcium-dependent potassium conductance and, in addition to fast TTX-sensitive sodium-dependent spikes, a slow non-inactivating sodium conductance which could generate long-lasting plateau potentials which were also TTX-sensitive (tetrodotoxin, 10^{-6} gm/ml). Upon electrical stimulation of the peri-mammillary region with a fine bipolar metal electrode, these neurons showed short-latency excitatory potentials followed by inhibitory potentials. At high stimulus strength a large prolonged secondary IPSP was also recorded.

The second type of cell was characterized by the presence of a low-threshold calcium spike and, following replacement of extracellular calcium with barium, by the presence of TTX-insensitive high threshold spikes. These neurons demonstrated only an early synaptic excitation following peri-mammillary stimulation. These two cell types were also distinguished after intracellular HRP injection. Those in the first category were usually fusiform, had medium-sized somata, and thick spiny dendrites. Those in the second group were smaller, with thin and sparsely spined dendrites.

Because the mammillary neurons project massively to the anterior nuclei of the thalamus (Powell & Cowan, J. Anat. 88: 489, 1954), their intrinsic electroresponsiveness may be central in the relaying of rhythmic synaptic input to these nuclei. In particular, the medial septum projects to the mammillary bodies (Swanson & Cowan, J. Comp. Neurol. 186: 621, 1979), and it is known to be a pacemaker for the theta rhythm (Gogolak et al., Brain Res. 7: 201, 1968). Thus, the possibility that the mammillo-thalamic pathway may be important in the propagation of the theta rhythm to the thalamus and cingulate cortex is to be emphasized. [Research supported by NS13742 from NINCDS].

148.9 **THE REGULATION OF ACTIVITY IN MIDBRAIN DOPAMINE NEURONS: ANALYSIS USING IN VITRO INTRACELLULAR RECORDINGS.** AA Grace Departments of Behavioral Neuroscience & Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Intracellular recordings were made from identified dopamine (DA) neurons of the substantia nigra (SN) and ventral tegmentum (VTA) of rat midbrain maintained in vitro. Intracellular staining with Lucifer yellow and HRP revealed three DA cell morphologies: In the SN, dorsal DA neuron somata were fusiform in shape with dendrites oriented along the compacta, whereas the more ventral multipolar DA cells had dendrites extending ventrally as well. In the VTA, dendrites of multipolar DA neurons were oriented in a radial pattern. The dendrites had sparse 3-15 um long protrusions and terminated in fork-like processes.

In contrast to the characteristic irregular or burst firing pattern of DA neurons recorded in vivo, DA cells in vitro fired exclusively in pacemaker patterns. The input resistance ranged from 150-350 megohms, or about 5x that found with in vivo recordings. Spontaneous spikes were driven by a sodium-dependent slow depolarization (SD). TTX administration revealed an underlying low threshold calcium spike (LTS, Llinas & Yarom, 1981) which, although inactivated at resting potentials, could be activated by depolarization from a hyperpolarized state. Thus, the LTS triggered by hyperpolarizing pulse rebound often led to spike discharge. However, larger hyperpolarizing pulses also activated a potassium current (I_A) which slowed the membrane repolarization, thereby blocking the rebound LTS and spike firing. A second calcium spike, the high threshold spike (HTS), was only observed after tetraethylammonium (TEA) administration, suggesting that HTSs are probably of dendritic origin (Llinas et al, 1984). Both action potentials and HTSs triggered afterhyperpolarizations (AHP), which also exhibited voltage-dependent properties.

Thus, DA cells have active currents similar to those of other mammalian neurons, with two exceptions: 1) the presence of prominent SDs driving spontaneous activity, and 2) the blockade of the LTS by strong hyperpolarizing pulses. Other cells with prominent LTSs, upon hyperpolarization, show decreased spontaneous activity coupled with enhanced excitation to depolarization (i.e., a greater "signal/noise" ratio). However, DA cell activity is modulated in an opposite manner to alterations in membrane potential. Thus, spontaneously firing DA neurons exhibit properties which serve to maintain ongoing spike activity (i.e., the LTS triggered from the rebound of a spike AHP would, in turn, activate the SD and subsequent spike), whereas hyperpolarized DA neurons are capable of maintaining their inactive state (i.e., decreased excitatory drive due to the strong anomalous rectification, coupled with attenuation of the LTS by I_A during depolarization). These factors could contribute to the bimodal distribution of DA cell activity. Thus, about 1/3 of DA cells are not spontaneously active in the absence of demand (Grace & Bunney, 1986); whereas spontaneously active DA cells fire at rates distributed normally about 4.6 Hz, with cells rarely firing at rates below 2 Hz. (Supported by USPHS MH42217 & MH30915)

148.10 THE CALCIUM CURRENT AND THE GLUTAMATE RESPONSES IN ISOLATED, IDENTIFIED SPINAL DORSAL HORN PROJECTION NEURONS. L.-Y.M. Huang. Marine Biomedical Institute and Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, TX 77550.

Spinothalamic and trigeminothalamic neurons in the spinal cord play important roles in transmitting information related to noxious stimuli. The understanding of the electrical properties of these cells and their responses to various amino acids will provide us with important information about how pain is mediated by the nervous system. We have reported the procedures to isolate and identify these cells and studied the voltage-dependent properties of Na,K channels (Soc. Neurosci. 12:1147, 1987). The properties of Ca currents and the responses to amino acid glutamate are described here. Slow inward current was isolated by blocking I_{Na} with 2 μM tetrodotoxin and inhibiting I_K by replacing intracellular K^+ with Cs^+. Ba or Ca was used as the current carrier in these experiments. The predominately slow inward current started to activate at -25 mV and did not inactivate substantially at the end of 100 msec depolarizing pulse. A second component of Ca current, which activated at more hyperpolarized potentials and inactivated relatively rapidly, was also observed. $CdCl_2$ was able to block, to different degrees, both types of these slow inward currents. These currents have many of the characteristics of the inward long lasting and transient Ca current observed in other preparations. Most of the neurons tested were sensitive to L-glutamate. The current voltage curve of the glutamate responses showed a slight rectification as the membrane was depolarized. The reversal potential of responses was closed to 0 mV. This response was blocked by the presence of 1 mM $MgCl_2$ similar to the N-methyl-D-aspartate (NMDA) seen in other neurons. Supported by NIH NS23061 and NS01050.

148.11 ELECTROPHYSIOLOGICAL PROPERTIES OF LATERAL DORSAL TEGMENTAL NEURONS IN VITRO. K.S. Wilcox, S.J. Grant#, G.R. Christoph. Neuroscience Section, E.I. duPont de Nemours and Company, Inc., Wilmington, DE 19898 and #Dept. Psychology, University of Delaware, Newark, DE 19716.

The lateral dorsal tegmental nucleus (LDT) is the origin of ascending cholinergic projections innervating numerous forebrain structures such as prefrontal cortex, habenula, and thalamus. Electrical stimulation of the LDT in vivo suppresses burst firing in the nucleus reticularis of the thalamus, suggesting that the LDT may affect sensory gating processes in the thalamus (Kayama et al. J. Neurophys. 56: 1310-1320, 1986). We investigated the electroresponsive characteristics of LDT neurons with standard intracellular recording techniques in a guinea pig brain slice preparation maintained in vitro. Microelectrodes were positioned with respect to visual landmarks in a region of the LDT which contains a high density of cholinergic perikarya. This region was determined by an enzymatic NADPH diaphorase stain (Vincent et al., Neurosci. Lett. 43: 31-36, 1983) in coronal sections through the LDT of other guinea pigs. LDT neurons included in the study all had membrane potentials greater than -55 mV and input resistances greater than 50 megohms. Most neurons fired action potentials in two modes: tonically and in bursts. With a background membrane potential less than -65 mV, depolarizing current pulses elicited TTX-sensitive, tonically firing action potentials, with frequency directly proportional to current intensity. At membrane potentials greater than -65mV, depolarization evoked a slow, TTX-insensitive wave of depolarization which served as a generator potential for a high frequency burst of fast spikes. These voltage-dependent characteristics suggest that, as in olivary, thalamic, and habenular neurons, activation of a low-threshold calcium conductance ($LT_{Ca(g)}$) underlies generation of burst activity in the LDT. Furthermore, the presence of $LT_{Ca(g)}$ in LDT neurons and the possibility that at least some of these neurons recorded from were cholinergic suggests that slight hyperpolarization of the membrane potential may serve to amplify responses to excitatory input and thereby enhance the release of acetylcholine in target regions.

148.12 **WHOLE CELL PATCH CLAMP RECORDING FROM DISSOCIATED CULTURES OF HYPOTHALAMIC NEURONS.** R.E. Petroski*, R. Ventimiglia, & H.M. Geller (SPON: M.T. Spoerlein). Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854.

We have developed methods for growing hypothalamic neurons in dissociated cell culture at low density. Neurons from these cultures have been shown to express tyrosine hydroxylase, several hypothalamic peptides, as well as β-adrenergic and GABA receptors. Because of their small size and precarious location, it has been difficult to perform voltage clamp analysis on hypothalamic neurons *in vivo* or in the *in vitro* slice or explant preparations. Our culture system permits the growth of isolated hypothalamic neurons, 10-25 μm in diameter, suitable for voltage clamp analysis by the whole cell patch clamp technique.

Hypothalami are dissected from E17 rat brains and plated on coverslips containing a monolayer of cortical astrocytes. Patch electrodes of 3-7 MΩ are used to obtain tight seals of 10-50 GΩ. Voltage clamp recording from neurons 3 to 12 days *in vitro* have been performed. Neurons exhibit typical resting membrane potentials of -55mV and input resistances of 200 to 1000 MΩ.

All neurons contain a delayed voltage-dependent outward current. This is blocked by addition of 30mM TEA as well as using Cs^+ as the major cation in the electrode. We have identified a voltage dependent inward current, which is blocked by 1μm TTX. The amplitude of this current in different neurons varies from 1000 pA to less than 100 pA. This does not appear to be a function of development as very young neurons may contain a robust inward current while some older neurons have shown no inward current. Many neurons exhibit a transient outward current immediately following the inward current. This current is facilitated by holding potentials of -80mV and is abolished at -40mV in some neurons. The I-V curve for this current displays the typical "N" shape of the A-current. In other cells, the transient outward current is not affected by resting membrane potential and the I-V curves run parallel to the delayed rectifier. This may be a Ca^{2+} dependent K^+ current.

These neurons do not easily lend themselves to categorization by the types of ion channels they exhibit. Rather, different neurons appear to display quantitative differences in the repertoire of currents they express. Continuing work on the development and pharmacology of these ion channels will be presented. (Supported by N.I.H. NS-19187).

148.13 SYNCHRONOUS ACTIVITY IN LOCUS COERULEUS NEURONS FROM NEONATAL RATS. M.J. Christie,* J.T. Williams and R.A. North. Vollum Institute, Oregon Health Sciences University, Portland, OR, 97201

Locus coeruleus (LC) neurons in brain slices from young rats display rhythmic variations in membrane potential, 5-15 mV in amplitude at a frequency of 0.3-3.0 Hz. These are TTX insensitive, dependent on extracellular calcium and appear to originate in the cellular dendrites (K.C. Marshall and J.T. Williams, Soc. Neurosci. Abstr. 12, 1390P, 1986). In the present study, simultaneous intracellular recordings were made from 59 pairs of LC neurons in submerged slices (300 - 400 μm, 37°C) prepared from young rats (1 day - 6 weeks). Cells impaled were separated by 50 - 300 μm. The rhythmic potentials increased in frequency and declined in amplitude with age, and became undetectable after 27 days. At ages less than 24 days, rhythmic activity was always synchronous between neurons in the same LC (N = 48). Pairs of neurons in opposite LC nuclei did not show this synchrony (N = 3). Spontaneous action potentials usually arose from the peak of the depolarizing phase of the rhythmic potentials, but were often asynchronous between pairs. From 24 to 27 days rhythmic activity, when present, was only partially synchronous (N = 8), but full synchrony could be restored by superfusion with tetraethylammonium (TEA) (10 mM) and 2 mM $BaCl_2$ (2 mM). At 40 days no synchronous rhythmic activity could be observed (3 pairs). Weak electrotonic coupling was found between 8 of 15 pairs of neurons less than 10 days old, but in 0 of 3 pairs greater than 10 days old: in the presence of TTX (600 nM), TEA (20 mM) and $MgCl_2$ (11.5 mM), hyperpolarizations (100 mV, 200 ms) evoked by direct current in one cell produced very small, slower hyperpolarizations (0.3-2 mV) in a second cell 50-150 μm away. These results demonstrate that TTX insensitive rhythmic activity is synchronous throughout the LC of neonatal rats. This synchrony declines with age, at least in slice preparations. Entrainment of rhythmic activity might be due in part to electrotonic coupling between neonatal LC neurons.

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148.14 INTRACELLULAR RECORDINGS FROM NORADRENERGIC LOCUS COERULEUS NEURONS IN UNRESTRAINED CATS: ELECTROPHYSIOLOGICAL CHARACTERIZATION DURING SLEEP. A. Hosseini and M.E. Trulson. Dept. Anat., Coll. Med., Texas A&M University, College Station, TX 77843.

The electrophysiological properties of noradrenergic (NE) locus coeruleus (LC) neurons have been characterized in anesthetized rats (Brain Res., 273, 1983, 237). LC neurons exhibit a pronounced post-action potential hyperpolarization followed by a slow interspike depolarization. This gradual depolarization continues to the next spontaneous spike. This pattern of activity appears to account for the automaticity in the discharge rate of LC neurons. It is well known that anesthetics dramatically alter the membrane properties of neurons (Trends Pharmacol. Sci., 5, 1984, 287). Furthermore, it is not possible to examine the membrane properties of neurons during behavior when the animal is anesthetized. Therefore, we developed a technique for recording the intracellular activity of single LC neurons in unrestrained cats. A plate designed to securely hold the slave cylinder of a Trent Wells motorized microdrive was cemented to the cat's skull under pentobarbital anesthesia. After complete recovery from the anesthesia the slave cylinder was attached to the baseplate. The electrode was a 2 mm (o.d.) Omega Dot glass tube beveled by the thick slurry method of Lederer et al. (Pflugers Arch. 381, 1979, 287) with an impedance of 15-30 mΩ and filled with 1 M potassium acetate. Six insect pins were positioned 3 mm apart in a hexagonal fashion around the LC to minimize pulsations. LC neurons were impaled by the use of a 2 msec 20 V DC pulse which was passed through the breakaway box of a M707 WP amplifier. Only intracellular recordings of neurons with stable spontaneous discharge rates for a given behavioral state (Rasmussen et al., Brain Res. 371, 1986, 324) and action potentials of >50 mV were studied. LC cells showed the slow (1.27 ± 0.10 spikes/sec during quiet waking), rhythmic discharge pattern previously described. A hyperpolarization of 4-5 mV occurred after each action potential during quiet waking, and there was a gradual ramp of depolarization preceding each spike. As the cat entered slow wave sleep the overall activity of LC units decreased significantly to 0.2 spikes/sec. During this phase of sleep, the hyperpolarization following an action potential was greater (12 - 14 mV) than during quiet waking periods. When the animal entered REM sleep, LC neurons became virtually silent (0.02 spikes/sec). The hyperpolarization following a spike during REM sleep was about the same (14 - 15 mV) and remained constant for prolonged periods of time. During the few seconds immediately preceding another spike during sleep, there was a rapid depolarization to 6 - 8 mV, and then a slow ramp of polarization immediately preceding the spike.

AUDITORY SYSTEM V

149.1 **CALMODULIN INHIBITORS BLOCK ADAPTATION IN VESTIBULAR HAIR CELLS** D.P.Corey, W.J.Smith*, B.A.Barres and W.J.Koroshetz, Neuroscience Group, Howard Hughes Medical Institute, and Department of Neurology, Massachusetts General Hospital; Program in Neuroscience, Harvard Medical School, Boston, MA

Hair cells in the bullfrog sacculus respond to displacement of their ciliary bundles with the opening of ion channels in the tips of the cilia. There is evidence that these channels respond directly to mechanical stress, and that stress is communicated to the channels by fine filamentous links between cilia (Hudspeth, 1982; Corey and Hudspeth, 1983; Pickles, *et al.*, 1984). In response to a maintained positive displacement of the ciliary bundle, channels open, but then spontaneously close over several tens of milliseconds; the process serves as an adaptation mechanism for hair cells (Eatock, Corey and Hudspeth, *in press*). At the termination of the stimulus, channels close, but then some reopen during the recovery from adaptation.

If the mechanical model for transduction is correct, this adaptation must correspond to a relaxation of the stress applied to the channel, either through a reduction in the spring constant of the link, or through a change in the relative attachment points. Recovery from positive adaptation must correspond to a tensioning process, that reestablishes stress on the channel protein. Changes in bundle stiffness occurring over the same timecourse suggest a similar model (Howard and Hudspeth, 1986).

What molecular constituents of the transduction mechanism mediate the adaptation? It is known that adaptation is accelerated by increased calcium in the endolymph, possibly acting at an intracellular site. We have used the *in vitro* microphonic preparation of the bullfrog sacculus to explore the site of calcium action.

Inhibitors of calmodulin substantially decreased the adaptation rate, at concentrations similar to those required to inhibit calmodulin. The adaptation rate was decreased to less than half the control rate by trifluoperazine (at 50 μM), by W-7 but not the inactive analog W-5 (both at 10 μM), and by calmidizolium (at 5 μM). Higher concentrations almost completely abolished adaptation, but also caused a reduction in total receptor current. All the calmodulin inhibitors also shifted the resting position of the displacement-response curve (DRC) towards negative displacements--corresponding to increased channel activation and thus to increased tension. Lowering endolymphatic calcium produced the same effect as calmodulin inhibitors on both the adaptation rates and on the resting position of the DRC.

Calmodulin in dissociated bullfrog hair cells was localized with a sheep antiserum to calmodulin and a secondary antibody coupled to glucose oxidase. Calmodulin-like immunoreactivity was distributed throughout the cytoplasm and cuticular plate, as in cochlear hair cells (Flock, *et al.*, 1986), but was conspicuously absent from the ciliary bundles. Thus calcium's action on the adaptation process may occur via calmodulin in the cuticular plate, or it may occur through an unidentified calcium binding protein, possibly within the bundle. Calmodulin inhibitors are not particularly specific, and could be inhibiting a calcium-binding protein other than calmodulin.

149.2 DIRECT EVIDENCE FOR ELECTRICAL TUNING OF CHICK COCHLEAR HAIR CELLS. P.A. Fuchs and M.G. Evans*. Dept. of Physiology, Univ. Colorado Sch. Med., Denver, CO 80262.

Recent studies have suggested that cochlear tuning mechanisms may differ in mammals and lower vertebrates. In birds, direct observation of basilar membrane motion has revealed mechanical tuning similar to that seen in mammals. However other, indirect evidence has raised the possibility of electrical tuning of hair cells as seen in turtles and frogs. We examined this latter possibility by making direct electrical recordings from single hair cells isolated from specific regions of the chick cochlea: the apical-most 200 μm where low frequency sounds (<100 Hz) are encoded ('apical cells'), and 200 μm wide sections ranging 1 to 2 mm from the apical tip ('basal cells') where sound frequencies of 400 to 1000 Hz are encoded. Single tall (inner) cells were isolated from the cochleas of 2-6 week old Leghorns using a combination of enzymatic and mechanical dissociation procedures. Tight-seal whole-cell recordings were made at room temperature (22-25°C).

Apical cells had resting potentials near -74 mV. Injection of depolarizing current elicited action potentials and low frequency (5-20 Hz) voltage oscillations that were smaller in amplitude and sinusoidal in shape. In voltage clamp these cells exhibited inward currents at voltages negative to -70 mV, and both early inward, and larger, slower outward currents in the voltage range -50 to +30 mV. Thus the steady-state I-V relation showed both inward and outward rectification and had an 'N-shape' at positive potentials, indicating outward current dependent on Ca entry. Basal cells had resting potentials near -57 mV, and injection of depolarizing current caused small high frequency (100-250 Hz) voltage oscillations. Action potentials were never seen. In voltage clamp basal cells had little or no inward rectifier current, and depolarization above -50 mV elicited large, rapidly activating outward currents, again resulting in an N-shaped steady-state I-V relation. Net inward current usually was not seen in these cells.

The N-shaped I-V relation, and other experiments showing the calcium dependence and sensitivity to tetra-ethyl ammonium of the dominant outward current, indicated that this was a calcium-activated potassium current. In addition to being 2-3 fold larger, basal cell outward currents also activated 20 times more rapidly than did those of apical cells. Kinetic variations in calcium-activated potassium current are thought to underlie electrical tuning of hair cells in turtles and frogs. Thus the bird cochlea may attain its frequency selectivity through a combination of mechanical and electrical tuning. (Supported by grants NS21454 and NS01007 from the NIH).

149.3 ACTION POTENTIALS AND VOLTAGE OSCILLATIONS IN HAIR CELLS FROM THE ALLIGATOR COCHLEA. M.G. Evans* and P.A. Fuchs. Department of Physiology, Univ. of Colorado Sch. Med., Denver, CO 80262.

We have used the tight-seal whole-cell recording technique to record from hair cells isolated from the apical half of the alligator cochlea. Five alligators (A. mississippiensis), 2 months old and 40-50 cm long were used. After decapitation and removal of the cochlea from the otic capsule, single hair cells were isolated by a combination of enzymatic and mechanical dissociation. Experiments were done at room temperature (22-25°C).

All of the successful recordings were from tall hair cells. Resting potentials ranged from -40 to -80 mV. Depolarizing current injections from the resting potential gave rise to two types of response. These were either single action potentials (spikes) that depolarized the cell to a value between 0 and -25mV or decaying voltage oscillations (rings) whose frequency varied from cell to cell (25-160 Hz). Overall, spiking cells were isolated from segments nearer the apex (low frequency end) of the cochlea than were ringing cells, and they usually had larger resting potentials.

In voltage clamp the spiking cells had transient inward followed by noisy outward currents in response to depolarizing voltage jumps from -70 mV. The inward current was found to be a Na current since it was dependent on external Na and was blocked by tetrodotoxin (100 nM). The major component of the outward current appeared to be a Ca-activated K current since it was dependent on external Ca and was blocked by external Cd (1 mM), tetra-ethyl ammonium (TEA, 20 mM) or by replacing internal K with Cs.

The ringing cells had much smaller inward currents under voltage clamp and often had larger outward currents than the spiking cells. The sensitivity of both inward and outward currents to external Ca and Ba, and of the outward current to external TEA or internal Cs, indicated that Ca influx through Ca channels activated the outward K current.

With the exception of the Na current, the currents recorded in alligator hair cells are similar to those recorded in electrically-tuned hair cells from other species. The ringing and spiking responses are especially similar to those seen in the morphologically similar chick cochlea (see P.A. Fuchs and M.G. Evans, this meeting). Since there is evidence for mechanical tuning in both birds and crocodilians, it is possible that they achieve frequency selectivity through a combination of electrical and mechanical tuning. (Supported by grants NS21454 and NS01007 from the NIH).

149.4 **REGENERATION OF SENSORY HAIR CELLS IN THE COCHLEA OF THE CHICKEN.** J.T. Corwin and D.A. Cotanche. Dept. of Zoology and Békésy Lab. of Neurobiology, University of Hawaii, Honolulu, HI 96822 and Dept. of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425

In birds and mammals the production of sensory hair cells in the cochlea normally ceases before birth, so it has been assumed that any subsequent loss of hair cells must result in a hearing loss that is irreversible. However, hair cells in the ears of lower vertebrates are produced throughout life, and in the lateral line system these cells can be replaced by regeneration. Recent ultrastructural investigations of sound induced damage in birds have produced indirect evidence suggesting that hair cells in the cochlea also can be replaced by regeneration. Here we report that autoradiographic labelling of newly replicated DNA has confirmed the occurence of hair cell regeneration in the mature cochleae of chickens. Damage in the cochlear sensory epithelium was produced by exposing pairs of ten-day-old chickens to pure tone acoustic stimulation at 1.5 kHz and 120 dB SPL for 48 hr. Immediately after exposure one chicken from each pair was overanesthetized and its cochleae were fixed in 1% OsO_4, and prepared for scanning electron microscopy. In this way the location and the extent of the damage produced by the sound exposure were assessed for each experiment. The other bird from each pair received ^{3}H-thymidine delivered continuously for seven days from two osmotic minipumps that were implanted subcutaneously. The time of pump implantation was varied in different experiments: from just prior to the period of sound exposure, to four days after the sound exposure. All implanted chickens were allowed to recover for ten days after the sound exposure, then their cochleae were fixed, sectioned, and processed for autoradiography. Controls chickens received radioactive thymidine but were not exposed to acoustic overstimulation. Scanning EM of cochleae that were fixed immediately after the sound exposure confirmed that the stimulus caused death and extrusion of hair cells from a limited area in the sensory epithelium. Autoradiographic localization of labelled DNA demonstrated that the damage in that region stimulated the mitotic replication of cells remaining in the sensory epithelium. In the region of damage the nuclei of hair cells and supporting cells were both labelled by ^{3}H-thymidine. Supporting cells which normally would be mitotically quiescent after embryogenesis are spared by this level of acoustic trauma, and it appears that those cells can proliferate, giving rise to progeny which can differentiate either as supporting cells or as replacement hair cells. If similar regeneration could be induced in human ears this might allow recovery from sensorineural deafness that has generally been considered irreversible and untreatable. (Supported by NINCDS and the Deafness Research Foundation)

149.5 **A MONOCLONAL ANTIBODY THAT LABELS SENSORY HAIR CELLS AND NEURONS IN AUDITORY, VESTIBULAR, AND LATERAL LINE SYSTEMS.** H.I. Kornblum, B. Trevarrow, and J.T. Corwin. Békésy Lab. of Neurobiology and Dept. of Zoology, Univ. of Hawaii, Honolulu, HI 96822, and Institute of Neuroscience, University of Oregon, Eugene, OR 97403

Investigations of hair cell development and function have been hampered by the lack of a cell-type specific marker. Znl, a monoclonal antibody raised against zebrafish (Brachydanio rerio), has been reported to label a variety of neural cell types in zebrafish larvae, including sensory hair cells in the otic capsule. We report that this antibody also recognizes hair cells and neurons of the inner ear and lateral line system in the urodele amphibian, Ambystoma mexicanum. Immunocytochemical methods labelled hair cells and neurons, but not supporting cells or accessory structures in the sensory epithelia of juveniles and adults. Labelling was cytoplasmic and was not associated with recognizable organelles or filaments. Labelling was also present in processes and cell bodies of neurons in the VIIIth cranial nerve and the lateral line nerves. Immunoreactive processes were also detectable in the dorsolateral medulla oblongata at the approximate location of primary octavolateralis nuclei and coursing transversely through the reticular formation. Immunoreactive cells were detected in other CNS regions as well.

Regeneration experiments were performed to determine if Znl would label newly differentiated hair cells. Juvenile axolotls were anesthetized, and the tip of the tail was amputated. The tail and its lateral line neuromasts regenerated, and were fixed at different times after amputation. Znl labelling was detectable in newly formed neuromasts within two weeks of amputation. This labelling was confined to hair cells. In at least one neuromast labelling was confined to a single newly differentiated hair cell. The intensity of labelling appeared to be correlated with cell age. Staining of hair cells within newly formed neuromasts was less intense than that of cells in mature neuromasts. In the sacculus, hair cells in the growth zone at the edge of the epithelium were lightly labelled, whereas the older hair cells in the center were heavily labelled.

Znl is a useful marker to distinguish hair cells from supporting cells in both mature and developing sensory epithelia of the auditory, vestibular, and lateral line systems. The similar staining in otic and lateral line hair cells affirms the close relationship between these systems. Although the function of the antigen recognized by Znl is unknown, its presence in receptor cells and neurons in the octavolateralis systems indicates that it may have a function specific to these modalities.

(Supported by NIH Awards to HIK, JTC, and C.B. Kimmel)

149.6 DC AND CM GRADIENTS IN THE GUINEA PIG COCHLEA. M. Zidanic*[1,2], W.E. Brownell[2], and P. Dulguerov*[2]. [1]Dept. of Neuroscience, Univ. of Florida, Gainesville, FL.; [2]Dept. of Otolaryngology-HNS, Johns Hopkins University, Baltimore, MD 20205.

We are studying cochlear physiology by characterizing extracellular current pathways in silence and how they are modulated by sound stimulation. Micropipets are advanced along a 600 μm radial track that begins outside the spiral ligament (SL) and extends to a point near the modiolus in either scala tympani (ST) or scala vestibuli (SV) of the second turn. DC and stimulus evoked potential waveforms are signal averaged at each depth in response to low frequency tones. Radial potential gradients are computed by taking the spatial derivative of the field potentials.

Standing currents (or DC gradients) directed into the spiral ligament are present in both ST and SV. The peak of the radial DC gradient appears to be within the SL and has a magnitude of 5-10 mV/mm in ST and 10-15 mV/mm in SV. During the presentation of a low frequency tone burst, these DC gradients are sinusoidally modulated. This is a result of magitude and phase gradients of the cochlear microphonic (CM) that exist in the radial dimension. The fundamental component of the modulation of the DC gradient will be referred to as the CM gradient. The CM gradient is of the same magnitude in SV and ST, about 3-7 mV/mm. However, a phase difference of 180 degrees exists between the CM gradient in SV versus ST, indicating that the standing currents are modulated in opposite directions in ST and SV at a given point in time during the tone burst. This relationship holds for all frequencies and intensities tested, covering a range 50-1500 Hz and 60-90 dB SPL.

An intriguing difference between the potential gradient profiles in SV and ST lies in the relationship of the CM gradient to the DC gradient. In SV, the peak of the CM gradient occurs at the same location in the track as the DC gradient peak. Thus, the standing current that flows into the SL from SV is sometimes reduced by as much as 50%, but never changes direction. However, the peak of the CM gradient in ST occurs at a point 160-200 μm modiolar to the DC gradient peak. In 3 experiments, the CM gradient was 3-5 mV/mm larger than the DC gradient at the location of the CM gradient peak. The CM and DC gradients were of nearly equal magnitudes at the CM gradient peak in 3 other experiments. We hypothesize that these variations in ST potential gradient profiles result from electrode tracks with quite different orientations or distances relative to the basilar membrane. A two-dimensional current density analysis technique will be utilized to test this hypothesis.

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149.7 RESPONSES OF SINGLE AUDITORY NERVE FIBERS IN THE MONGOLIAN GERBIL. K.K. Ohlemiller and S.M. Echteler. Auditory Physiology Laboratory. Department of Neurobiology and Physiology. Northwestern University, Evanston, IL 60201.

Evidence from the cat indicates that the spontaneous firing rate (SR) of auditory nerve fibers is linked to some aspects of their response to tones. Compared to high-SR fibers, low-SR fibers generally exhibit a higher threshold (Liberman, J. Acoust. Soc. Am., 63:442, 1978), a wider dynamic range, and greater firing rate at saturation (Evans and Palmer, Exp. Brain Res., 40:115, 1980). These findings suggest that low-SR fibers are more resistant to saturation of their response, and may preserve frequency selectivity of the auditory nerve at high intensities (Shofner and Sachs, Hearing Res., 21:91, 1986). The idea that low-SR fibers comprise a distinct class has been generalized to other mammals; however, only the relation of threshold to SR has been extended to other species (Schmiedt, Assn. Res. Otolaryngol. Abstr., 5:12, 1982; Geisler et al., J. Neurophysiol., 37:1156, 1974).

As part of a normative study of single fiber responses in the Mongolian gerbil, we have examined the relation of SR to threshold, dynamic range, and saturation rate at the characteristic frequency (CF). The auditory nerve was accessed through a hole drilled in the round window antrum at the base of the cochlea (Sokolich and Smith, J. Acoust. Soc. Am., 54:283, 1973). A PDP 11/34 computer controlled stimulus parameters and counted intervals. In our tuning curve paradigm threshold was defined as the intensity producing a 2-spike increase in a 50 msec stimulus period. A wide-band noise burst served as the search stimulus.

We find that threshold is only weakly correlated with SR. While the average threshold for fibers of SR below 2 sp/sec is higher than for other fibers, only about one half of these low-SR fibers have thresholds above 20 dB SPL. Saturation rates depend principally on CF, rather than SR. Fibers of CF above 4 kHz have the highest saturation rates, followed by fibers of CF below 2 kHz. Dynamic range appears more closely related to threshold than to SR. The neural audiogram exhibits a notch between 2 and 5 kHz. Fibers with CFs in this range have, on average, higher thresholds, higher spontaneous rates, and lower saturation rates. Tuning curve shape and Q_{10} for these fibers are not indicative of noise-induced damage.

Our data suggest that low-SR fibers (SR < 2 sp/sec) play a role in extending the dynamic range of the gerbil auditory nerve, yet only when a low SR is accompanied by a high threshold. The extended dynamic range of these fibers derives from a gently-sloping rate-intensity function, not a higher saturation rate. Such a link between threshold and dynamic range is predicted by the model of Sachs and Abbas (J. Acoust. Soc. Am., 56:1835, 1974).

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149.8 RADIAL AFFERENT TERMINATION PATTERNS IN THE GUINEA PIG COCHLEA. J.H. Siegel. Depts. of Communication Sciences and Disorders and Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

Our recent success in intracellular recording from the spiral ganglion cells (radial afferents) innervating inner hair cells (IHC) of the apical turns of the guinea pig cochlea (Siegel, J.H. and Dallos, P., Hearing Res., 22:245, 1986) has stimulated our interest in better characterizing the synaptic termination patterns of the afferents in this species. The unmyelinated portions of the afferent dendrites within the organ of Corti were serially reconstructed from 0.25 µm sections viewed with an A.E.I. EM-7 microscope at 1 MeV (HVEM facility, University of Wisconsin, Madison).

An average of 12 different afferents contacted one IHC. In most cases, the afferent ran unbranched to a nearby hair cell, forming a single simple synaptic junction. The dendrites of these ganglion cells typically had relatively large diameters and terminated on the IHC at all points around its circumference. A minority of the afferents formed more complex synapses with either more than one presynaptic dense body in the hair cell or a single presynaptic body which contacted the presynaptic membrane at two places. The diameters of the dendrites of these cells were among the smallest encountered. They tended to innervate the hair cell on the side facing the modiolus and sometimes branched.

The termination pattern of radial afferents in the guinea pig appears similar to that reported for the cat (Liberman, M.C., Hearing Res., 3:45, 1980). The most prominent exception is that each active zone at complex IHC afferent synapses in the guinea pig appears to exhibit a presynaptic dense body, whereas complex synapses in the cat appear to exhibit only one presynaptic body, regardless of the number of active zones. Branching also appears more frequent than in the cat. The possible correspondence between the two types of synaptic complex and populations of single eighth-nerve units with low and high rates of spontaneous discharge is discussed.

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149.9 ONTOGENIC CHANGES IN COCHLEAR INNERVATION FOLLOWING THE ONSET OF HEARING IN MONGOLIAN GERBILS. S.M. Echteler. Auditory Physiology Laboratory. Dept. of Neurobiol. and Physiol., Northwestern Univ., Evanston, IL 60201.

Previous tracing studies of mammalian cochlear neurons have mapped the pattern of hair cell innervation either during early postnatal life, well before the onset of hearing, or in adulthood. In the present study we provide information on the pattern of innervation of cochlear receptors during the initial stages of hearing in the gerbil, a rodent with a prolonged period of postnatal cochlear maturation.

Horseradish peroxidase was injected via an insect pin ("000") into the cochlear nerve of gerbils beginning at the onset of hearing (12 days after birth) (Woolf and Ryan, Hearing Res., 13:277, 1984). After four to eight hours, animals were perfused through the heart and cochlea with 2.5% glutaraldehyde in 0.1 M phosphate buffer. Following decalcification (0.1 M EDTA) for 2-4 days, cochleae were reacted with diaminobenzidine, embedded in epon, and viewed as surface preparations.

Cochleae examined thus far, ranging in age from 12 to 15 days postnatal, exhibit a pronounced basal to apical gradient in the maturity of inner hair cell (IHC) innervation. Radial fibers projecting to IHCs within the base (high frequency region) of a 13 day old gerbil cochlea have already achieved a mature morphology, with well defined terminal boutons contacting one or two adjacent receptors. Neurons projecting to IHCs within the second and third cochlear turns, however, appear less mature. Many of these neurons exhibit growth cone-like enlargements from which emmanate multiply- branched neuritic processes innervating adjacent or widely spaced IHCs. The relative immaturity of the second and third cochlear turns is also reflected by the presence of kinocilia on IHCs within these regions, contrasting, once again, with basal IHCs, which show a lower incidence of kinocilia at 13 days of age.

HRP injections into the auditory nerves of 16-30 day old animals are now underway to resolve when IHC innervation of the middle and apical cochlear turns becomes mature. These preliminary findings suggest, however, that changes in cochlear innervation occur after the onset of hearing in gerbils. Moreover, the timecourse of these changes corresponds well with the ontogeny of neural frequency tuning in this species (Woolf and Ryan, Dev. Brain Res., 17:131, 1985).

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149.10 DEVELOPMENT OF ACETYLCHOLINESTERASE-STAINED NERVE FIBERS IN THE MOUSE ORGAN OF CORTI. H.M. Sobkowicz, M.R. Emmerling* and C.V. Levenick*. Dept. of Neurology, Univ. of Wis, Madison, WI 53706.

Acetylcholinesterase (AChE)-positive innervation was studied in the light and electron microscope, using Karnovsky and Roots, and Topilko methods. With light microscope faintly stained fibers were first seen in one-day old animals. The fibers enter the organ in the intraganglionic bundle, from which they radiate upward via radial bundles. By the 4th day, fibers reach inner hair cells. Here, the fibers divide into two groups. The first group of fibers, and usually the earliest, grows close to the basilar membrane. Most of these fibers form in front of the inner pillars the earliest spiral path in the organ. They then cross as tunnel fibers between tightly apposed inner and outer pillars and enter the outer hair cell region. The tunnel bundles are radially orientated and regularly spaced, some of them ultimately reach the third row of outer hair cells. Individual tunnel bundles appear to innervate outer hair cells in all three rows, within a narrow vertical sector. The first outer spiral bundle appears at this time. The second group of fibers grows up to the inner hair cells, to form the inner spiral plexus and bundle. This bundle is initially formed in short segments and may be discontionuous even in the 6-day old animal. By the 7th postnatal day, numerous AChE-positive fibers and endings are present in the outer hair cell region, and all three outer spiral bundles are formed. The innervation appears to be complete by the 12th day. Electron microscopical studies indicate that AChE-positive and negative fibers enter the organ together. The AChE-positive fibers tend to become positioned just above the border cell of Held; while AChE-negative fibers collect closer to the inner hair cell. Most of AChE-positive fibers are about 0.25 microns in diameter, they show distinct microtubules and occasional mitochondria, but no synaptic vesicles. In addition, a large population of AChE-positive fibers collects in front of inner pillars. The inner spiral plexus contains vesiculated nerve endings, both free or synaptically engaged, which may be either AChE-positive or negative. In our present material, all vesiculated endings forming synapses with inner hair cells were AChE-negative. In a 6-day old animal, both vesiculated and nonvesiculated nerve endings on outer hair cells may stain for AChE. In conclusion: The AChE-positive innervation of outer hair cell develops around birth, independently of and prior to the AChE-positive projections to the afferent system of inner hair cells. The initial projections of tunnel fibers are distinctly radially oriented, generating a columnar pattern of innervation. The enzymatic characterization of developing neuronal endings may precede their ultrastructural differentiation. (NIH NS 15061)

149.11 GABA-LIKE IMMUNOREACTIVITY IN THE ORGAN OF CORTI OF THE DEVELOPING MOUSE. D.S. WHITLON*, H.M. SOBKOWICZ & B.K. AUGUST* (SPON: J. Chan) Dept. Neurology, Univ. of Wis. Madison, WI. 53706.

Surface preparations of cochleas from postnatal mice (ages: newborn, 1,4,8,12, and 15 days) were stained immunohistochemically using an anti-GABA antibody (Wenthold, R.J. et al. Brain Research (1986) 380:7-18).

In the mouse, a few GABA-positive fibers displaying numerous varicosities are already present around birth, throughout the entire length of cochlea. The growing fibers run and ramify in the intraganglionic bundle and their individual paths can be followed with the light microscope. The fibers give off a few collaterals that grow toward the hair cell region as well as toward the spiral ganglion. Sometimes, the collaterals from both regions can be traced back to the same fiber in the intraganglionic bundle. Some end-collaterals seem to wrap and to terminate around spiral neurons. With age, GABA-containing fibers, albeit sparse, can be frequently identified in radial bundles. By the 8th day, the stained radial fibers reach the inner hair cells and form a distinct inner spiral plexus and bundle. A discrete spiral bundle is also formed, by GABA-containing fibers, between the inner hair cells and inner pillars. Tunnel fibers are seen to originate from the latter bundle. By the 12th day, the bundles of tunnel fibers enter the tunnel of Corti at the intervals of one to six pillars, the fibers fan out within small sectors of the outer hair cell region. They arborize and terminate on the outer hair cells of all three rows. The fibers may provide from one to multiple endings.

In conclusion: The GABA-containing neuronal system in the cochlea of the mouse begins to differentiate around birth and continues for at least 12 postnatal days. The GABA-positive neuronal system appears to project to both types of sensory cells and to their afferent neurons. (Supported by NIH Grant NS15061.)

149.12 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF T II CELLS IN THE SPIRAL GANGLION OF THE RAT. A. Hafidi*, R. Romand, and B. Onteniente, Laboratoire de Neurobiologie, Université de Clermont II, 63170 Aubière, France and Laboratoire de Physiologie, Université de Bordeaux I, 33405 Talence Cédex, France.

The spiral ganglion of the cochlea in mammals, contains two type of cells which were differentiated on structural and ultrastructural characteristics of their cell bodies and their connections to the receptor cells. One type of cell which represents only 5% of the whole population can be differentiated by a dense packing of neurofilaments in their perikarion and are called T II cells.

Neurofilaments are the neuron specific class of intermediate filaments. In mammals they are composed of three immunochemically distinct subunit polypeptides with an apparent molecular weight of approximately 200 KiloDaltons (NF 200 KD), 160 KiloDaltons (NF 160 KD) and 68 KiloDaltons (NF 68 KD). A previous study (Berglund and Ruygo 1986, Brain research 383 : 327-332) with a neurofilament antibody which recognized the NF 200 KD and perhaps another protein (Godsave et al. 1986; J. Embryol. exp. Morphol., 97 : 201-223) showed the specific reaction of T II cells by light microscopy.

In our study we used neurofilament monoclonal antibodies and monoclonal antibodies against each neurofilament subunit in order to determine their presence in T II cells. We used the indirect immunofluorescence and the PAP methods for the light microscopic observation while using an antibody against non phosphorilated NF 200 KD, and two others antibodies against phosphorilated NF 68 KD and NF 160 KD.

Some ganglionic cells presented a strong immunofluorescence with the neurofilament antibody. The shape, the position inside the ganglion and the number of reacting cells corresponded well to T II cells found by classical methods. As previously observed, only a few cells in the spiral ganglion reacted with antibodies against the three neurofilament subunits, 68, 160 and 200 KD. The ganglion cell processes, irrespective of cell types, reacted strongly to various antibodies. Peripheral processes could be traced to inner hair cells and outer hair cells. Experiments to characterize at the electron microscopic level the immunohistochemical reaction of antibodies against the three filament subunits in T II cells are at present being undertaken.

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149.13 INHIBITION OF INNER EAR ORNITHINE DECARBOXYLASE BY NEOMYCIN IN-VITRO. Charles M. Henley III, Sanford C. Bledsoe, and Jochen Schacht. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109 U.S.A.

Ornithine decarboxylase (ODC), a key enzyme in polyamine synthesis, was quantitated in homogenates and subcellular fractions of inner ear tissues from both the guinea pig and the rat. Specific activities of ODC, defined as α-difluoromethylornithine (DFMO)-sensitive decarboxylation of ^{14}C-ornithine, in homogenates of combined inner ear tissues were: guinea pig = 22 ± 1 pmoles CO_2 produced/hr/mg protein, and rat = 134 ± 24. In the guinea pig, homogenates of lateral wall tissues (stria vascularis and spiral ligament) had specific activities of 62 ± 5, those of the organ of Corti (plus VIIIth nerve) 34 ± 10. Subcellular fractionation showed the enzyme associated with the postmitochondrial supernatant. Specific activities in combined inner ear tissues were guinea pig = 44 ± 4 and rat = 133 ± 30. Lateral wall tissues yielded specific activities of 62 ± 25 and organ of Corti = 64 ± 41.

The ototoxic aminoglycoside, neomycin, produced dose-dependent inhibition of ODC in the supernatant fraction with half-maximal inhibition observed at 50 μM drug and almost complete inhibition at 100 μM. Neomycin inhibition of cochlear ODC complements our earlier findings of aminoglycoside inhibition of renal ODC. We are presently investigating the effects of systemic administration of neomycin on auditory brainstem responses and ODC from cochlear, renal, liver and brain tissues from the guinea pig. Since DFMO and neomycin can cause hearing loss in patients and experimental animals it is suggested that inhibition of ODC may be an important factor in the ototoxicity of these drugs.

149.14 QUININE REDUCES NOXIOUS EFFECTS OF LOOP DIURETICS ON COCHLEAR FUNCTION. L.P. Rybak, C. Whitworth*. Dept. of Surgery and Pharmacology, SIU Sch. of Med., Springfield, Illinois, 62708.

Loop diuretics are known to have ototoxic effects in man and animals. These drugs cause a reduction of cochlear function and edema of the stria vascularis. Loop diuretics are weak organic acids, and we have previously shown that prior administration of other organic acids reduced the noxious cochlear effects of furosemide (Rybak and Whitworth, Hear Res 26:89-93, 1987). Studies of the choroid plexus by other investigators have shown that quinine inhibits the uptake of organic acids such as riboflavin (Spector, J Clin Invest, 66:821, 1980) and PAH (Suzuki et al, JPET, 239:927, 1986). Therefore we decided to investigate the interaction of quinine with the loop diuretics furosemide and ethacrynic acid to determine whether the cochlear effects of the latter were attenuated by pretreatment with quinine. Adult chinchillas weighing 400-600 grams were anesthetized with katamine 45 mg/kg IM followed by pentobarbital 30 mg/kg IM. The head was fixed in a head holder. The auditory bulla was opened and a microelectrode was inserted through the round window to measure endocochlear potential (EP). A tungsten wire was placed on the round window membrane to measure eighth nerve action potential (AP) responses to click stimuli. Control animals received 0.5 ml saline while experimental animals were injected with quinine HCl 25 mg/kg IV thirty minutes prior to diuretic injection. Furosemide 25 mg/kg IV or ethacrynic acid 15 mg/kg IV was then injected. Control animals were found to have a large drop in EP following furosemide to only 28.0 + 7.7% of initial value, or ethacrynic acid to 27.7 + 13.0% of initial value. In addition, a considerable elevation of AP threshold was observed following each diuretic in the controls. Animals pretreated with quinine were found to have a significantly smaller reduction of EP following furosemide resulting in values 81.9 + 5.6% of initial value, or ethacrynic acid to values 71.9 ± 9.5% of initial value. A much smaller reduction of AP (N_1) amplitude of 44.3 ± 13.3% and 41.6 ± 13.0% respectively as compared to 25.2 ± 9.8% and 0%, respectively, in controls. No significant differences in urine volumes were noted between experimental and control groups. Quinine is known to cause nonspecific changes in the membranes of epithelial cells which may cause alterations of the transport of organic anions by such tissues. Such an effect on epithelial cells in the cochlea could cause a reduction of uptake of loop diuretics in this organ, resulting in reduced toxicity. (Supported by NIH grant #5R01 NS 22530-02).

149.15 COCHLEAR MICROPHONIC POTENTIALS TO VERY LOUD ACOUSTIC STIMULI ARE ENHANCED BY THE MIDDLE EAR REFLEX.
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Electrodes were chronically implanted in the middle ear of rats to measure cochlear microphonics (CM) as well as electromyograms of the stapedius muscle.

CM unaffected by the middle ear reflex - measured during the first 5 ms - increase with increasing stimulus intensity up to a sound pressure level of 100 dB SPL (at 10 kHz). At intensities higher than 100 dB SPL CM show a successive decrease with increasing intensity.

For intensities between 85 and 100 dB SPL (at 10 kHz) after a latency of 10 - 20 ms CM amplitude is decreased by the middle ear reflex; this is expected from the known attenuation due to reflex activity. At sound intensities above 100 dB SPL the CM amplitude is enhanced by the middle ear reflex! This enhancement occurs after a latency of 5 - 10 ms.

The outer hair cells in the inner ear are the main source of CM. The decrease of the initial CM to very loud acoustic stimulation is probably due to a decrease of the receptor potential of outer hair cells. The input/output function of outer hair cells shows a maximum at about 100 dB SPL, and a decrease to still louder stimulation (Dallos,P., Cheatham,M.A., 1975, J.Acoust.Soc.Am. 60,510-512). CM increase due to middle ear reflex activity when very loud stimuli are used can therefore be explained by the following mechanism. Due to stimulation above the maximum of the input/output function CM show a low amplitude before reflex activity. Than after a short latency the input to the inner ear is attenuated by the middle ear reflex, the receptor potential of the outer hair cells is shifted back towards the maximum, which enhances CM.

At very loud acoustic stimulation the middle ear reflex seems to serve as a gain control, which prevents overstimulation of the receptor cells in the inner ear.

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149.16 COCHLEAR BLOOD FLOW IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS.
W.S. Quirk, M.J. Bademian*, H.A. Dengerink*, and J.W. Wright. Department of Psychology, Washington State University, Pullman, WA 99163-4380.

Noise-induced hearing loss has been proposed to be the result of insufficient blood supply to the cochlea (Borg, 1981). Studies of human hypertensive patients (Johnsson and Hansson, 1977;) and the spontaneously hypertensive rat (SHR) model of human essential hypertension (Axelsson, 1983) have identified a relationship between hypertension and noise-induced hearing loss. However, there is no direct experimental evidence describing the physiological mechanisms involved. Previous investigations in our laboratory have measured significant increases in plasma angiotensin II (AII) levels in rats (Wright, 1985). Increases in plasma AII concentrations result in elevated systemic blood pressure in mammalian species (Ramsey, 1982). In an initial experiment these levels of AII were approximated through intracarotid infusion (0, 1, 10, 100, 1000 pmol/kg/min for 5 min) in normotensive Wistar-Kyoto (WKY) and SHR. WKY cochlear blood flow (CBF) increased in response to elevations in systemic blood pressure except at the highest dose utilized (1000 pmol/kg/min) where a decrease in CBF was evident despite continued elevated systemic blood pressure. In SHR a decrement in CBF was apparent despite continuous elevations in blood pressure at every dose utilized.

A second experiment stemmed from the observation that many treatments of otopathologies similar to those approximated in the present study depend on blood transport of therapeutic agents or expansion of blood volume to increase CBF. Therefore we employed two clinical agents, Mannitol (20%) and Dextran 70 (6%), in order to evaluate the effectiveness of these compounds to increase CBF. The data indicate a decrement in CBF in SHR despite elevated systemic blood pressure and expanded blood volume in response to both Mannitol and Dextran infusion.

Taken as a whole these results are interpreted to indicate that there may be a form of autoregulation in SHR during elevations of systemic blood pressure. Since this response is evident in response to infusion of the vasoconstrictive peptide AII as well as plasma volume expanding substances, it is not likely that the mechanisms involved in the mediation of cochlear autoregulation are activated simply by expansion of blood volume. Preliminary data from our laboratory using a pretreatment of the angiotensin receptor antagonist sarile (Sar^1,Ile^8-AII) show a decrease in CBF and systemic blood pressure in response to infusion of AII in both strains, when compared to saline pretreatment. These data indicate that endothelial angiotensin receptors of the cochlear vasculature may, in part, mediate the autoregulatory response in WKY and SHR.

149.17 PHYSIOLOGICAL CORRELATES OF BEHAVIORAL MASKING.
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Acoustic behaviors, such as the mating behavior of frogs, are performed in natural environments in which there is a great deal of interfering background noise. Previous behavioral experiments have shown how effective some anurans are at extracting important acoustic information from extraneous noise. The question remains, however, as to whether their acuity is derived from sharp peripheral filtering or whether a central process mediates the behavior. To investigate this question we have recorded from single units in the eighth nerve and the torus semicircularis (midbrain) of the bullfrog in order to study how cells respond to acoustic signals imbedded in a background of continuous white noise.

Bullfrogs (Rana catesbeiana)were anesthesized with Sodium pentobarbitol. The eighth nerve was exposed through a small hole in the roof of the mouth. For recording from the midbrain, the skull above the optic tectum was carefully removed. Acoustic stimuli (closed-field) were delivered ipsilaterally for eighth nerve recording and contralaterally for midbrain recording.

When a single auditory unit was isolated its Best Excitatory Frequency (BEF), threshold to continuous white noise, and tuning curve were determined. The response to tone bursts at its BEF and threshold intensity was then measured, and continuous white noise was added beginning at threshold level and increasing in 2 dB (spectrum level) steps until the response was masked. The signal-to-noise ratio at the masked threshold (critical ratio) was calculated based on masked thresholds determined in two ways. The masked thresholds were determined on the basis of spike rate increases during the tone burst, and they were also calculated on the basis of the degree to which the response was synchronized (phase-locked) to the stimulus waveform. Physiological results from both the eighth nerve and midbrain were then compared to the results of previous behavioral studies.

Critical ratios (CRs) determined using both rate and synchronization criteria for the eighth nerve closely approximate the CR's determined behaviorally. At any given frequency, however, the critical ratios based on the synchronization coefficient were lower than those based on spike rate. Moreover, the sharpness of tuning of individual units were similar to the animal's tuning determined behaviorally. Cells in the midbrain showed more variability. While some cells were more sensitive (had lower critical ratios) than the behavioral measure, others had higher CR's. We conclude that the bullfrog's acuity in discriminating signals from noise is based primarily on peripheral filtering. [Supported by NIH].

149.18 **COCHLEAR IMPLANTS FOR SENSORINEURAL DEAFNESS: RECEPTOR CODING IMPROVES SPEECH DISCRIMINATION.**
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Cochlear implants (cf. Miyamoto, et al., J. Ind. Med. Assn., 1982, 75, 174) can be viewed as artificial receptors that transduce acoustic energy into bioelectric signals in the auditory nerve. Transduction in natural auditory receptors produces bioelectric signals whose waveform markedly differs from the acoustic waveform (Russell and Sellick, J. Physiol., 1978, 284, 261). This waveform change could: (A) degrade sensory information and thus limit perceptual performance or (B) efficiently code information and hence facilitate performance.

This question led us to do controlled experiments on the effect of receptor coding on cochlear implant performance with speech. The control condition was the commonly-used House implant (described by Fretz & Fravel, Ear & Hearing, 1985, 6, 14S); it creates a waveform that follows the acoustic waveform. The experimental condition added a receptor-coded signal processor (described in Wasserman, Bull. Psychon. Soc., 1981, 18, 161). Within-patient tests were run in an acoustic booth with visual and auditory isolation. Stimuli were prerecorded; testing was computer controlled in a two-alternative forced-choice design. Performance was objectively assessed by computer-monitored patient-controlled response keys.

Word articulation was tested with an adaptation of the House, et al. test (J. Acoust. Soc. Amer., 1965, 37, 158); it presented pairs of monosyllabic words as visual text. One of these words was presented aurally and the patient chose the better of the two possibilities. Receptor coding produced a consistent substantial increase in word discrimination.

Sentence comprehension was examined with a modification of the SPIN test (Kalikow, et al., J. Acoust. Soc. Amer., 1977, 61, 1337). A sentence (minus its last word) was presented acoustically with no visual cues. Two words were presented as visual text with no auditory cue. The patient chose the written word that best completed the audible sentence. Since the patient never heard the word, the patient had to hear out the meaning of the sentence. Many patients cannot perform above chance in this task. Nevertheless, receptor coding can bring a patient whose control performance is at chance up to a substantial, clearly significant level of comprehension.

These data suggest that receptor transduction efficiently codes afferent information for analysis by the brain.

149.19 SURGICAL IMPLANTATION AND BIOCOMPATIBILITY OF CNS AUDITORY PROSTHESIS. J. K. Niparko*, J.J. Zappia*, X. Xue*, D. J. Anderson, R. A. Altschuler. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109

Presently employed cochlear implants are not applicable to significant numbers of deaf individuals with bilateral cochlear dysgenesis, ossification or cochlear nerve ablation. Neuroprosthesis placement proximal to the cochlear might achieve activation of the auditory tract in such cases. We are therefore examining the feasibility of implanting the modiolar portion of the cochlear nerve (Md) and the cochlear nucleus (Cn) with microelectrodes. The present histological studies determined the accuracy of placement and the biocompatibility of these electrodes with and without stimulation.

Probes were fabricated on a silicon substrate using thin film techniques. Md and Cn implantations were performed with both wire-tethered and untethered microelectrode probes in anesthetized guinea pigs. Md implants were placed through a post-auricular, transbullar approach to the cochlea. The lateral wall of the cochlea was drilled out exposing the bony modiolus which was penetrated to allow passage of the microelectrode into the cochlear nerve. Cn implantation was accomplished through a posterior craniotomy. The cerebellum was exposed and the paraflocculus retracted or aspirated to expose the pontomedullary junction. Microelectrodes were then inserted in a position ventromedial to the cochleovestibular nerve insertion. Nonstimulated animals were fixed with mixed aldehydes 4 to 8 weeks after implantation. Tissues were examined for accuracy of electrode placement and tissue reaction.

Md implants were consistently placed into the modiolar portion of the cochlear nerve. Loss of spiral ganglion cells occurred in approximately half of the Md implanted animals. Other Md implanted animals demonstrated essentially normal cytoarchitecture. Fibrosis was found lining the implant and wires (when tethered). Cn implants were successfully placed in the ventral cochlear nucleus. A thin rim of gliosis lining the electrode tract and otherwise normal cytoarchitecture was observed in wire-tethered and untethered Cn implanted animals. Electrophysiologic data suggests that physiologic activation of the central auditory system was obtained with Cn implant stimulation.

These results demonstrate tolerance to the Md and Cn implant procedure and implant biocompatibility in most cases. Assessment of tissue reaction with implant stimulation is currently in progress. This Research was supported by NIH Contracts # N01-NS-5-2387 and N01-NS-6-2309.

AUDITORY SYSTEM VI

150.1 FREQUENCY ORGANISATION OF THE AUDITORY MIDBRAIN IN THE BOB-WHITE QUAIL. S.W. Shaver*, L.Z. Wise* and B.J. Frost, (SPON: M.W. Donald), Dept. of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The ground-dwelling Bob-white quail (*Colinus virginianus*) has a complex vocal repertoire and relies heavily on auditory communication in its visually-restricted natural habitat. We have examined the frequency organisation of the midbrain auditory nucleus (nMLD) of this quail using conventional electrophysiological recording techniques in conjunction with 14-C-2-deoxglucose (2DG) metabolic mapping.

A physiological audiogram was generated for ketamine-anesthetised quails by plotting the envelope of multiple frequency tuning curves recorded from many different sites within nMLD. The region of greatest sensitivity in the audiogram is between 1 to 2 kHz, where unit thresholds are as low as 0 dB SPL. Thresholds increase sharply above 2.5 kHz with a slope of around 56 dB / octave, but rise more gradually below 1 kHz, with a slope of 24 dB / octave to 400 Hz. Below 400 Hz, thresholds remain fairly constant (at around 35 dB SPL) to the lower limit of our sound generating system (50 Hz). In common with other avian species, units with sharp frequency tuning are found in tonotopic sequence within the central nMLD, with low frequencies represented dorsally and higher frequencies ventrally. Frequency tuning appears to be broader in the lateral region of nMLD, which in the barn owl is broadly-tuned and contains a neural "map" of auditory space.

We also studied the organisation of iso-frequency laminae in nMLD by injecting the metabolic marker 2DG (150 uCi/kg) in awake quails during exposure to various sound stimuli. In addition to bands of 2DG label in the isofrequency laminae corresponding to the stimulus frequency, a region of dense label is consistently found in the antero-dorsal pole of nMLD, an area receiving input from brainstem nuclei innervated by fibres from the lagena (J.W. Conlee and T.N. Parks, Brain Res. 367:96-113). Many units in this region were highly active in our physiological studies, although we could not drive them with our stimuli. The anechoic chamber in which our experiments are performed is not protected from very low frequency sounds generated by the ventilation system of the building (< 50 Hz) nor from infrasound, and thus a very low frequency confounding stimulus may have been present during all our experiments. This circumstantial evidence, in conjunction with the fact that we were unable to identify a low frequency cut-off in our audiogram, has led us to hypothesise that the antero-dorsal region of quail nMLD is specialised for processing infrasound. Supported by NSERC Grant A0353 and MRC Grant MT7244.

150.2 DEVELOPMENT OF GABA-IMMUNOREACTIVE TERMINALS IN CHICK BRAIN STEM AUDITORY NUCLEI. R.A. Code, G.D. Burd and E.W Rubel. Dept. of Otolaryngology, Univ. of Washington Med. Sch., Seattle, WA 98195.

Two major classes of terminal endings have been described in both n. magnocellularis (NM) and n. laminaris (NL) of the chick brain stem. The excitatory input to NM is from the ipsilateral VIIIth nerve. NM, in turn, provides bilateral excitatory input to NL. The other major input to each nucleus is immunoreactive to glutamic acid decarboxylase (GAD) and gamma - aminobutyric acid (GABA). Development of GABA- immunoreactive (GABA-I) terminals was studied in NM and NL in embryos from E9 to E19 and in posthatch chicks up to 1 year of age. Twenty-three animals were perfused intracardially with buffered, mixed aldehydes and staged according to the Hamburger and Hamilton series. Vibratome sections were incubated overnight in rabbit anti-GABA antisera (Immunonuclear, Inc.), diluted 1:2000-1:4000, or in an affinity-purified rabbit anti-GABA antisera conjugated to keyhole limpet hemocyanin (KLH), diluted 1:1000. Similar staining was observed with either antisera. Control studies included: 1) incubation in normal goat serum without the GABA antiserum; 2) incubation in GABA antisera pre-adsorbed with GABA conjugated to bovine serum albumin (BSA), diluted 1:10, 1:100 or 1:1000. These controls resulted in the absence of GABA-I staining, indicating the antisera used are highly specific for GABA. Sections incubated in GABA antisera pre-adsorbed with either glutamine, glutamate, taurine, or ß-alanine resulted in staining that was similar to that in sections incubated in GABA antisera alone, indicating no cross-reactivity between the GABA antisera and these structurally similar amino acids. In posthatch chicks, GABA-I terminals were observed as puncta circumscribing neuronal soma in both NM and NL. More puncta appear to be present in lateral regions of both nuclei than in medial regions. In E17-19 embryos, GABA-I puncta are also present around cell bodies in NM and NL, but no mediolateral gradient is evident. Around E14-15, there is a transition in the staining pattern: some GABA-I puncta are present; however, most stained structures appear to be axons with varicosities along their length, pre-terminal axonal thickenings or ramifying terminal branches. At E12-14, fewer GABA-I puncta and fibers are present. From E9-11 there is little GABA-I staining in either NM or NL. In summary, the first appearance of GABA-I fibers appears to coincide with the development of inner ear function and synaptic activation of NM by VIIIth nerve fibers, while the appearance of puncta is correlated with the formation of mature end -bulbs of Held on NM cells. Thus, the ontogeny of presumed inhibitory inputs to chick auditory brain stem nuclei temporally correlates with, and could modulate development of excitatory auditory afferent structure and function. This research was supported by NIH grant NS24522 to EWR.

150.3 GLYCINERGIC PATHWAYS IN THE AUDITORY SYSTEM OF THE RAT.
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The high levels of glycine (GLY), the dense distribution of GLY receptors as well as electrophysiologic data suggest that this amino acid is a major inhibitory neurotransmitter in the mammalian brain stem. The GLY antagonist strychnine induces seizures in rats which may be triggered by acoustic stimuli. We have studied the glycinergic pathways in the auditory system likely to be involved in controlling sound sensitive seizures in rats using two approaches.

The first experiments utilized the property of glycinergic elements to uptake and retrogradely transport GLY into the corresponding neuronal somata. Iontophoretic deposits of [^{3}H] GLY were made in the ventral nucleus of the lateral lemniscus (VLL). Autoradiographic processing of this material showed labeling of some VLL neurons close to the injection site, and of the majority of nerve cells in the ipsilateral medial nucleus of the trapezoid body (MNTB). Accumulation of silver grains was less dense over neurons of the ventral nucleus of the trapezoid body (VNTB), the superior olivary complex (SO), and a subgroup of medium-sized cells in the region of the nucleus reticularis pontis oralis (NRPOo) on both sides. No labeled somata were found in either ventral cochlear nuclei, or the contralateral VLL. Injections into the thoracic spinal cord or the medullary reticular formation gave negative results in the nuclei under consideration.

A second series of experiments used the immunocytochemical localization of endogenous GLY with a polyclonal antibody, raised against a GLY-BSA conjugate, and the PAP technique. Immunoreactivity was very dense in most of the neurons in the MNTB. Moderate to low staining was observed in some cells of the VLL and SO. These nuclei also showed GLY-positive fibers and puncta. Clusters of labeled neurons were also found in the NRPOo. Control sections treated with the antiserum which had been preadsorbed with GLY-BSA conjugate and coprecipitated with an anti-BSA antibody, were negative.

These findings document the existence of glycinergic pathways from MNTB, SO, and NRPOo, which innervate VLL, as well as the presence of probably projecting GLY-immunoreactive cells in this latter nucleus. The results are supported by the demonstration of high density GLY receptors found in VLL and SO (Zarbin et al., J. Neurosci., 1981). Failure of this pathway may be important in the production of acoustic startle and/or audiogenic seizures in the rat.

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150.4 A COMPARISON OF GABA AND GLYCINE IMMUNOREACTIVITY IN THE GERBIL DORSAL COCHLEAR NUCLEUS
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Rabbit antibodies against glutaraldehyde conjugates of bovine serum albumin and GABA or glycine (GLY) (Wenthold et al., Brain Res. 380:7-, 1986; Neurosci. In press, 1987), have been utilized to identify GABA and GLY containing cell bodies, terminals and processes in the cochlear nucleus in 20 um frozen, 1-3 um plastic sections and thin sections of mixed aldehyde fixed brains of young adult Mongolian gerbils (Meriones unguiculatus).

In frozen sections processed immunocytochemically and visualized with the Vectastain avidin-biotin-peroxidase complex (ABC) method, differences in the patterns of cell body labeling were very distinct. Incubations with A/GABA labeled small to medium cells preferentially located in the fusiform (FL) and molecular layers (ML) of the DCN, while A/GLY incubations preferentially labeled medium sized and larger cells in the FL and deep layers (DL). Postembedding incubations of adjacent 3 um thick sections with the two antibodies allowed some neurons to be identified as labeling with both. With the antibodies and incubation conditions used the most intense GABA staining was much heavier than the most intense GLY staining. No cells stained intensely for both. Most heavily GABA labeled cells showed light GLY labeling. Many cells which were heavily GLY labeled showed only faint GABA labeling. Electron microscopic examination of material incubated before embedding, visualized with the ABC method, or incubated in the thin section, visualized with goat anti-rabbit IgG-gold, allowed the identification of a number of heavily GABA labeled cartwheel neurons. Several heavily GABA labeled small neurons have been identified as Golgi cells light microscopically, & at least one electron microscopically, by their characteristic size and lumpy soma. At least 3 stellate neurons have been shown at the EM level to be GABA immunoreactive.

In the ML, GABA labeled synaptic terminals were found synapsing on large unlabeled spiny dendrites (presumably belonging either to fusiform or cartwheel neurons) but not necessarily on the spines. A few heavily labeled synaptic terminals were found on labeled cartwheel neuron somata. GABA labeled terminals were most heavily concentrated in the ML & FL, the regions in which cartwheel axons distribute their terminals. Some labeled terminals were found on unlabeled fusiform neurons. Some unmyelinated and thinly myelinated fine axons in the ML were labeled.

In A/GLY incubated material the distribution of labeled synaptic terminals was much more uniform across the ML, FL & DL--a pattern similar to that observed autoradiographically following uptake of H3-GLY by fresh brain slices.

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150.5 GABA AND GLYCINE IMMUNOREACTIVITY IN THE GUINEA PIG SUPERIOR OLIVARY COMPLEX.
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GABA and glycine (GLY) are believed to be major inhibitory neurotransmitters in the CNS, and may play important roles in the auditory brainstem. This study describes the distribution of GABA and GLY immunoreactivity in the nuclei of the superior olivary complex (SOC), an initial site for the convergence of binaural input and the locale of the neurons of the olivocochlear system.

Guinea pigs were anaesthetized and perfused with mixed aldehyde fixative (4% paraformaldehyde, 0.2% glutaraldehyde in phosphate buffer for GABA; 1.25% glutaraldehyde, 1% paraformaldehyde in phosphate buffer for GLY). Immunoperoxidase immunocytochemistry was performed on free-floating vibrotome sections using antibodies to GABA (1:1500) and GLY (1:1000). Alternate sections were prepared for light microscopy while the remainder were prepared for subsequent electron microscopic evaluation.

Roughly one-half of the neurons within the neuropil of the lateral superior olive (LSO) exhibited GABA immunoractive (IR) labeling; the size of these neurons ranged from 15-25 μm, and their shape varied from round/polygonal to fusiform/oval. GABA IR axons were observed in the envelope surrounding the LSO, and within its neuropil. A very small number of GABA IR puncta were apposed to the perikarya of most LSO neurons. A population of LSO neurons with morphological features similar to those of the GABA IR neurons displayed light GLY IR labeling. The neuropil of the LSO also contained an abundance of densely GLY IR axons and puncta.

In the medial superior olive (MSO), the vast majority of the neurons showed neither GABA nor GLY IR labeling. Rarely an intensely GABA IR MSO neuron, 30 μm in length, was observed among the neurons within the central zone of the nucleus. The neuropil of the MSO likewise contained only sparse distributions of GABA or GLY IR processes.

The superior paraolivary nucleus (SPN) contained small populations each of moderately and lightly stained oval/fusiform neurons 20-25 μm in length. The SPN also contained larger (20-30 μm) polygonal neurons lightly stained with GLY antibody. Like the LSO, the neuropil of the SPN was densely labeled with GLY IR axons and puncta; much less so with GABA.

The principal and elongate neurons of the medial nucleus of the trapezoid body (MNTB) exhibited GLY IR staining. The principal neurons are thought to provide inhibitory input to neurons of the LSO; as such they are most likely the source of the extensive GLY immunoreactivity found within the LSO neuropil. In addition, a small population of MNTB neurons labeled lightly with GABA antibody; these neurons were polygonal, and ranged in size from 20-25 μm.

The ventral and lateral nuclei of the trapezoid body (VNTB and LNTB, respectively) contained small numbers of neurons which were the most intensely GABA IR in the SOC. They were oval and 15-20 μm in length. Larger (25-30 μm), more irregularly shaped, lightly GABA IR neurons were also identified in these nuclei. The VNTB was defined by an intensely GABA IR neuropil. Both the VNTB and LNTB contained 15-20 μm GLY IR neurons similar, morphologically, to the intense GABA IR neurons observed in this region.

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150.6 CO-LOCALIZATION OF GLYCINE AND GABA IN THE COCHLEAR NUCLEUS.
M.D. Oberdorfer*, M.H. Parakkal*, R.A. Altschuler and R.J. Wenthold, (SPON: J. Fex). Lab. of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892 and Kresge Hearing Res. Inst. Univ. of MI, Ann Arbor, MI 48109.

Recent findings indicate that GABA and glycine are major inhibitory neurotransmitters in the cochlear nucleus. Immunocytochemical studies localizing glycine, GABA, GAD and the postsynaptic glycine receptor have been used to identify GABAergic and glycinergic synapses throughout the cochlear nucleus. GABA and glycine immunoreactive cell bodies are largely restricted to the dorsal cochlear nucleus (DCN) while most immunoreactivity in the ventral cochlear nucleus is present in terminals and fibers. The overlapping distributions of the GABA and glycine immunoreactivities suggested that they may be co-contained in some cases within the same neural elements. In the present study, we have addressed this question using antibodies against GABA, GAD, glycine and the glycine receptor.

Antibodies against GABA and glycine were made in rabbits or guinea pigs (Wenthold et al. Brain Res. 380, 7(1986); Neuroscience, In Press). Antibodies against GAD were provided by Dr. Irwin J. Kopin, and antibodies against the glycine receptor were provided by Dr. Heinrich Betz. For studies localizing GABA and glycine, guinea pigs were perfused with 4% paraformaldehyde containing 0.25% glutaraldehyde, and for those localizing GAD or the glycine receptor, 4% paraformaldehyde containing 0.1% glutaraldehyde. Vibratome sections were processed as previously described (see above).

Immunofluorescence studies showed co-localization of GABA and glycine in cell bodies in the superficial DCN. With this approach double labeled puncta, suggestive of presynaptic terminals, were seen throughout the cochlear nucleus, but concentrated in the posteroventral cochlear nucleus (PVCN). This analysis was extended to the ultrastructural level in order to determine if glycine receptor immunoreactivity is found postsynaptic to terminals containing GAD or GABA immunoreactivity. Three classes of terminals were found in the DCN and the PVCN, those that were immunoreative for GABA/GAD only, those immunoreactive for glycine receptor only, and those immunoreactive for both GABA/GAD and the glycine receptor. These findings suggest that GABA and glycine may be present in the same cell bodies and terminals in the cochlear nucleus. Futhermore, glycine receptor immunoreactivity is found postsynaptic of terminals containing GABA/GAD immunoreactivity. It remains to be determined if such terminals also contain glycine.

150.7 LOSS OF GABA-IMMUNOREACTIVE NEURONS IN THE INFERIOR COLLICULUS OF THE AGED RAT. D.M. Caspary and B.A. Lawhorn*. Dept. Pharmacology, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, IL 62708 USA.

The inferior colliculus (IC) is an important auditory structure receiving ascending inputs from the cochlear nuclei (CN), superior olivary complex (SOC), nuclei of the lateral lemniscus (NLL) as well as descending inputs from auditory cortex. A major neurotransmitter role in IC for the inhibitory amino acid, gamma-aminobutyric acid (GABA), is supported by neurochemical, immunocytochemical and neuropharmacologic studies. Temporal response patterns and binaural inhibition reflect inhibitory synaptic interactions in CN, SOC, NLL as well as processing occurring within the IC itself. Intracellular recordings from IC neurons in vivo and in vitro display ipsp's which can be blocked by picrotoxin while bicuculline can block extracellularly recorded binaural inhibition (Kuwada et al., 1980; Moiseff, 1985; Smith, 1986; Faingold et al., 1986). These studies suggest that synaptic inhibition occurs in the IC and that GABA may be involved.

The present study used an affinity-purified antibody against a GABA conjugate (kindly provided by Dr. R.J. Wenthold) to immunolabel neurons and terminals in the IC. Seven pairs of mature (2-8 mo.) and aged (18-29 mo.) Fisher 344 rats and an additional six mature controls were studied. Maps of GABA-positive neurons in the ventral-lateral portion of the central nucleus of the IC were generated, using computer morphometry, to obtain cell counts. Both labeled puncta and neurons were observed throughout the central nucleus of the IC. Immunostained terminals were observed on both large and small neurons. A significant reduction (33%) in the number of GABA-positive neurons was observed in the aged animals when compared to their matched controls (151 neurons/mm^2 vs. 101 neurons/mm^2; $p<0.01$). When the seven aged animals were compared to all 13 controls a 45% reduction in the number of GABA immunoreactive neurons was observed (183/mm^2 vs. 101/mm^2; $p<0.005$). Preliminary observations from two mature-aged pairs suggest that a major proportion of the reduction in the total number of Nissl stained cells can be accounted for by the loss of GABA-positive neurons. The two-dimensional area of GABA-labeled IC neurons displaying nuclei and nucleoli was measured and no age related differences were observed (mean area 225 u^2). Areas of the GABA-positive cells displayed a broad distribution of neuronal sizes comparable to reported sizes for three subpopulations of IC stellate (multipolar) cells. At the upper size range of GABA-positive neurons fusiform profiles were also observed. The present study suggests that in one area of IC in the Fisher 344 rat an age-related loss of GABA mediated inhibitory processes may occur.
(Supported by Deafness Research Foundation and NS15640.)

150.8 **POSTNATAL DEVELOPMENT OF GLYCINE IMMUNOREACTIVITY IN THE RAT LOWER AUDITORY BRAINSTEM.** **C.R.Snead*, R.A.Altschuler, R.H.Helfert, R.J.Wenthold+. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109; and +Laboratory of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892.**

There is considerable evidence supporting the hypothesis that GABA and Glycine are important inhibitory neurotransmitters in the mammalian central nervous system. Both immunocytochemical and physiological studies have produced evidence of glycinergic activity in the auditory brainstem. In the present study, the pattern of development of glycine immunoreactivity was investigated in the rat at the light microscopic level.

Long-Evans ("hooded") rats were examined at two or three day intervals from birth to 21 days after birth and at 28 postnatal days. The mothers were used as adult controls. All animals were anesthetized with chloral hydrate and fixed by cardiac perfusion with a mixed aldehyde fixative (either 4% paraformaldehyde/0.2% gluteraldehyde or 1% paraformaldehyde/1.25% gluteraldehyde in sodium buffer. Thirty to fifty micron sections were cut on a cryostat or vibratome, incubated 18-36 hours with antibody to glycine, and followed by immunoperoxidase staining.

The pattern of development of glycine immunoreactivity was similar to that previously reported for GABA labeling in the rat pup, with soma in and terminal labeling appearing during the second postnatal week and rapidly increasing in the third week. Both the 21 and 28 day old animals exhibited a pattern of immunoreactivity similar to the adult pattern, although there was evidence of subsequent growth of the lower auditory brainstem nuclei and in absolute numbers of labeled cells and terminals.

(Supported by NIH grant NS21440).

150.9 **EFFECTS OF SURGICAL LESIONS UPON AMINO ACID CONCENTRATIONS IN CAT COCHLEAR NUCLEUS**

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Relations of amino acids to neural pathways in the cochlear nucleus are being quantitatively evaluated by a combined lesion and microchemical approach. Surgical cuts were placed in anesthetized cats to unilaterally transect either all centrifugal pathways to the cochlear nucleus by a complete cut just medial to it, just olivocochlear bundle and dorsal acoustic stria pathways by a dorsal cut, or interconnections between rostral and caudal parts of the cochlear nucleus by a transverse intra-nuclear cut. Cats were decapitated 8-10 days after surgery and tissue containing each cochlear nucleus isolated and rapidly frozen. Frozen sections were cut and freeze dried. Cochlear nucleus samples are obtained by microdissection of freeze-dried sections, weighed (0.2-1 µg), then chemically analyzed. Amino acids in each sample are measured by fluorescence of ortho-phthalaldehyde-derivatized amino acids separated by high performance liquid chromatography (HPLC). Activities of some enzymes of amino acid metabolism (glutaminase and aspartate aminotransferase) have also been measured for some additional samples via fluorometric enzymatic microassays.

Within the rostral part of the anteroventral cochlear nucleus (AVCN), GABA concentrations were reduced to approximately half on the lesion side following complete cuts, but were not clearly affected by dorsal or intra-nuclear cuts. Aspartate concentrations appeared to be reduced slightly (by about 20%) on the lesion side, following complete or dorsal cuts, but were not clearly affected by intra-nuclear cuts. Glutamine concentrations were consistently elevated in the lesion-side rostral AVCN following all cuts, by as much as two-fold following complete cuts. Concentrations of other amino acids - glycine, glutamate, taurine, alanine - were not clearly affected by any of the cuts. Aspartate aminotransferase and glutaminase activities were reduced by about 30% in the lesion-side rostral AVCN following the complete cuts, but were not clearly affected by the dorsal cuts.

The results could be consistent with centrifugal GABA-rich and aspartate-rich pathways to the cat rostral AVCN, entering mostly via ventral (presumably trapezoid body) and dorsal (olivocochlear tract or dorsal acoustic stria) routes, respectively. The increased glutamine concentrations on the lesion side may relate to gliosis in the regions of fiber degeneration, since glial cells tend to have relatively high glutamine concentrations.
[Supported by NIH Grant NS17176.]

150.10 CHOLINERGIC, GABAERGIC, AND NORADRENERGIC INPUT TO COCHLEAR GRANULE CELLS IN THE GUINEA PIG AND MONKEY. J.K. Moore. Dept. of Anatomical Sciences, SUNY at Stony Brook, NY 11794.

The cochlear granule cell area receives input from several non-primary afferent systems. Among those systems which can be identified on the basis of transmitter-related enzymes are (1) cholinergic axons and terminals, arising partly from descending systems and partly from intrinsic cochlear sources; (2) GABAergic terminals, potentially representing terminals of either GABA-positive cochlear interneurons or brainstem efferent GABAergic axons; and (3) noradrenergic axons, most likely originating in the locus coeruleus. Because previous observations have indicated that there is marked morphological change in the cochlear granule cell population in primates (Moore, J. Comp. Neurol. 193:609-629, 1980), comparative histochemical studies of the cochlear nuclei were done in the guinea pig and the macaque monkey. Noradenergic and GABAergic afferents were investigated immunohistochemically by the avidin-biotin method, using antibodies to dopamine-β-hydroxylase (DBH), the synthetic enzyme for noradrenalin, and glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA. Cholinergic axons and terminals were identified histochemically, using the Koelle indirect staining method for acetyl cholinesterase (AChE), the degradative enzyme for acetyl choline. In both guinea pig and monkey brainstems, all three types of histochemical staining demonstrated fascicles of reactive axons running between the brainstem and the cochlear nuclei in the acoustic striae and subpeduncular route. In the monkey, the size of the granule cell area is greatly reduced, but terminal staining of all three types was seen in this area. However, the density of reactive elements was less than that in the guinea pig and the marginal area of reactivity was less sharply bounded from the underlying magnocellular part of the cochlear nuclei. In both guinea pig and monkey AChE histochemistry produced a diffuse granular reaction product in the extracellular space of the granule cell layer: AChE staining in the remainder of the nucleus was limited to faint cytoplasmic staining of the larger neurons. In the GAD material, the granule cell layer contains numerous discrete immunoreactive terminals, as well as scattered dense clusters of terminals. In the DBH material, the immunoreactive structures were fine varicose axons crossing the deeper of the nuclei and forming a moderately dense plexus of axons in the peripheral granule cell layer. These results suggest that the macaque cochlear granule cell system, despite morphological change, retains the mammalian function of relaying nonprimary input into the cochlear nuclei.

This project was supported by the Deafness Research Foundation. Monkey brainstems were obtained from Dr. A. Hendrickson. The antibody to GAD was kindly provided by Dr. I. Kopin.

150.11 AN *IN VITRO* PREPARATION OF THE AUDITORY BRAINSTEM IN THE BAT, *EPTESICUS FUSCUS*. J.M. Zook and R.A. DiCaprio. Dept. of Zoological & Biomedical Science and College of Osteopathic Medicine, Ohio U., Athens, OH 45701.

We have developed a slice preparation of the auditory brainstem of the big brown bat, *Eptesicus fuscus*. The slice preparation presents advantages not only for intracellular recording stability but also for the intracellular labeling of large numbers of cell soma and cell processes. Our initial focus has been to exploit the anatomical aspects of the slice preparation while working out the necessary physiological parameters to pursue our main goal: characterization of the synaptic input to the cells that are the source of the olivocochlear bundle.

There are some specific benefits from the use of the bat in this preparation. The brainstem is quite compact and, with the relative hypertrophy of auditory nuclei, this means that the entire extent of the auditory brainstem from cochlear nucleus to inferior colliculus can be included in three to five consecutive 500 μm slices sectioned in the transverse plane. Sections are maintained in a chamber perfused with a temperature-regulated, oxygenated, physiological solution. Preliminary results also suggest that the brain of this poikilothermic mammal may be unusually tolerant to the hypothermic and hypoxic stresses of the slice preparation.

The olivocochlear bundle (OCB) can be studied to particular advantage with the slice preparation. It is possible to selectively target the cells in the superior olivary complex which give rise to this fiber tract. A fluorescent dye (Fast blue or Lucifer yellow) injected into the cochlea of intact subjects is carried by retrograde transport to the OCB-originating cells in the superior olive. After a few days survival these dye-filled cell soma in the superior olive can be localized in the slice by illumination with an appropriate excitation light source. The identity of cells penetrated can be readily confirmed by antidromic stimulation of the OCB bundle as it is separate from other auditory tracts. Penetrated cells can be distinguished by iontophoresis of a second fluorescent dye or horseradish peroxidase. At the end of recording sessions the slice can be lightly fixed and additional cells filled intracellularly by pressure injection with a contrasting label. The combination of iontophoretically-filled, characterized cells and diffusion-filled fixed cells gives us a rich population of cells for study of a specific functional group: the cells of the superior olive which originate the olivocochlear bundle. Filled cells and axons are drawn for comparison with golgi material prepared in this species. Future experiments will use the slice preparation to further characterize this cell population by exploring the morphology and physiology of the synaptic input to individually identified cells.

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150.12 HEARING AND THE AUDITORY BRAINSTEM IN A FOSSORIAL MAMMAL, THE POCKET GOPHER. R.S. Heffner[1], M.M. Richard*[2], and H.E. Heffner[1] [1]Dept. of Psychology, Univ. of Toledo, Toledo, OH 43606; [2]Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148

Because sound propagation underground and in tunnels is quite different from sound propagation above ground, it is likely that the selective pressures on hearing in underground mammals may be somewhat different from those encountered above ground. As part of an investigation into the selective pressures that have shaped mammalian hearing, we have examined the hearing abilities and central auditory neuroanatomy of a fossorial species, the pocket gopher, Geomys bursarius. The pocket gopher, which is the North American rodent most specialized for underground living, rarely forages above ground but spends almost its entire lifetime in tunnels up to a meter below the surface.

Pocket gophers were tested for both sensitivity to pure tones and for the ability to localize brief noise bursts in the azimuthal plane using the conditioned avoidance procedure. The hearing ability of the pocket gopher is unusual in that it is the least sensitive of any mammal yet tested. In addition, the gopher has the poorest high-frequency sensitivity among rodents and, unlike elephants and humans which have also lost high-frequency hearing, the gopher has not appreciably extended its low-frequency hearing. Finally, the sound-localization acuity of the pocket gopher is the poorest yet encountered in mammals—perhaps not unexpected in a species inhabiting tunnels.

The auditory brainstem was examined using thionin and protargol stains. The dorsal cochlear nucleus is well developed and the ventral cochlear nucleus appears similar to that of other small rodents. The medial superior olive is large, resembling that of gerbils and kangaroo rats, species with similar low-frequency hearing. The lateral superior olive is present but not as well developed as in most rodents, a result which corresponds to the pocket gopher's restricted high-frequency sensitivity.

In conclusion, compared to mammals in general including rodents which live below-ground but forage on the surface, the hearing of the pocket gopher appears to be degenerate but its auditory brainstem has retained an essentially rodent-like character.

(Supported by PHS grant NS17850.)

150.13 A COMPUTER MODEL FOR PERIODICITY ANALYSIS IN THE AUDITORY MIDBRAIN BASED ON PHYSIOLOGICAL PROPERTIES AND CONNECTIVITIES OF UNITS IN THE COCHLEAR NUCLEUS. G. Langner, J. Decker*, M. Günther*, and B. Hose Inst. of Zoology, TH - Darmstadt, 61 Darmstadt, FRG

Periodicity pitch is a perception evoked by periodic envelopes of acoustic signals covering a range of envelope frequencies from about 20 to 1000 Hz. There are good reasons to believe that pitch perception in this range is based on temporal coding mechanisms. For example, units in the auditory midbrain of many species are tuned to envelope frequencies up to 1000 Hz (Langner,G., Exp. Brain Res., 44:450, 1981; Schreiner, C. and Langner, G., Soc. Neurosci. Abstr., 10:395, 1984; Batra, R. et al., Soc. Neurosci. Abstr., 12: 361.3, 1986). Response properties of units in the midbrain of Guinea fowl and cat were found to be compatible with a cross correlation model (Langner, G., Exp. Brain Res., 52: 133, 1983). One component of this model is a coincidence unit with two inputs, one of them phase coupled to the envelope and the other one to the fine structure of the signal. The fine structure is coded in a reduced way similar to Wever's volley principle. The delays which are necessary for any correlation mechanism are provided in this case by intrinsic oscillations triggered by the envelope fluctuations. This model explains the effect of the signal fine structure on the preference of a coincidence unit for a certain envelope frequency as well as on the pitch perceived by human subjects.

A computer model was developed which consists of a trigger unit (corresponding to on- type cells of the VCN), oscillator units (corresponding to chopper-type cells of the VCN) and a reducer circuit (corresponding to type IV-cells and inhibitory cells of the DCN) and finally a coincidence unit (corresponding to a cell in the midbrain). All components of the model have properties and connectivities which are to a first approximation realized by the actual cell types. The model predicts additional response features for the included cell types and requires some additional connections not yet demonstrated anatomically. It simulates temporal tuning properties of the midbrain units and their complex transient response behavior after stimulus onset. The model reproduces this transient effect using as input the transient temporal responses of auditory nerve fibers.

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151.1 PROJECTIONS OF GLOBULAR BUSHY CELLS IN THE CAT.
P.H. SMITH, L.H. CARNEY*, and T.C.T. YIN, Dept. of Neurophysiology, University of Wisconsin-Madison, Madison, WI 53706.

In order to identify synaptic termination sites of globular bushy cells, we have done intra-axonal recording, physiological characterization, and subsequent HRP injection into single globular bushy cell axons in the trapezoid body (TB) of the cat. Entry of the microelectrode tip into a single axon was signaled by a 40-65 mV DC shift and 30-55 mV action potentials. A threshold tuning curve was used to determine the fiber's characteristic frequency (CF). The presence of a primary-like with notch (including onset with low sustained rate) PST in response to short tones at CF and a coefficient of variation within the appropriate range (Young, 1986) were used to identify axons of globular bushy cells physiologically. HRP was injected into these axons. After 12-36 hours survival, the cat was perfused and the tissue was processed using DAB with nickel-cobalt intensification. Morphologically, cells were verified as globular bushy cells by the location and features of their HRP-labeled somata within the posterior anteroventral cochlear nucleus or nerve root area, and the lack of axon collateral branches within the cochlear nucleus.

The main axons of labeled globular bushy cells leave the cochlear nucleus, traveling in the ventral component of the TB, and always give off one or more collaterals before crossing the midline. These collaterals branch frequently as they course dorsally, with terminal endings often in close apposition to cell bodies in the posterior periolivary nucleus, lateral nucleus of the trapezoid body and dorsal periolivary nuclei. Major sites of termination are regions containing somata whose axons project to the ipsilateral cochlear nucleus (Adams, 1983). For terminals studied thus far at the EM level, HRP-filled collateral enlargements contain large round synaptic vesicles. The main axons proceed across the midline and may branch up to 4 times contralaterally. One and occasionally two of the branches end in large calyces of Held in the medial nucleus of the trapezoid body. In addition, other contralateral branches, when they can be followed to termination, end in periolivary nuclei and the ventral nucleus of the lateral lemniscus.

151.2 **DEVELOPMENT OF PROJECTIONS FROM THE HINDBRAIN TO THE INFERIOR COLLICULUS.**
F.H. WILLARD AND G.F. MARTIN. Depts. of Anatomy, Uni. of New England, Biddeford, ME 04005 and Ohio State Uni., Columbus, OH 43210.

The inferior colliculus (IC) receives axons from most auditory regions of the hindbrain. We have studied the development of these projections and their topographic order using the HRP technique to retrogradely label neurons in pouch-young opossums. The opossum is an ideal animal for studying early development since it is born in an immature state, 12 days after conception.

The IC of young opossums, ranging in age from estimated postnatal day (EPND) 5 to 33, received either iontophoretic injections or placements of crystalline HRP. Approximately 24 hours later the brains were removed, sectioned and reacted for HRP. The youngest animals to show retrogradely labeled neurons in all of the appropriate auditory nuclei were operated on EPND 15. However, we found labeled neurons in the superior olivary nuclei and in a precochlear band destined for the dorsal cochlear nucleus (DCN) as early as EPND 5. Retrogradely labeled neurons were first seen in the nuclei of the lateral lemniscus in EPND 10 animals and in the ventral cochlear nucleus (VCN) at EPND 15. In a EPND 20 opossum, an injection limited to the medial border of the central nucleus of the IC resulted in the labeling of neurons in a restricted column along the medial border of the DCN and VCN, suggesting a topographic relationship. Finally, in the EPND 25-33 animals it was possible to place small, disc-shaped injections, oriented in the plane of the fibro-dendritic laminae of the central nucleus. Such injections resulted in well defined columns of labeled neurons in the cochlear nuclei. Injections centered in the ventromedial laminae of the central nucleus labeled neurons in the medial portion of the cochlear nuclei, those centered dorsolaterally labeled neurons in laterally positioned columns.

These results suggest that most hindbrain auditory nuclei have established projections to the IC by EPND 15 and that topographic order in the cochleocollicular connection is detectable shortly thereafter. Well defined topography exists by EPND 33. All of these events appear to take place prior to the onset of detectable hearing or eighth nerve activity as reported by Larsell and et al. (Arch. Otolaryngol. 40:233, 1944). Supported by BNS 8309245 and NS-16165.

151.3 MORPHOLOGICAL SUBSTRATES FOR BINAURAL INTERACTIONS IN THE MIDBRAIN: AFFERENTS TO THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS.
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The dorsal nucleus of the lateral lemniscus (DNLL) may provide a major, inhibitory input to the inferior colliculus (Shneiderman and Oliver, this volume; Adams and Mugnaini, Brain Res. Bull. 13: 585-590, 1984). Yet, the circuitry and the synaptic organization of the DNLL are not well known.

In this study, the inputs to the DNLL in the cat were examined with anterograde and retrograde axonal transport methods at the light microscopic level, and the reciprocal projections of the DNLL were studied with electron microscopic autoradiography. Fibers from anteroventral cochlear nucleus (AVCN) and lateral superior olive (LSO) terminated bilaterally in DNLL, while medial superior olivary inputs were ipsilateral. The contralateral AVCN always provided a heavy input to the DNLL. However, only the dorsolateral part of the DNLL received inputs from the ipsilateral AVCN. No inputs from the dorsal cochlear nucleus were found. The afferent axons formed horizontal layers which paralleled the dendritic fields of DNLL neurons. Inputs were topographical and are probably tonotopically organized. Although most inputs distributed evenly across the DNLL, inputs from the contralateral LSO were heaviest medially and from AVCN laterally.

The reciprocal, crossed projection between the DNLL also had a laminar pattern and dorsal-to-ventral topography that corresponded to injections restricted in either the dorsal or ventral part of the contralateral nucleus. When a cocktail of WGA-HRP and 3H-leucine was injected in the DNLL, the location of the heaviest anterograde labeling contralaterally corresponded to the location of the most intensely WGA-HRP-labeled cells. At the electron microscopic level, most labeled axonal endings from the contralateral DNLL contained pleomorphic vesicles and formed symmetric synapses. These endings were labeled more than 5 times background and constituted 22-32% of all boutons with pleomorphic vesicles. Numerous synaptic contacts were made by labeled endings on medium and large-sized dendrites and on somata. A few endings with round synaptic vesicles appeared to be labeled in one sample.

These data suggest that the reciprocal projections of the DNLL may be inhibitory. Since most ascending inputs to the DNLL may be excitatory, the responses of DNLL cells may be influenced by the laterality of acoustic stimulation. If the neurons in the DNLL on one side are more strongly driven, the crossed projection may suppress activity in the DNLL of the other side.

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151.4 AFFERENT INPUT TO MEDIAL SYSTEM OLIVOCOCHLEAR NEURONS FROM POSTEROVENTRAL COCHLEAR NUCLEUS IN GUINEA PIG.
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We have previously used the PHA-L (Phaseolus vulgaris leucoagglutinin) anterograde tract-tracing technique to demonstrate neural projections from the posteroventral cochlear nucleus (PVCN) to the superior olivary complex (SOC) in guinea pig. In the present study, we examined whether or not olivocochlear (OC) neurons, located in medial periolivary regions of SOC, are targets of PVCN neural projections.

Iontophoretic injections of PHA-L were made into PVCN in order to label the terminal endings of its projection neurons. Six days later, OC neurons were retrogradely labeled by perfusing HRP through the cochlea. Animals were sacrificed 24 hours after the HRP perfusions and the brain sections were processed to simultaneously visualize both labels. To determine the laterality of the PVCN afferent projections to, and the efferent projections from, OC neurons, both the PHA-L injection and HRP perfusion were done unilaterally in each case with the HRP perfusion either ipsilateral or contralateral to the PHA-L injection. By definition, medial system OC neurons were those HRP-labeled neurons located in dorsomedial (DMPO) and ventromedial (VMPO) periolivary and preolivary (PREO) regions of SOC.

Light microscopic examination revealed PHA-L-immunoreactive terminal varicosities of the PVCN projection neurons contacting medial system OC neurons located in DMPO, VMPO, and PREO regions. Most of the varicosities were en passant type endings making contact with dendritic processes. Additionally, we discovered unique patterns with regard to the laterality of the afferent/efferent projections. The majority of afferent input to the medial OC system originated in the contralateral PVCN and ended upon contralaterally-projecting neurons. This provides evidence that this OC system primarily projects back to the same cochlea from which it received sensory input, thus defining a recurrent cochlear pathway. Conversely, PVCN also projected to other medial system OC neurons that projected to the cochlea contralateral to their PVCN input, demonstrating the existence of a pathway linking the two cochleas. Overall, these results provide the first direct evidence of two cochlear pathways, each originating in one cochlea and projecting to either the same or the opposite cochlea. Both pathways consist of PVCN to medial OC system projections which may provide the anatomical substrate by which the CNS modulates sensory transduction at the auditory periphery.

151.5 BIOCHEMICAL BASIS FOR THE ACOUSTIC CHIASM? K.A. Hutson*, K.K. Glendenning and R.B. Masterton. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

The 'acoustic chiasm' allows the two hemifields of auditory space to become represented in the contralateral side of the upper levels of the neuraxis (Jenkins & Masterton, '82). Because the lateral superior olive is the one binaural nucleus in the hindbrain common to all mammals, it is the prime candidate to be the neural substrate of the 'chiasmatic' process.

However, the cells of LSO are known to be of the EI type: meaning that stimulation of the ipsilateral ear excites and contralateral stimulation inhibits; while the responses at the collicular targets of LSO are almost entirely of the IE type. This clear reversal in laterality from EI at LSO to IE at colliculus occurs despite both LSO's projecting directly, and nearly equally, to both colliculi. Thus, the question arises: how can a virtually complete functional reversal in interaural dominance occur in the presence of only a 50% anatomical decussation.

One obvious possibility is that not all of LSO's projections are excitatory. In the manner of Hunt, Künzle & Cuénod ('75), Streit ('80) and Rustioni & Cuénod ('82) we injected 3H-glycine in one colliculus of otherwise normal cats, sacrificed them from 1 to 48 hrs later, and processed sections through the hindbrain with conventional autoradiographic procedures.

The results show that as little as one hour after the injection, some fibers and cells of the lateral lemniscus become intensely labeled. After 6 hrs, many cells in ipsilateral LSO are intensely labeled. This labeling of cells in ipsilateral LSO stands in sharp contrast to the complete absence of labeling of cells in other auditory nuclei, whether ipsilateral or contralateral to the injection, even after 48 hr survivals. We compared the percentage of cells labeled with glycine with that obtained from an HRP injection in the colliculus. The close approximation of the two percentages suggests that every cell in LSO with an axon ending in the ipsilateral colliculus may be labelable by the retrograde transport of glycine.

It can be noted that if this technique is specific to glycinergic cells (Hunt, Streit, Knecht & Cuénod, '77) and that being glycinergic they are inhibitory in their effect at the colliculus, the curious disappearance of EI responsive cells between hindbrain and midbrain would be resolved. Half of LSO's EI cells reverse their allegiance (from EI to IE) by crossing the midline. The other half reverse their allegiance by remaining ipsilateral but being, themselves, inhibitory: in this sense, the acoustic chiasm is a biochemical 'decussation'.
(NINCDS 18877)

151.6 THE CHEMICAL ACOUSTIC CHIASM: ASYMMETRIC GLYCINE IMMUNOREACTIVITY IN THE BILATERAL PROJECTION FROM THE LATERAL SUPERIOR OLIVE TO THE INFERIOR COLLICULUS IN THE CAT.
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Principal neurons in the lateral superior olive (LSO) are excited by ipsilateral and inhibited by contralateral sound. The LSO, in turn, projects equally to both inferior colliculi (IC). Despite this bilateral projection from LSO, only contralateral stimulation is excitatory to most cells in the IC. Hutson et al. (this volume) suggest that neurochemically distinct excitatory and inhibitory projections from the LSO are the basis for this functional acoustic chiasm. They describe the uptake and retrograde transport of ^{3}H-glycine from the IC by principal cells in LSO, but only ipsilaterally. To verify this neurochemically asymmetric projection, we compared the glycine immunoreactivity of LSO cells following large, unilateral HRP injections in the IC. Glycine immunoreactivity was determined by postembedding PAP histochemistry on semi-thin, Epon-embedded sections.

In the superior olivary complex, the principal cells of the medial trapezoid nucleus were intensely immunoreactive (Gly+), as were their axons projecting to the LSO. Cells in the medial superior olive were unstained, but, like the principal cells in the medial trapezoid nucleus, they had few Gly+ perisomatic endings. In contrast, the cell bodies and proximal dendrites of LSO principal cells were heavily encrusted with GLY+ endings. Of these LSO cells, 40% had Gly+ somata and these were evenly distributed in the LSO.

Of the LSO cells retrogradely labeled from the contralateral IC, less than 1% were GLY+. In the ipsilateral LSO, two populations of retrogradely labeled cells were distinguished: many (>60%) were GLY+ and these occurred throughout the LSO. The remaining ipsilateral projection neurons were GLY- and concentrated in the lateral half of LSO. Retrogradely labeled marginal and hilar cells surrounding LSO had a similar immunostaining pattern.

We conclude that three distinct neuronal populations project to the IC from the LSO. 1) The entire crossed projection to the IC is Gly- and is presumably excitatory. 2) The uncrossed pathway includes a presumed inhibitory projection, which consists of all of the Gly+ principal cells. These results agree with Hutson et al. and suggest a neurochemical basis for the acoustic chiasm and for some of the binaural properties of IC neurons. 3) The remaining neurons of the uncrossed projection are Gly- and are confined to the lateral half of the LSO (frequencies below 10-12 kHz). This latter projection also may be excitatory; its existence implies that sounds below 10-12 kHz are subject to additional processing in the IC. (Supported by NIH grant NS14347)

151.7 CONVERGENCE OF COMMISSURAL AND CORTICOFUGAL INPUTS TO THE RAT INFERIOR COLLICULUS: TERMINAL AXONAL FIELDS AND CELLS OF ORIGIN. Kate D. Games and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The inferior colliculus, a primary station in the ascending auditory system, receives substantial corticofugal and commissural input. We examined the subdivisions of the rat inferior colliculus (IC) in Nissl and Golgi preparations and used horseradish peroxidase (HRP) to study commissural axons and cells of origin, and the auditory corticofugal projection, to the rat auditory midbrain. Pressure and iontophoretic HRP injections were made in the central and external nuclei of the IC, and reacted with the heavy metal-intensified DAB-$CoCl_2$ or TMB chromogens. Autoradiographic studies with [^{3}H]leucine or experiments with the anterograde transport of HRP seen with TMB were also used to study the auditory cortical projections. Our goals were to: *(1)* study the relation of anterogradely labeled axons and terminals to the pattern of retrogradely labeled cells, and *(2)* discern whether there is overlap or segregation of commissural axons with input from the auditory cortex (K.D. Games and J.A. Winer, *Exp. Brain Res.*, 1986: submitted).

Three major subdivisions of the inferior colliculus were identified. These resembled the architectonic plans proposed by Ramón y Cajal (guinea pig; *Trab. Lab. Invest. Biol.*, 1902, **1**:207-227) and Geniec and Morest (human; *Acta Oto-Laryngol.*, 1971, **suppl. 295**:1-33). The central nucleus, characterized by fibro-dendritic laminae, was bounded laterally by the external nucleus and dorsally by the dorsal cortex. Every subdivision sent and received commissural projections. The dorsomedial IC, including parts of the dorsal cortex, central nucleus, and adjacent intercollicular tegmentum, received the heaviest commissural input but contained few retrogradely labeled cells of origin. Other parts of the IC received comparatively sparse terminations even after HRP injections which labeled all parts of the contralateral cochlear nucleus. Input from the ipsilateral auditory cortex largely overlapped with the commissural projections in the dorsomedial IC, but not in the central nucleus. Commissural axons passing through the dorsomedial IC toward the central and external nuclei formed thin fascicles largely orthogonal to the fibro-dendritic, presumptive isofrequency lamina of the central nucleus. These axons had few branches, and terminated in delicate boutons. The ventral part of the central nucleus was sparsely labeled by either IC or cortical injections.

These observations suggest that the commissural, corticofugal, and cochlear afferents are partially segregated (*e.g.*, corticofugal versus cochlear) and partly overlapping (*e.g.*, corticofugal versus commissural). Thus, both parallel and convergent acoustic pathways are represented in the inferior colliculus.

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151.8 THE RAT MEDIAL GENICULATE COMPLEX: NUCLEAR ARCHITECTURE, NEURONAL ORGANIZATION, AND NEOCORTICAL CONNECTIONS. Stuart J. Cheff, David T. Larue, and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We studied the anatomy of the medial geniculate body in adult rats in Nissl and plastic-embedded preparations, in Golgi impregnations, and after horseradish peroxidase injections in the subdivisions of auditory cortex. We wished to integrate the results of these methods and to compare the chief organizational features of rat medial geniculate body with previous findings in the opossum (D.K. Morest and J.A. Winer, *Adv. Anat. Embryol. Cell Biol.*, 1986, **98**:1-96), cat (J.A. Winer, *Adv. Anat. Embryol. Cell Biol.*, 1985, **86**:1-98), bat (J.J. Wenstrup and J.A Winer, *Proc. Soc. Neurosci.*, 1987, **13**: in press), and human (J.A. Winer, *Hearing Res.*, 1984, **15**:225-247).

We found that most, if not all, major divisions recognized in these species could be delineated in the rat. In particular, the *ventral division* was large and contained tufted principal neurons arranged in fibro-dendritic laminae; these cells projected to auditory cortex in a topographic sequence: laterally situated thalamic cells sent their axons to the caudal aspect of the primary cortical field, while more medial cells projected more rostrally. In the *dorsal division*, two classes of thalamocortical relay cells--one with a bushy and the other with a radiating dendritic branching pattern--were seen; these were devoid of any conspicuous topography in their cortical projection, which lay caudodorsal to the primary field, nor was there evidence of fibro-dendritic laminar organization. The *medial division* contained several types of medium-sized neurons and larger cells, which projected to auditory and non-auditory cortex. In every thalamic division, small neurons were also recognized; these had a few slender dendrites, sparse, often short appendages, and were sometimes labeled by cortical injections. The architectonic parcellation proposed here is consistent with findings of glutamic acid decarboxylase (GAD) immunoreactivity of neurons and puncta (D.T. Larue and J.A. Winer, *Proc. Soc. Neurosci.*, 1987, **13**: in press), and with the distribution of corticothalamic axon terminals in autoradiographic studies (J.A. Winer and D.T. Larue, *J. Comp. Neurol.*, 1987, **257**:282-315). Compared to the cat, the main differences in the rat are the small size of the dorsal and medial divisions, the relative paucity of GAD-positive neurons, and the comparative reduction in the volume of neuropil. The tufted relay neurons in the ventral division appear to have been conserved in evolution and are readily recognized in marsupials, carnivores, insectivores, primates, and rodents as a fundamental feature of the lemniscal auditory pathway.

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151.9 ARCHITECTURE OF GAD-IMMUNOREACTIVE DENDRITES IN RAT MEDIAL GENICULATE COMPLEX: A POSSIBLE SUBSTRATE FOR DENDRODENDRITIC RELATIONS. David T. Larue and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

Since presynaptic dendrites are an important feature in the intrinsic organization of the ventral (lemniscal) division of the cat medial geniculate body (D.K. Morest, *J. Comp. Neurol.*, 1975, **162**:157-194), we wondered whether comparable structures might occur in the rat ventral division, and what their source might be. We studied glutamate decarboxylase immunostained dendrites from adult rats perfused with a formalin-zinc salicylate solution. Free-floating, 25-µm-thick frozen sections were processed by either the peroxidase anti-peroxidase or avidin-biotin procedures at a 1:2000 dilution of primary antiserum. Thalamic subdivisions were defined in Nissl, Golgi, or plastic-embedded preparations, and on the basis of axoplasmic transport studies (J.A. Winer and D.T. Larue, *J. Comp. Neurol.*, 1987, **257**:282-315; S.J. Cheff, D.T. Larue, and J.A. Winer, *Proc. Soc. Neurosci.*, 1987, **13**: in press).

Immunopositive neurons comprise only a small proportion of *ventral division* neurons, their primary dendrites may be 100-300 µm long and their trunks tapered gradually and branched sparsely, terminating in fine sprays (<0.5 µm thick) that form elaborate nests and were often capped with varicose expansions or bouton-like appendages. These dendrites often occurred in close proximity to other dendrites, especially those of larger non-immunoreactive neurons, and, on occasion, near neural somata. Most immunopositive neurons were less than 10 µm in diameter. In the *dorsal division* the number of immunopositive neurons was smaller, cells received few perisomatic puncta, and dendrites were rarely immunopositive beyond their initial 50 µm. *Medial division* immunoreactive neurons were varied in size, received many perisomatic puncta, and had sparse dendritic staining. Control sections incubated in pre-immune serum were free of specific immunostaining.

The form, number, and distribution of puncta differed significantly among thalamic divisions: in the *ventral division*, they were small, numerous, and mainly peridendritic; in the *dorsal division*, they were far finer, exceedingly sparse, and scattered; in the *medial division*, they were large and coarse, as numerous as in the ventral division, and terminated both upon neural somata and in the neuropil. These patterns no doubt embody different forms of GAD-labeled circuitry within the auditory thalamus.

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151.10 BRAINSTEM LOCATIONS OF PHYSIOLOGICALLY CHARACTERIZED STAPEDIUS MOTONEURONS IN CAT : SINGLE UNIT LABELING. S.R. Vacher*, J.B. Kobler and J.J. Guinan,Jr., Eaton Peabody Laboratory, Mass. Eye & Ear Infirmary, Boston, MA 02114; EECS DEPT., M.I.T..

Stapedius motoneurons in the cat are widely distributed around the facial nucleus and the superior olivary complex. From the effects of partial lesions of the stapedius motoneuron pool, it has been suggested that stapedius motoneurons in different brainstem locations might have different inputs and responses properties (McCue, M.P.,and Guinan, J.J., *Soc. Neurosci. Abstr.*, 9:1085, 1983). Recordings from single stapedius motoneuron axons (Kobler, J.B., Vacher, S.R., Guinan, J.J., *Soc. Neurosci. Abstr.*, 11:288, 1985) have shown that these motoneurons can be divided into 5 categories : 1)"Contralateral" units (10%) responded only to sound contralateral to the recording, 2)"Ipsilateral" units (28%) responded only to ipsilateral sounds, 3) "Binaural And" units (5%) responded only when sounds were delivered to both ears, 4)"Binaural-Or" units (44%) responded to sound delivered to either ear, 5)"Tonically active" units (13%) were active even in the absence of delivered sound. The purpose of the present study was to determine whether these electrophysiological categories of stapedius motoneurons are related to the location or morphology of cell bodies in the brainstem.

Cats were anesthetized with ketamine and paralyzed with gallamine. Single axons of stapedius motoneurons were recorded in fascicles which course from the facial nerve to the stapedius muscle. The category of each motoneuron was determined by its response to 100 msec. monaural and binaural tone bursts presented over a wide range of frequencies. Motoneurons were intracellularly injected with horseradish peroxidase (HRP). Labeled cell bodies were visualized with Tetramethylbenzidine in transverse sections of the brainstem.

Twenty-nine physiologically-identified stapedius motoneurons were labeled : four "Contralateral", eight "Ipsilateral", three "Binaural And", twelve "Binaural-Or" and one "Tonically active". The distribution of the thresholds at 1 kHz for these 29 labeled motoneurons was similar to the distribution obtained for all stapedius motoneurons from which we have recorded. Labeled motoneurons were found in all of the previously identified regions of the brainstem which contain stapedius motoneurons. However, motoneurons within a single category were found in relatively circumscribed locations. All four "Contralateral" motoneurons were found ventromedial to the facial nucleus. "Ipsilateral" motoneurons (7/8) were distributed in a layer between the facial nucleus and the superior olivary complex (SOC); one was dorsal to the anterior tip of the SOC. The three "Binaural-And" motoneurons were located dorsal to the SOC. Most of the "Binaural-Or" motoneurons (11/12) were located around the facial nucleus (ventral and dorsal to it); one was dorsal to the SOC. The "Tonically Active" motoneuron was found lateral to the facial nucleus. The cell bodies of the "Contralateral" motoneurons had the largest profile areas (827 μm^2 to 342 μm^2), while the "Binaural And" and "Tonically Active" motoneurons had the smallest areas (233 μm^2 to 292 μm^2). The areas for the other categories of units were distributed between the above extremes.

This work shows that stapedius motoneurons with distinctive electrophysiological properties have similar locations in the brainstem with limited overlap between categories. The results are consistent with the idea that the stapedius motoneuron pool is divided into groups which are spatially segregated in the brainstem and receive different patterns of inputs from the two ears. *(supported by Fogarty International Center 3 F05 TWO 3605-01S1 and NIH grant PO1NS13126).*

151.11 TONICALLY ACTIVE MOTONEURONS INNERVATING THE STAPEDIUS MUSCLE OF THE CAT J.B. Kobler, S.R. Vacher, and J.J. Guinan, Jr., Dept. of Otology and Laryngology, Harvard Medical School, Boston, MA 02115, Eaton-Peabody Laboratory, Mass. Eye & Ear Infirmary, Boston, MA 02114, and Dept. of Electrical Engineering and Computer Science, MIT, Cambridge, MA 02139 USA

Most work on the stapedius muscle has focused on its reflex contractions in response to loud sounds. Preliminary data have been presented indicating that some stapedius motoneurons exhibit activity in the absence of sound (Kobler et al., *Soc. Neurosci. Abstr.* 11:288, 1985); in addition, there have been electromyographic (EMG) recordings of tonic activity in the human stapedius muscle (e.g. Salomon and Starr, *Acta Neurol. Scand.* 39:161 1963). We now provide further direct evidence that tonically active units constitute a discrete sub-group of the stapedius motoneuron pool with special properties.

Experiments were performed on adult cats anesthetized initially with pentothal (5 mg/kg) followed either by decerebration or intermittent i.v. doses of ketamine. Recordings were made from single axons within small fascicles which course on the lateral surface of the muscle. In order to supress contractions of the muscle, the animals were paralyzed with gallamine and artificially respirated. Calibrated earphones were sealed in each external acoustic meatus and the animals were placed in a sound-proof chamber.

More than 1200 stapedius motoneurons were studied in 63 cats. Thirteen percent were found to have some tonic activity (TA) in silence. TA rates varied from <1 spike/sec to 90 spikes/sec with a mean rate of 12 spikes/sec. The intervals between spikes were regular as demonstrated in interval histograms; however, slow fluctuations in TA rates were frequently observed. When tested with 100 ms 1 kHz tone bursts, approximately 95% of units with TA responded to stimulation of either ear whereas only 50% of units without TA responded to stimulation of either ear. In four cats with many TA units, the mean contralateral threshold was lower for TA units than for non-TA units ($p<.05$); for two of these cats the ipsilateral thresholds were also significantly lower ($p<.05$).

In six cats a pair of electrodes was placed on the internal genu of the facial nerve in the floor of the fourth ventricle to stimulate the central portion of the stapedius motoneuron axons. By measuring orthodromic spike latencies following shocks, axonal conduction velocities were computed. Units with TA were found to have significantly slower mean conduction velocities (9.6±2.7 m/sec, N=27) than units without TA (18.3±5.1 m/sec, N=239; $p<.001$). This observation suggests that TA motoneurons have small diameter axons, consistent with the fact that the amplitude of TA unit spikes are relatively low and that these units are often lost quickly. We have also observed that in stapedius EMG recordings the electrical potentials of tonically active muscle units are usually of smaller amplitude than those recruited at high sound levels.

According to reports of stapedius muscle fiber histochemistry (e.g. Teig and Dahl, *Histochemie* 29:1 1972), about 20% of the fibers have characteristics of slow twitch muscle fibers. Given the well documented correlations between activity pattern and muscle fiber type, a reasonable hypothesis is that motoneurons with tonic activity innervate these slow muscle fibers, providing a tension in the muscle which persists in the absence of acoustic stimulation. It remains to be seen whether this background tension is a significant factor in the transmission of sound through the middle ear and to what extent this tonic activity is a function of the state of the animal or its past history of sound stimulation. *Supported by NIH grant PO1 NS13126 and Fogarty International Center 3 F05 TWO 3605-0151.*

152.1 THE COMPLETE SEQUENCE OF A cDNA CODING FOR BETA-NERVE GROWTH FACTOR FROM *MASTOMYS NATALENSIS* M. Fahnestock and R. A. Bell*. SRI International, Menlo Park, California 94025.

Nerve growth factor (NGF) from the submaxillary gland of the male mouse is a 7S complex consisting of three subunits: the βNGF dimer which contains the nerve growth promoting activity; the γ subunit, a member of the kallikrein family of trypsin-like proteases which is implicated in the cleavage of a carboxy-terminal dipeptide from the βNGF subunit; and the α subunit, a kallikrein highly homologous to γNGF but with no enzymatic activity. The female mouse contains much lower levels of these proteins than the male.

Mastomys natalensis is an African rat with high levels of βNGF in both male and female submaxillary glands. Preliminary evidence indicated the *Mastomys* NGF is isolated as a 5.1S complex containing α and β subunits but no γ subunits (Fahnestock et al., J. Cell Biochem. Suppl. 8B, p. 104, 1984). These results suggested that *Mastomys* might contain an altered β subunit, specifically a β subunit lacking the carboxy-terminal dipeptide cleavage site. In order to address this question, we isolated and sequenced a full-length cDNA clone coding for βNGF from *Mastomys*.

Poly A^+ mRNA was purified from male *Mastomys* submaxillary glands using guanidine thiocyanate. Northern analysis of this mRNA using mouse βNGF cDNA (M. Selby and W. J. Rutter, UCSF) as a probe demonstrated a single, strongly-hybridizing band at 1.3kb. The *Mastomys* mRNA was used as a template to synthesize double-stranded cDNA which was inserted into λgt10. This library was screened with the mouse βNGF probe.

A single, full-length cDNA clone coding for *Mastomys* βNGF was selected, and the sequence was determined using the chain termination method. The *Mastomys* βNGF sequence is 90% homologous to the mouse βNGF sequence. In addition, the *Mastomys* βNGF contains the same carboxy-terminal sequence as mouse, Arg-Arg-Gly, which the γ subunit processes in mouse. The sequence demonstrates that mouse and *Mastomys* βNGFs are extremely similar, and suggests that *Mastomys* could contain a γ-like protein that processes the carboxy-terminal dipeptide of its βNGF.

152.2 THE NGF COMPLEX FROM THE AFRICAN RAT *MASTOMYS NATALENSIS*. T.S. Burcham* and E. M. Shooter (SPON: D. Shelton). Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The submaxillary glands of the rat *M. natalensis* are a rich source of NGF (Aloe *et al. Exp. Cell Res.*,133:475, 1981). Unlike the mouse, both sexes of *M. natalensis* have submaxillary glands which contain NGF. The male *M. natalensis* and the male mouse have similar w/w quantities of NGF, and the submaxillary glands of the female have approx. half the NGF of the male. NGF has been found in many different cells of many species, yet in none of these instances has an NGF complex been fully characterized. Clearly the NGF from some sources exists as a high molecular weight complex, such as that from guinea pig prostate. We have therefore undertaken a study of the NGF complex from *M. natalensis* to further understand the biological role of the NGF complex and the α and γ subunits.

M. natalensis NGF complex was purified similarly to mouse 7S NGF (Varon *et al. Biochemistry*, 7:1296, 1968). Submaxillary glands from both male and female *M. natalensis* rats (age > 60 d), were processed separately. The glands were homogenized in 10 vols. of 10 μM $ZnCl_2$ at 4°C. The homogenate was filtered and centrifuged at 27,000xg for 15 min. The supernatant was centrifuged again at 100,000xg for 1 h. The supernatant was then concentrated by lyophilization. The lyophilizate was dissolved in 50 mM Tris-Cl/10 μM $ZnCl_2$ (pH 7.4 @ 25°C) and chromatographed on Sephadex G100. The fractions containing NGF activity were pooled, and subjected to DEAE ion-exchange chromatography. The 80 mM NaCl peak was concentrated by ultrafiltration, and then applied to a Sephacryl S400 column. Previous work continued purification with chromatofocusing, and found that the *M. natalensis* NGF complex contained only the α and β subunits. Results which will be presented indicate that the *M. natalensis* NGF complex is >95% pure after the S400 step (as determined by FPLC and non-denaturing-gel electrophoresis) and that chromatofocusing actually results in partial breakdown of the NGF complex into an αβ subunit. NGF and gamma activity were followed throughout the entire purification, and in every instance the peak in NGF activity was also a peak in γ esterase activity. The presence of the α subunit was evidenced by a positive immunoblot assay. When the stability of the *M. natalensis* NGF complex was examined by using the procedure of Palmer and Neet (*J. Biol Chem.* 255:5170, 1980), esterase activity was similar to that of the free γ subunit, suggesting that the NGF protein does not, as in mouse 7S NGF, inhibit γ activity.

A cDNA library has been constructed of both *M. natalensis* male and female poly(A)$^+$ RNA, and the library was screened using mouse NGF cDNA. Clones corresponding to *M. natalensis* βNGF, have been obtained and sequenced. Comparison of the *M. natalensis* β NGF sequence to that of other species will be shown. (This work supported in part by NIH NS07967-02.)

152.3 RETROVIRUS MEDIATED GENE TRANFER OF BETA-NERVE GROWTH FACTOR INTO MOUSE PITUITARY LINE AtT-20. M.P. Short*, D. Wolf*, C. Richer-Landsberg, C.Cepko* and X.O. Breakefield (SPON: W. Koroshetz) Molecular Neurogenetics Division, E.K. Shriver Center, Waltham, MA 02254; Genetics Dept. and Neuroscience Program (Neurology), Harvard Medical School, Boston, MA 02114.

Expression of the biologically active beta subunit of mouse nerve growth factor (beta-NGF) was conferred onto cultured AtT-20 mouse pituitary cells via a replication defective retroviral vector. The retroviral vector, derived from pLJ, includes a full length cDNA for beta-NGF (kindly provided by Dr. A. Ullrich) under the control of the retroviral LTR promoter, and a bacterial neomycin-resistance gene under the control of the SV40 early promoter. The cDNA for beta-NGF corresponds to the shorter mRNA species produced by most tissue which receive sympathetic innervation (Edwards, R.H., Selby, M.J. and Rutter, W.J.,Nature, 319:784, 1986). The retroviral vector was packaged in psi 2 cells. AtT-20 cells which produce essentially no endogenous beta-NGF, were then infected with this viral vector and cloned under neomycin-selection. Clones were evaluated for release of biologically active beta-NGF into the medium by neurite outgrowth by PC-12 cells. The biological activity released was relatively small, 1-10 beta-NGF beta per mg cell protein over 24 hr. Immune precipitation and SDS-polyacrylamide gel electrophoresis of labelled proteins from the media showed that the immunoreactive beta-NGF secreted from cells co-migrated with authentic mature beta-NGF, apparent MW 13000. The amount released over a 24 hr period was increased 2-3 fold by the presence of 10 nM epidermal growth factor or phorbol ester, which have been shown to increase transcription from the retroviral LTR promoter (Elsholtz, H.P. et al., Science 234:155 2, 1986). Following a 24 hr labelling period and replacement of medium, release over several hours was increased by addition of the secretagogue 1 mM 8-bromocyclic AMP, suggesting that at least some beta-NGF was stored in secretory vesicles. These studies, together with those on cultured cells lacking secretory granules, e.g. L cells (Brachet, P. Dicou, E., Exp. Cell Res., 150:234 1984), indicate that the beta-NGF precursor synthesized from the shorter mRNA species can be processed and secreted through either the regulated or constitutive pathway (kelly, R.B., Science 230:25, 1985). This vector thus provides a potential means of conferring beta-NGF expression onto a number of different cell types in culture and in vivo.

152.4 EXPRESSION OF THE cDNA FOR MOUSE BETA-NERVE GROWTH FACTOR (NGF) IN E. COLI. G.-L. Hu* and K. E. Neet (SPON: S. Rochel-Landau). Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

Nerve Growth Factor fulfills an essential role in the development and maintenance of neurons in the sympathetic, sensory, and (shown most recently) central nervous systems. The biologically active beta-subunit is a 13,000 Da polypeptide chain that contains no carbohydrate, possesses three intrachain disulfide bonds, and self-associates into a noncovalent dimer under most native conditions. Relatively little information is available on the structure/function relationships of this important neuronotrophic protein. Manipulation of the structure of NGF by site specific mutagenesis is a promising procedure with which to obtain more detailed information on its structural requirements. Expression of the cDNA for mouse beta-NGF in a bacterial system is the initial step in this process.

An expression vector for NGF was constructed from a pBR322 plasmid vector containing a 1068 basepair insert of nearly full length preprobeta-NGF [Scott, et al. Nature **302**, 538 (1983)] by excising a 373 base pair fragment (HaeII to PstI) encoding the entire mature NGF sequence. The fragment was inserted by blunt end ligation into a plasmid expression vector, pAS1 [Rosenberg, et al. Meth. Enzymol. **101**, 123 (1983)], which contains strong phage transcriptional and translational control elements that provide for regulated expression in E. coli. One recombinant plasmid, pAS1-NGF1(H2), was selected and shown to contain the insert by restriction mapping, southern blot analysis, and nucleotide sequencing.

The NGF protein was produced in E. coli transfected with the pAS1-NGF1(H2) by temperature induction and purified by ammonium sulfate precipitation and ion exchange chromatography. A purified fraction was obtained that demonstrated the authenticity of the expressed protein by appropriate behavior on cation exchange column, by reactivity with both monoclonal and polyclonal antibodies specific for beta-NGF, by size analysis on reducing SDS gel electrophoresis followed by immunotransblots, and, by biological activity that was inhibitable by NGF specific antibodies in the neurite outgrowth assay with PC12 cells.

The current system results in relatively poor yields of bioactive, recombinant NGF protein from E. coli. The low yield may be a result of any of the following: (a) low rates of synthesis of this particular protein, (b) proteolysis of the newly synthesized protein, (c) lack of tolerance of the foreign protein by the bacteria, or (d) the requirement for three disulfides to be correctly formed in the mature NGF. Efforts are underway to evaluate these problems and increase the amounts of NGF obtained. Nevertheless, the current expression of biologically active protein is significant and promises to open new lines of research on the structure and function of NGF. (Supported by NIH Grant NS24380.)

152.5 MULTIPLE MODES OF REGULATION OF β-NERVE GROWTH FACTOR SYNTHESIS IN C6 GLIOMA CELLS. J.P.Schwartz and W.Turner* Lab. of Neuronal Growth and Regeneration, NINCDS, NIH, Bethesda, MD 20892.

Many studies have demonstrated synthesis and release of nerve growth factor (NGF) by glial cells as well as glioma cell lines. We have investigated the regulation of synthesis of NGF in C6 glioma cells by measuring NGF mRNA (using a cDNA probe provided by A.Ullrich, Nature 303:821,1983), as well as NGF content by radioimmunoassay. Northern blot analysis shows a single NGF-hybridizing mRNA, 1.35 kb in size. Both NGF mRNA and NGF protein are increased by agents which elevate intracellular cyclic AMP, such as β-adrenergic agonists and forskolin. NGF mRNA is maximally increased by 2-3 hrs following either 100 nM isoproterenol (IP) or 5 μM forskolin - cyclic AMP is maximally elevated within 15-30 min. The rapidity of the increase of NGF mRNA suggests that regulation is occurring at the level of gene transcription: nuclear run-off assays are being carried out to verify this. The effect of the two agents, IP and forskolin, is not additive, supporting the idea that β-adrenergic regulation of NGF mRNA occurs via changes in cyclic AMP. NGF protein is significantly increased by 2 hrs and reaches a maximal content by 6 hrs - a proportional amount of NGF is released into the culture medium. All effects of IP are blocked by the β-adrenergic blocker propranolol, but not by either of two α-adrenergic blockers, phentolamine or phenoxybenzamine. The glucocorticoid dexamethasone has a small effect on NGF synthesis when added in the presence of dialyzed serum (to remove endogenous steroids) and potentiates the effect of IP. Dexamethasone does not change the cyclic AMP content of C6 glioma cells. The effects of other agents which may affect NGF content in animals, including T_3 and the ganglioside GM1, are being tested on C6 glioma cells as well as on primary cultures of normal glia. These results support the hypothesis that neurons may be able to regulate their own supply of NGF via a variety of neurotransmitter receptors known to be localized on glia.

152.6 K-252a: A SPECIFIC INHIBITOR OF THE ACTION OF NERVE GROWTH FACTOR ON PC12 CELLS. S. Koizumi,* M. L. Contreras, Y. Matsuda,* P. Lazarovici,* and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, Bethesda, MD 20892 and Kyowa Hakko Kogyo Co., Ltd. Tokyo 194, Japan.

K-252a, an alkaloid-like kinase inhibitor isolated from the culture broth of Nocardiopsis sp., selectively inhibits the actions of nerve growth factor on PC12 cells. At a concentration of 200 nM K-252a prevents, in a completely reversible fashion, neurite generation initiated by nerve growth factor. Under comparable conditions, K-252a has no effect on the generation of neurites produced by fibroblast growth factor, nor on the outgrowth produced by dibutyryl cAMP. K-252a also inhibits the induction of ornithine decarboxylase by nerve growth factor, but stimulates ornithine decarboxylase induction by epidermal growth factor and by a number of other effectors. Stimulation of phosphatidylinositol breakdown by nerve growth factor is similarly inhibited by K-252a, while stimulation by epidermal growth factor is enhanced. K-252a blocks nerve growth factor-induced heterodown regulation of the epidermal growth factor receptor, but not epidermal growth factor-induced homodown regulation of the epidermal growth factor receptor. Although the mechanism by which K-252a acts is not known, it appears not to be acting directly at the receptor because binding of NGF to its receptor is unaffected by the presence of 200 nM K-252a. It also does not seem to be acting by inhibiting either protein kinase C or cAMP dependent kinase because ornithine decarboxylase induction by either tumor promoters or dibutyryl cAMP is either unaffected or, in fact, stimulated by K-252a. K-252a, then, provides a new tool for the dissection and study of nerve growth factor-requiring processes in PC12 cells.

152.7 EVALUATION OF THE POTENTIAL ROLE OF PROTEIN KINASE C AND CALCIUM IN THE NGF INDUCTION OF ORNITHINE DECARBOXYLASE IN PC12 CELLS. D.S. Reinhold* and K.E. Neet. Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106.

The rat pheochromocytoma cell line PC12 undergoes a number of cellular responses when treated with nerve growth factor (NGF). Among these responses is the generation of neurites and the induction of ornithine decarboxylase activity (ODC). We have investigated the possible role of protein kinase C and calcium as second messengers in the NGF mediated induction of ODC in this cell line.

PC12 cells treated with 1nM NGF (concentration at which maximal effect is observed) for 5 hours at 37° showed a 20-50 fold induction in ODC activity over control cells, while cells treated with the phorbol ester phorbol 12-myristate 13-acetate (PMA) at concentrations of 50nM or higher produce about a 3-10 fold induction of the enzyme. The induction by each agent was inhibited by either cycloheximide or actinomycin D. However, when the protein kinase C activity of the PC12 cells was down regulated by incubation with 1uM PMA for 24 hours, the induction of ODC by PMA was completely inhibited, while the induction by NGF was not greatly affected. In addition, the maximal induction by NGF in untreated cells was significantly increased when 50nM PMA was added simultaneously with the NGF, suggesting the possibility that the two stimulants were acting through different but additive pathways. The mutant cell line $PC12^{nnr5}$ of Green et al.(Green et al., J. Cell Biol. 102, 830-843, 1986) was also tested for induction of ODC activity and it was found that PMA treatment caused about a 3 fold induction of ODC in these cells, while NGF had no effect on the enzyme levels. We interpret these data to mean that protein kinase C is not responsible for NGF induction of ornithine decarboxylase activity in PC12 cells. In addition to the induction of ODC, protein kinase C does not appear to play a role in NGF binding (both fast and slow) or internalization of bound NGF since down regulation of the kinase by PMA did not affect any of these parameters.

The possible role of phosphatidylinositol metabolism and calcium in the induction process has also been investigated. When cells were incubated with NGF in the presence of 20mM lithium, the induction of ODC activity was completely inhibited. The addition of 3.6mM EGTA or the calmodulin inhibitor W-7 (100uM) to the media also produced a complete inhibition of the NGF induction. Both the EGTA and W-7 inhibitions were partially, if not totally, reversible. These data suggest that both the phosphatidylinositol pathway and calcium may be important in the induction process. (Supported by an American Heart Association Northeast Ohio Affiliate Postdoctoral Fellowship #30-965.)

152.8 EARLY REGULATION BY NGF OF GENE SEQUENCES IN PC12 CELLS. F.Tirone and E.M.Shooter, Department of Neurobiology. Stanford University, School of Medicine, Stanford, CA. 94305.

Nerve growth factor (NGF) is a protein that acts as a regulator of the development and survival of sympathetic and some sensory neurons and as a differentiative factor for chromaffin cells. An in vitro model for the differentiative action of NGF is represented by the clonal cell line PC12, derived from a rat pheochromocytoma. In PC12 the shift from an endocrine to a neuronal phenotype is moreover partially mimicked by analogues of cyclic AMP (cAMP), which acts synergistically with NGF (Gunning,P.W., et al., J.Neurosci., 1:1805, 1981). In PC12 cells cAMP analogues modulate the synthesis and the phosphorylation of the same set of proteins as NGF, elicit the early outgrowth of short neurites and share with NGF the ability to increase the RNA levels of some genes. Moreover, the differentiative effect of NGF is dependent on RNA transcription.

In view of this, we decided to study the process of PC12 cells differentiation by NGF at molecular level, and to evaluate the role played in this by cAMP. The study has been undertaken analysing changes in gene expression due to NGF at the early time periods of its action, when the regulation by cAMP appears to be more likely. We constructed a complementary DNA (cDNA) library of 8000 clones from RNA of PC12 cells treated 1 hour with NGF(100 ng/ml).This library has been screened by hybridizing the bacterial colonies with single strand cDNA probes either from the same RNA used for the library or from RNA of control PC12 cells . In this way we were able to isolate four different clones whose levels of RNA were highly increased by NGF. The RNAs of all four clones were similarly increased by epidermal growth factor (EGF, 5ng/ml) while dibutyryl cAMP (dBcAMP,1 mM) affected only three of them. Northern analysis revealed that the four clones hybridized to four different species of RNA of 3.2, 2.7, 2.6 and 2.1 Kb.The 3.2 and 2.6 kb mRNA were maximally induced by NGF after 1 hour of treatment and then rapidly decreased, while the 2.7 and 2.1 kb mRNA levels showed a maximal induction at 2 hours , followed by a slower decrease. Three of the RNA species were induced as well by dBcAMP (the exception being the 2.1 kb), with a temporal pattern closely similar to the one observed following NGF induction. Partial sequence analysis of these clones, as well as Southern hybridization, did not show homologies with proto-oncogenes. These data show a similarity between NGF- and EGF-induced early gene expression and suggest that in PC12 cells the early increases of RNA levels observed in response to NGF occur, at least in part, through pathways not cAMP-dependent. We are currently evaluating the relevance of these findings in the process of differentiation by further characterizing the four clones.

152.9 ACTIVATION OF PROTEIN KINASE C IN PC12 CELLS MIMICS THE NGF INDUCTION OF THE CELL SURFACE THY-1 GLYCOPROTEIN. F.S. Walsh and P. Doherty. Institute of Neurology, Queen Square, London WC1N 3BG.

The polypeptide hormone nerve growth factor (NGF) has been shown in vitro to elicit a wide variety of cellular responses including promoting the survival and morphological differentiation of neurons dissociated from embryonic sensory and sympathetic ganglia. We have recently shown that NGF treatment of PC12 cells results directly in a 4-fold increase in Thy-1 gene transcription with accumulation of the mRNA species encoding Thy-1 readily detectable within 24hr of growth factor addition (Dickson et al, 1986, EMBO J. 5:3449-3453). In an attempt to elucidate the second messengers involved in the action of NGF, the effect of activators of protein kinase C on the expression of Thy-1 glycoprotein and the neural cell adhesion molecule (N-CAM) has been determined.

The addition of NGF to PC12 cells induced an approximate doubling in the cell surface expression of the Thy-1 glycoprotein and N-CAM after 24hr of culture. Half-maximal induction of Thy-1 was apparent at NGF concentrations (~0.1ng/ml) that had little effect on N-CAM expression. The protein kinase C activators, 12-O-tetradecanoylphorbol-13-acetate (TPA) and phorbol 12,13-dibutyrate (PdBu), were found to mimic the induction of Thy-1, but not N-CAM. When phorbol esters were added together with a maximally active concentration of NGF, their effects on Thy-1 expression were not additive. Treatment with phorbol esters did not mimic NGF induced neurite outgrowth, thus increased expression of Thy-1 is not consequential to morphological differentiation nor does it trigger the latter. The polyadenylation inhibitor cordycepin inhibited NGF and phorbol ester induced increases in Thy-1 expression in a similar fashion. Treatment of PC12 cells with phorbol esters under conditions reported to inhibit protein kinase C activity were found to inhibit NGF induction of Thy-1, but not N-CAM. Treatment of PC12 cells with cholera toxin, an agent that increases intracellular cAMP concentration was without effect on Thy-1 or N-CAM expression. In contrast cholera toxin (0.1-10ng/ml) was found to inhibit the NGF induction of the Thy-1 glycoprotein (Doherty and Walsh, 1987, J. Neurochem, in press).

Our results suggest that NGF can activate the transcription of genes encoding cell surface glycoproteins via at least two independent pathways, one of which directly involves activation of protein kinase C.

152.10 NERVE GROWTH FACTOR ACTIVATES A MAP 2 KINASE IN PC12 CELLS. G.E. Landreth, C. McCabe*, D.S. Smith* and C.K. Gittinger*. Dept. of Neurology, Medical University of South Carolina, Charleston, SC 29425.

Nerve Growth Factor (NGF) acts to direct the morphological and biochemical differentiation of the clonal pheochromocytoma line, PC12. The molecular mechanisms subserving the biological effects of NGF are unknown. We have investigated the ability of NGF to rapidly activate protein kinases, since these enzymes mediate many rapid hormonal effects.

NGF rapidly activated a protein kinase which phosphorylated the microtubule associated protein MAP 2. NGF transiently activated the enzyme, eliciting maximal activity within 5 min of NGF addition which diminished with longer periods of exposure. The NGF-stimulated phosphorylation of MAP 2 was similar to the phosphorylation of an endogenous PC12 cytoskeletal protein, pp250 (J.C.B. 100:677, 1985). MAP 2 competitively inhibited the labeling of pp250. The MAP 2 kinase, like the previously characterized pp250 kinase, had a marked preference for Mn^{2+} over Mg^{2+} and was inhibited by Na^{+}, suggesting the same enzyme species was involved.

The NGF-stimulated MAP 2 kinase remained associated with the detergent-insoluble cytoskeleton following extraction with 0.15% Triton X-100; however, the enzyme was found in both crude nuclear and soluble fractions following subcellular fractionation of PC12 cells. The MAP 2 kinase was isolated following chromatography on DE-52, eluting with 200 mM NaCl. The NGF-stimulated MAP 2 kinase activity did not require Ca^{2+} and was not inhibited by a specific inhibitor of cAMP-dependent kinase.

PC12 cells undergo a dramatic reorganization of their cell surfaces as an immediate consequence of NGF receptor occupancy. The rapid activation of a protein kinase which phosphorylates cytoskeletal proteins may play a role in these events.

152.11 NERVE GROWTH FACTOR-INDUCED CHANGES IN PROTEIN KINASE C LEVELS AND ACTIVITY IN PC12 CELLS. T. Hama, F. L. Huang,* Y. Yoshida,* K.-P. Huang,* and G. Guroff. Section on Growth Factors and Section on Metabolic Regulation, National Institute of Child Health and Human Development, Bethesda, MD 20892

Nerve growth factor (NGF) induces morphological and biochemical changes in its target cells. Among the biochemical changes are alterations in the phosphorylation of several cellular proteins. The phosphorylation of one of these, Nsp100, a cytoplasmic protein, is decreased after treatment of the cells with NGF. We have previously shown (Proc. Natl. Acad. Sci. USA 83, 2353 (1986)) that protein kinase C is involved in the cascade leading to the decrease in Nsp100 phosphorylation. The present experiments explore the short-term and long-term alterations in protein kinase C following NGF treatment.

PC12 cells were treated with NGF (50 ng/ml) for various periods of time between 30 minutes and 7 days. After the treatment, cells were collected and homogenized in 0.25 M sucrose containing 20 mM Tris-HCl, pH 7.5, 5 mM EGTA, 2 mM EDTA, 0.5 mM PMSF, and 50 mM mercaptoethanol. The extract was centrifuged at 100,000 xg and the cytosol collected. The pellet was extracted with 5% NP 40 in the buffer described above. Protein kinase C activity was measured using H3 histone as substrate. Alternatively, the protein kinase C level was examined by Western blots using polyclonal antibodies against protein kinase C or monospecific antibodies against the various isozyme forms of the enzyme.

Thirty to 60 minutes after NGF treatment the protein kinase C activity in the cytosol fraction was 3 to 4-fold elevated. The activity returned to baseline after several hours. Western blots of the same samples showed only a moderate (less than 2-fold) increase in protein kinase C content. Long-term treatment with nerve growth factor (1-2 days) increased the protein kinase C content, as seen in Western blots, about 2-fold. Using antibodies monospecific for the various isozyme forms, it was observed that the cytosolic kinase C is mainly type III, and it is the type III that is increased upon long-term NGF treatment.

From these data we conclude that the short-term increase in cytosolic kinase C activity is due to activation of existing kinase C, and the long-term increase is due to increased kinase C content. These results suggest that protein kinase C has a signaling function in the mechanism of action of NGF and, later, an important role in determining the neuronal characteristics of PC12 cells.

153.1 **DIFFERENTIAL EFFECT OF LOFEXIDINE (LOF) ON FOOD INTAKE IN RABBITS AND PRIMATES.** N.L. Katz, N. Sobaski* J. Young*, D. McGinness*, J.M. Davis and R. F. Schlemmer, Jr., Dept. of Pharmacodynamics, Univ. of Ill. at Chicago and Ill. State Psych. Inst., Chicago, IL 60612.

It is well established that central noradrenergic systems regulate feeding and eating behavior. For example, the central noradrenoreceptor agonist clonidine (CLON) stimulates food intake in both rabbits (Katz et al., Pharmacol. Biochem. Behav., 22:649, 1985) and Stumptail macaque monkeys (Schlemmer et al., Psychopharmacology, 61:233, 1979). Drugs which enhance the urge to eat may be useful in treating anorexia nervosa (Casper et al., Psychiat. Res., in press). With this in mind, we tested the effect of LOF, a drug structurally similar to CLON, on food intake in rabbits and primates.

LOF, in doses ranging from 0.005 to 0.02 mg/kg, failed to increase food intake above baseline control levels during the first and second hour following intramuscular injections into 10 Male New Zealand rabbits. Rabbits treated with 0.02 mg/kg of LOF ate less food over a 24 hour time span in comparison with saline-treated controls. The same rabbits given 0.01 mg/kg of CLON exhibited an increase in feeding behavior significantly greater than baseline control levels after one hour as expected. Doses of LOF of 0.05 mg/kg and greater induced a loss of postural tone, and some of the animals lost their righting reflex.

Twelve Stumptail macaque monkeys were injected intramuscularly with saline and LOF in doses ranging from 0.01 to 0.08 mg/kg. The amount of food eaten was measured 4½ hr. following the injections. At each dose tested, LOF significantly increased food intake above saline-levels. The effect of each dose on food intake was comparable, and no dose differed significantly from any other. A dose of 0.08 mg/kg of CLON increased food intake in the monkeys to the same extent as the LOF.

LOF exhibited a differential effect on food intake in rabbits and primates. It appeared that LOF, in contrast to CLON, had a more marked sedative effect in rabbits. This is the opposite of results of Graf et al., (Arzneim-Forsch, 32(II):931, 1982) who reported that LOF, in contrast to CLON, had less marked central sedative side effects in mice and rats.

153.2 INITIAL AMPHETAMINE ANOREXIA: RELEVANCE FOR TOLERANCE STUDIES. R. Eikelboom and H. Looy*. Department of Psychology, Queens University, Kingston, Canada, K7L 3N6.

While amphetamine (AMPH) anorexic tolerance has received considerable attention only rarely is the initial AMPH anorexia analyzed. But changes in the initial effects of AMPH can confound interpretations of tolerance.

Sensitization of AMPH anorexic effect has been reported to occur (Antelman et al., 1981), but the effect of prior AMPH on baseline milk consumption is unknown. Adult male Sprague-Dawley rats maintained at 80% of ad libitum weight were given a half hour experience with a sweetened milk/water (1:3) solution (milk). Animals then received 10 injections (IP) of 3 mg/kg d-amphetamine sulfate (A, n=17) or saline (S, n=16) over 6 days. Twenty-four hours after the last injection half the animals in each group were injected with AMPH and the other half with saline. A half hour later rats had 30 min milk access. An ANOVA revealed significant AMPH history and current drug effects but no interaction (A-A = 5.6±1.7 (SEM), A-S = 15.1±1.4, S-A = 13.8±3.4, S-S = 20.3±0.9 g consumed). Though prior AMPH has an effect on consumption, there does not appear to be any sensitization of AMPH anorexia.

Experiment 2 looked at weight manipulations. Half the rats (n=17) were maintained at 80% while the remainder (n=17) were not deprived. Animals received 4 daily 30 min exposures to milk. On the next two days saline or AMPH (3 mg/kg) injections (counterbalancing order) were followed half hour later by milk. An ANOVA of milk consumption for the AMPH and the preceding day found that the weight manipulation and the AMPH injection had a significant effect but the interaction was not significant (80-S = 31.1±1.0, 80-A = 10.2±1.8, 100-S = 27.5±1.0, 100-A = 3.2±0.8 g of milk consumption). AMPH anorexia appeared unaffected by weight manipulations.

Experiment 3 investigated milk familiarity. After 48 h of continuous milk rats were deprived to 80% and given a daily half hour of milk. On days 1 and 2 group 1 (n=11) was injected with 3 mg/kg AMPH or saline (counterbalanced) a half hour before milk. Group 2 was injected on days 8 and 9, and group 3 on days 19 and 20. ANOVA revealed significant drug, familarity and interaction effects. Consumption increased with saline (21.5±1.7, 30.5±2.2, and 34.3±1.7 g for groups 1, 2, and 3) but remained stable after AMPH (13.2±1.8, 14.2±3.2 and 13.5±3.6 g respectively). Thus only relative to baseline did the anorexic potency of AMPH increase with familiarity. These findings emphasize the role of baseline consumption in determining AMPH anorexia. Supported by grants from NSERC and OMH.

153.3 **COCAINE REDUCES THE EFFECTS OF α2 BLOCHADE OF NORADRENERGIC STIMULATION WITHIN THE PARAVENTRICULAR NUCLEUS OF THE RAT.** A. Clark* and P. Winn* (SPON: R. Pitman). Dept. of Psychology, University of St. Andrews, St. Andrews, Fife, Scotland KY16 9JU.

Infusion of noradrenaline (NA) into the hypothalamic paraventricular nucleus (PVN) induces feeding (Leibowitz, S. Pharm. Biochem. and Behav. 8: 153-175 1975). Selective pharmacological blockade appeared to indicate that this stimulation acted through post-synaptic α2 receptors (Goldman, C., Marino, L., and Leibowitz, S. Europ. J. Pharm. 115: 11-19 1985) as 6-OHDA lesion of pre-synaptic terminals, inhibition of NA synthesis, β and α1 blockade, were ineffective in inhibiting feeding. Studies of the peripheral sympathetic nervous system show that the response of a preparation to endogenously and exogenously released NA differs when in the presence of selective α-blockade. (Langer, S., Pharmacol. Rev. 32.45: 337-362 1981) suggesting a differential distribution of post-synaptic α1 and α2 receptors with regard to the synapse and its component re-uptake mechanism. A similar hypothesis has been used to explain recent blockade effects to tyramine-induced NA release (Luccelli, A., Barbone, M., and Grana, E., Pharm. Res. Comm. 17,8: 787-801 1985). Using the PVN as a model NA system we hypothesised that exogenously released transmitter exists at higher concentrations surrounding extra-synaptic receptors than at those protected by the pre-synaptic re-uptake mechanism. Were this the case, the extra-synaptic receptor would be of the α2 type. We also predicted that α2 blockade of the response to exogenous NA would be affected if the re-uptake mechanism were to be inhibited, allowing equal concentrations of NA to reach each receptor subtype. Using cocaine (40nmoles in 0.5ul) to block the re-uptake mechanism we found a 100% reduction in the potency of idazoxan (α2 selective) blockade. Idazoxan alone produced a 60% reduction in NA induced feeding. No changes were found in response to prazosin (α1-selective) blockade, nor did the presence of cocaine significantly alter the response to NA alone. We therefore conclude that some of the actions of exogenously administered NA may be due to its differential concentrations at receptor sub-types and thus that pharmacological identification of the receptors effecting a response should account for such topographically induced variations.

153.4 EFFECTS OF SELECTED ANTIDEPRESSANTS ON RAT FOOD INTAKE AND BODY ENERGY BALANCE OF RATS. G. Bautz*, S. Kowalik*, N. Spirt*, and L.A. Campfield (SPON. J. Sepinwall). Neurobiology and Obesity Research, Hoffmann-La Roche, Inc., Nutley, NJ 07110

Recent studies have emphasized 5-HT as a modulator of feeding and body energy storage. In order to further examine this hypothesis, we have compared the effects of five antidepressant drugs that alter 5-HT synaptic concentrations on food intake (FI), body weight (BW) and feed efficiency (FE). Sixty male Charles River rats (CD) were housed individually in metabolic cages with a 12:12 dark/light schedule. Water and powdered chow were available ad lib. and FI and BW were measured daily. After a 10 day baseline period, imipramine (I), amitriptyline (A), tranylcypromine (T), phenelzine (P), and fluoxetine (F) were presented as a food admix at a projected daily dose of 20 mg/kg for 13 days. An additional group received two daily doses of 10 mg/kg of imipramine by gavage (IG). Each of the drugs significantly reduced FI during the 13 day drug treatment period except A (% decrease: I-25%; IG-24%; T-30%; P-7.5%; F-18%). The same four drugs also reduced the rate of BW gain while A had no effect on BW (% decrease: I-46%; IG-36%; T-96%; P-25%; F-55%). FI and the rate of BW gain were maximally reduced during the first week by three of the drugs, with P having its effect during days 8-13. Analysis of FE during treatment demonstrated a significant 28% decrease in weight gained/gram of food eaten for I and a lack of effect for A. IG produced a significant 38% decrease in FE during the first week of dosing, however this effect was not present during days 8-13. Both MAO inhibitors (T and P) reduced FE during treatment. Although T reduced FE by 95%, signs of taste or food aversion was observed. The P group also demonstrated a significant 19% decrease in FE. In contrast to the T treatment, P had its major effect during days 8-13 of treatment. F significantly decreased FE by 45% during treatment. Blood levels of I in the I and IG groups at sacrifice were within the human therapeutic range. These results demonstrate that the five antidepressant drugs studied have differential effects on FI, BW and FE. Given that all five drugs used in this study can increase synaptic 5-HT, these results suggest that subtle differences on the determinates of 5-HT synaptic concentration and/or alternative mechanisms may underlie the observed differential effects on energy balance.

153.5 INTERACTIONS BETWEEN FOOD DEPRIVATION, MAINTENANCE OF TOLERANCE DEVELOPED TO AMPHETAMINE ANOREXIA AND CROSS TOLERANCE TO HALOPERIDOL CATALEPSY: NEUROCHEMICAL AND BEHAVIOURAL IMPLICATIONS. Angela Streather & Riley E. Hinson.* Dept. of Psychology, Univ. of Western Ontario, London, Ontario, N6A 5C2, Canada.

The maintenance of tolerance to AMP anorexia as a function of the level of food deprivation was investigated, as well as the effects of tolerance maintenance on HAL catalepsy. One hundred & twenty rats were administered 15 tolerance induction sessions where 3 mg/kg AMP ip was followed 20 min later by 30 min access to milk. Sixteen control rats received SAL instead of AMP during tolerance induction. Following tolerance induction, the 120 rats that had developed tolerance to AMP anorexia were divided into 12 groups whose mean milk intakes did not differ (~23 ml). Six of these groups continued to be maintained at 85% *ad lib* while the other 6 groups were allowed free access to food. One 85% and one 100% group was assigned to each of the following "extinction" treatments: 2 groups received milk 24h after AMP (AMP-MILK), 2 groups received AMP but no milk (AMP-0), 2 groups received saline followed by milk (SAL-MILK), 2 groups received saline but no milk (SAL-0), 2 groups received milk but no injections (0-MILK), 2 groups received no milk and no injections (0-0). The 2 saline control groups continued to receive saline followed by milk (CONTROL). After 14 training sessions (28 days) all animals received an AMP anorexia test. It was found that all rats in the 100% *ad lib* groups had lost tolerance to AMP anorexia. No loss of tolerance to AMP anorexia was found in the groups that had been maintained at 85% *ad lib*. Subsequently, a HAL catalepsy test (1.25 mg/kg HAL ip) was administered. Catalepsy was tested 45 minutes after HAL injection. The results showed that the animals that had retained tolerance to AMP anorexia were significantly less cataleptic than the animals that had lost tolerance.

These findings indicate that the level of food deprivation is the most important factor in the maintenance or loss of tolerance to amphetamine anorexia. Furthermore, the results demonstrate that the maintenance or loss of tolerance to AMP anorexia is reflected in the amount of catalepsy induced by HAL.

153.6 FEEDING AND PUMPING BEHAVIORS IN ADULT LAMPREYS. R. Kawasaki* and C.M. Rovainen, Dept. of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110

Parasitic species of lampreys feed by attaching to fish with their suckers, by forming a hole in the skin by a combination of tissue-liquefying saliva and rasping by the apicalis, and then by sucking and swallowing fluids and/or fragments of tissues. The goal of these experiments is to characterize sucking behaviors, including feeding, by pressure and EMG recordings in restrained spinal unanesthetized animals.

Adult silver lampreys, Ichthymoyzon unicuspis, were tested in a large chamber of lake water near 10°. Their suckers were placed over a hole in a plexiglass plate or onto a pithed goldfish. Vacuum in the sucker was monitored with a pressure transducer through the hole or through a syringe needle. Electomyograms were obtained with paired Teflon-coated silver wires, 125μm diameter, inserted as small hooks into target muscles. Minced goldfish muscle and skin were extracted with saline as test solutions. These and control volumes of lake water were injected under the lip of the sucker through a syringe needle into the sucker cavity.

The feeding behavior recorded in the present experiments is long-lasting (hours), has low frequency cycles of bi- or triphasic pressure changes (to -50-100cm H_2O) (see record below), is initiated by injections of fish extracts into the sucker and pharynx and terminated by injections of lake water, and has distinctive "radular" movements of the apicals. "Attack marks" were observed on a pithed goldfish following attachment and feeding behavior by the lamprey. In contrast to feeding, pumping behavior is characterized by high frequency cycles of monophasic vacuum, is initiated by fluid in the sucker and terminated at resting vacuum or volume, and has repetitive rostrocaudal movements by the apicalis. In conclusion, one distinctive type of periodic suction in the lamprey corresponds to a feeding behavior.

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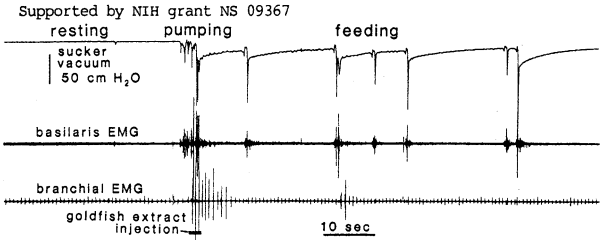

153.7 ATTENTUATION OF THE SEVERE EFFECTS OF FOOD-RESTRICTED INDUCED ANOREXIA IN VASOPRESSIN-DEFICIENT RATS BY SHORT, SEPARATED FEEDING PERIODS. C. H. Wideman and H. M. Murphy, John Carroll University, Cleveland, OH 44118

Brattleboro rats, which lack the hormone vasopressin, have the condition of diabetes insipidus and are referred to as DI rats. DI animals have been shown to be more susceptible to stress than normal animals. For example, DI rats with 22h of food restriction survive for a significantly shorter period of time, have a greater decrease in body weight, and develop significantly more ulcers than control animals (Wideman & Murphy, 1986). In that study, the animals subjected to 22h of food restriction were allowed 2h continuous access to food each day. The purpose of the present study was to determine whether or not two separate feeding periods, each with 1h access to food, would attenuate the deleterious effects noted in DI rats subjected to 22h of continuous food restriction. The subjects were 20 male homozygous DI rats and 20 male Long-Evans (LE) rats that were 5 wks of age and weighed between 80 and 100 g. Animals were randomly divided into 2 subgroups of 10 rats each. Group 1 rats (DI & LE) had ad-lib access to food (control group) and group 2 rats (DI & LE) were food restricted for 22h (experimental group). All rats were placed on a 12h light-12h dark cycle. The animals were subjected to a 7 day habituation period with ad-lib access to food and water, followed by a 9 day experimental period in which control animals had ad-lib access to food and water and experimental animals had ad-lib access to water and 2h access to food each day. These rats were fed during the 2nd and 8th hours of the light cycle. Results of this experiment in which the 2h feeding time was split, were the opposite of results where the 2h feeding time was consecutive for DI rats. DI food-restricted animals in this experiment were better able to maintain body weight and ate and drank significantly more than DI animals in the 2h consecutive feeding situation. In addition, with the split-feeding paradigm, all rats survived throughout the testing period. In comparing DI and LE food-restricted animals, there were no significant differences in % decrease in body weight or % decrease in water intake. DI animals had a significantly lower % decrease in food intake than did LE animals. By consuming a significantly greater % of food, DI rats maintained a similar weight loss pattern as compared to their LE counterparts. In addition, DI food-restricted rats developed significantly more ulcers than did LE food-restricted rats. The results of this study demonstrate that food restriction and vasopressin deficiency do not necessarily lead to severe anorexia and the eventual demise of the organism if the feeding periods provided are sufficiently separated so as to provide some time for digestion of food from the previous feeding period. Anorexia can be significantly attenuated in DI animals by short, separated feeding periods.

153.8 SCHEDULE-INDUCED POLYDIPSIA: ABSENCE OF POLYDIPSIA WITH SCHEDULED DELIVERY OF FOOD POWDER. D. Mumby*, C. H. M. Beck and T. J. S. Huh*. Department of Psychology, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Food deprived rats develop excessive drinking on an intermittent (60-s) schedule of food pellet delivery but not on an identical schedule of food powder delivery. Detailed coding of behavior revealed that the rats receiving powder did not develop any non-drinking behaviors to an excessive degree relative to the behavior of all rats on the first day of testing. Rats switched from powder to pellets, gradually developed polydipsia whereas rats changed from pellets to powder abruptly reduced drinking. In tests in which massed food was delivered, the powder rats appeared less aroused than the pellet rats as indicated by their lower bout frequency of rearing, locomotion, and investigation relative to that of the pellet rats. Pellets were eaten first by both groups in a preference test, in which limited amounts of both pellets and powder were presented simultaneously at the outset.

A number of factors were discounted as potentially contributing to the differences in behavior of the rats receiving powder and those receiving pellets. A cross-over design, using the rats as their own controls, eliminated the possibility that individual differences between members of the two groups could have accounted for the group differences. The food and water delivery mechanisms were the same for both groups. The powder was made from ground, sieved pellets thus eliminating flavor, nutrient content and moisture content as contributing factors to group differences. Equating the duration of access to the food and water mechanisms for the two groups, did not alter group differences.

In summary, schedule-induced polydipsia in rats is in reality, not simply schedule-induced. This polydipsia, known for its excessiveness and persistence, was abruptly terminated in rats without changing the amount of food eaten, the body weight deficit, or the schedule of reinforcement.

153.9 A CHRONIC STARVATION PARADIGM IN FEMALE RATS: STUDIES ON FEEDING, DRINKING, AND BODY WEIGHT REGULATION. J.K. Nishita, M.A. Robinson*, H.L. Sweatt*, M.A. Fichett*, E.H. Ellinwood, Jr., and W.J.K. Rockwell*. Dept. of Psychiatry, Duke Univ. Med. Cntr., Durham, N.C. 27710.

A novel paradigm was designed to study the effects of chronic starvation in developing female rats. In this paradigm, rats were required to crawl through Plexiglass tubes in order to obtain both food and water. Each experimental cage was divided in half by a Plexiglass wall that separated the food bin fixed to one side of the cage from the water source fixed to the opposite side of the cage. Two, 12-cm long tubes embedded in the wall were the only passageways from one end of the cage to the other. If a rat could not squeeze through the tube passageway, it was denied either food or water until its body size would permit the rat to pass across the divider. The onset and the severity of starvation was manipulated by varying the diameter of the tubes.

Sprague-Dawley female rats were time-bred and their litters were culled to 8 pups (6F:2M). Littermates were weighed every 5 days and weaned on postpartum Day 21. Pairs of females were randomly assigned to one of three experimental conditions (SM = small tube, LG = large tube, NT = no tube) on Day 25 for the remainder of the study. Vaginal opening was determined, and daily vaginal smears, body weights, and ingestive measures were taken up to Day 60. All rats easily adapted to the experimental tube apparatus since initially the tube size did not impede passage. Vaginal opening was similar in all pairs of rats (range: Day 33-39) and estrous cycles were not disrupted. Body weight differences between SM vs. NT groups were significantly different as early as Day 40 ($p<.03$), and by Day 43, body weights of SM vs. LG groups were also significantly different ($p<.01$). On Day 58, body weights for LG rats were significantly different from those of NT rats ($p<.04$). Significant changes in food intakes for each pair of rats paralleled those for body weight changes (e.g., On Day 58: SM = 22.6 $\pm$ 1.0g, LG = 34.4 $\pm$ 2.8g, NT = 41.1 $\pm$ 2.8g; $F(2,3) = 15.5$, $p<.03$; Mean $\pm$ SEM). Water intakes, however were not statistically significant (SM = 44.0 $\pm$ 2.0ml, LG = 63.5 $\pm$ 13.5ml, NT = 74.5 $\pm$ 1.0ml).

Previous studies of chronic starvation in the rat have used experimenter-controlled food deprivation and/or diet manipulation. Our paradigm includes the additional feature of permitting the rat to freely adjust its ingestive behavior according to induced body size contraints. Our paradigm may be particularly valuable in developmental studies of long-term malnutrition or clinical disease, such as anorexia nervosa.

153.10 EFFECT OF PURIFIED HUMAN SATIETIN ON MEAL PATTERNS OF RATS. V.E. Mendel and T.W. Castonguay Depts. An. Physiol., An. Sci., Nutr. Sci. and Food Intake Lab., Univ. of Calif., Davis, CA 95616.

Purified human satietin (p hSAT) has been shown to reduce food intake of both fasted (96 hr., J. Knoll, 1975) and non-fasted rats (Mendel, 1986). Semipurified human satietin has been shown to be aversive in rats (Bellinger and Mendel, 1985). If satietin makes animals sick, one would predict that both meal size and meal frequency during the day and night would be reduced in animals injected with satietin.

Twenty-four male Sprague-Dawley rats were divided into two groups (Phase I and Phase II). Phases I and II were run in sequence (14 days for each phase). In both phases, six rats were randomly selected to receive 2mg/kg p hSAT i.p. for 7 consecutive days. All rats received 1 ml sterile saline (St.S) i.p. for 7 days before p hSAT; control rats were injected with St.S. for all 14 days. Each phase (12 rats each) were placed in our individualized, computer monitored, feeding modules to continously measure food intake. Daily water intake was measured manually.

Daily food intake (FI) was reduced on average 22% by2mg/kg/rat, water intake was not significantly reduced. FI reduction occurred largely as the result of a 15.2% reduction of night meal frequency (NMF). Night meal size was not significantly reduced ($P>0.05$). Day meal pattern was unaffected by p hSAT.

These data suggest that purified satietin is not causing nausea because day meal frequency and size were unaffected as was night meal size. Purified satietin appears to affect only NMF.

153.11 DIET-INDUCED CHRONIC ELEVATIONS IN HEART RATE IN SHR AND SPRAGUE-DAWLEY RATS. V.L. Williams*, R.J. Contreras, J.F. Lorden, J.E. Cox, D.C. Tucker. Dept. of Psychology, University of Alabama, Birmingham, AL 35294.

Both genetic and environmental factors are known to influence cardiovascular function. We used Spontaneously Hypertensive Rats (SHR) and normotensive Sprague-Dawley rats to examine the effects of a high fat/high sucrose diet on both heart rate and blood pressure over a 10-week period. Male 60-day old rats were given either a high fat mash and sweetened milk (HF/M) diet or Agway Prolab chow pellets. Blood pressure and heart rate were measured weekly using tail plethysmography under light ether anesthesia. Body weight increased in the Sprague-Dawley rats fed the HF/M diet in comparison with pellet-fed Sprague-Dawley rats. This effect emerged during week 7 and persisted, with HF/M animals weighing 18% more than controls at week 10. The HF/M diet was not as effective in increasing body weight in the SHR rats: weights were 8% higher in HF/M animals at week 10.

The HF/M diet resulted in higher heart rates in both strains ($p<.0001$), although the highest heart rates were seen in the SHR strain ($p<.0001$). This increase persisted for the duration of the study. Differences between strains in response to the HF/M diet were apparent during the early weeks of treatment. Heart rate was the same (X=449$\pm$15 BPM) in both Sprague-Dawley groups at week 1, while SHR's fed a HF/M diet exhibited higher heart rates (X=522$\pm$14 BPM) at week 1 than those fed the pellet diet (X=452$\pm$13 BPM).

Despite the chronicity of differences in heart rate, we failed to see elevations in blood pressure due to the diet. In fact, blood pressure was lower in SHR animals fed the HF/M diet than in pellet-fed SHR controls ($p<.04$). As expected, the blood pressure for both genotypes increased with age ($p<.0001$), and the blood pressure levels of the SHR groups were higher than those of the two normotensive Sprague-Dawley groups ($p<.0002$). We suspect that the failure of the HF/M diet to induce higher blood pressure may be due to the unsaturated fat composition of the diet, which has been shown to protect against hypertension in other studies (Tobian et al, Hypertension, 1982). It is also possible that the HF/M diet will lead to higher blood pressure only in the presence of a more substantial degree of obesity than that observed in the present study or over a longer period of time. The increase in heart rate seen with the HF/M diet is consistent with the short term sympathetic activation shown to result from either a diet high in fat or sucrose as shown in previous studies from other laboratories. Thus, the chronic changes in heart rate seen in the present study may be due to a synergistic effect of both the fat and sucrose constituents of our HF/M diet in promoting sympathetic activation.

This research was supported by NIH Grant HL-38630 to R.J. Contreras.

153.12 ESTROGEN INDUCED ANOREXIA IN THE RAT: AN LICL COMPARISON SUGGESTS IT IS ESTROGEN INDUCED NAUSEA. C.R. Gustavson and J.C. Gustavson*. Psychiatric Ethology Laboratory. University of Texas Medical Branch, Galveston, Texas 77550.

Suppression of food intake after administration of estrogenic compounds is a broadly observed, well documented laboratory phenomena, and the correspondence between high estrogen periods with low food intake has been noted in many different free ranging vertebrates and invertebrates, during a variety of activities, e.g., migration, gestation, hibernation, incubation, lactation and courtship. Adaptive arguments for these observations abound, and have led to many speculations about conceptual CNS mechanisms (e.g., lowered set point) which may account for these observations. Sensitivity of the hypothalamic/pituitary axis to estrogen, and experimental evidence linking hypothalamic structures to food intake are sufficient reasons to base speculations upon, however no data we have reviewed provides more than phenomenological association.

We administered estradiol cypionate in peanut oil (0.4mg/kg body weight, i.m.), lithium chloride (LiCl, 2% body weight, 0.15m solution in tap water, i.p.), or peanut oil to 60 female Sprague Dawley rats 2 hours before, or immediately after, a 15 min. opportunity to consume apple juice. Every 2 hours after the first apple juice opportunity, for 8 hours, apple juice, grape juice, or both were presented for 1/2 hour. The amount of juice consumed was recorded following each presentation. Forty eight hours after juice was first presented, apple and grape juice were made available for 1/2 hour to each rat. The rats were immediately sacrificed. Uteri were rated for estrogen exposure. Blood was collected for estrogen determination.

Rats administered estrogen had enlarged, vascularized uteri compared to LiCl and oil injected animals indicating estrogen injections had the expected reproductive tract impact. Juice intake was suppressed similarly in rats given estrogen and LiCl injections compared to the oil injected rats. Juice intake suppression was delayed and prolonged in the estrogen injected animals compared to LiCl injected subjects. Apple juice intake at the end of the experiment was significantly and similarly lower in rats given estrogen or LiCl injections immediately after their first apple juice exposure compared to rats given estrogen or LiCl 2 hours prior to the first apple juice exposure, or oil injections after the first apple juice exposure.

These results, like our earlier results, suggest estrogen is a potent agent to establish conditioned taste aversions, and is phenomenologically similar to LiCl. We suggest the CNS mechanisms behind estrogen induced anorexia may have more to do with nausea than satiety and set point.

153.13 FUNCTIONAL SPECIFICITY OF HIGH AND LOW AFFINITY CORTICOSTEROID RECEPTORS. L.D. Devenport, S.S. Dallas*, and T.L. Thomas. Department of Psychology, University of Oklahoma, Norman, OK 73019.

Corticosterone (Cort) is variously reported to suppress or stimulate food intake; similar contradictory results are commonly found for body weight (e.g., 1, 2). Our analysis of the problem suggested that the issue might be resolved in terms of dosage and the dual population of Cort receptors, for which much evidence has now accumulated (3). Both Type I and Type II receptors bind Cort, but with high and low affinity, respectively. If these receptor actions could be experimentally separated by relatively pure receptor ligands, then distinctive metabolic actions of each might be revealed.

Aldosterone (Aldo) binds principally with Type I, but the synthetic glucocorticoid, RU28362, binds almost exclusively with Type II receptors. Neither binds significantly with transcortin or albumin. We infused these steroids at doses of 0, 3.4, 17.2, and 86.2 nmol/d with the object of observing patterns of feeding and weight gain corresponding to Type I and Type II receptor activity. Miniature infusion devices (Alza Corp) delivered the hormones in polyethlene glycol to separate groups of adrenalectomized male rats (ns = 8-10) for 28 days. Sham operated intact controls were also included for reference. The effects of the two hormones were linear and opposite: Type I receptor stimulation progressively increased intake and weight gain while Type II stimulation suppressed intake and slowed weight gain.

These findings suggest that Cort itself exerts separate dose-dependent actions that are at first anabolic but progressively more catabolic as Type I receptors become saturated and Type II increasingly stimulated. The results may resolve some contradictory reports of glucocorticoid action. They also suggest that normal fluctuations of Cort may engage qualitatively different processes, basal and peak levels meeting the successive requirements of fuel deposition and later mobilization.

1. Physiol. Psychol., 1982, 10, 399.
2. Nutr & Behav., 1984, 2, 115.
3. Front. Neuroendocrin., 1986, 9, 169.

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153.14 ESTROGEN PRODUCES CONDITIONED TASTE AVERSIONS IN RATS WHICH ARE BLOCKED BY ANTIHISTAMINE. A.G.Rice*, A.Lopez* and J.Garcia* (SPON: Gary D. Novack), Department of Psychology, UCLA, Los Angeles, Ca. 90024.

Estrogen has many effects upon feeding behavior, metabolism, and body weight. Recently Gustavson has shown that an injection of estradiol cypionate when paired with saccharin flavored water produce conditioned taste aversions (CTA) for saccharin. Estrogen is known to produce nausea in humans and induce the release of histamine in reproductive tissue. Nausea and histamine are both conditions that when paired with a taste subsequently render that taste as unpalatable. People with endocrine disorders such as anorexia nervosa and PMS "sweet cravers" exhibit feeding and taste abnormalities for which the specific cause is not known. It may be that some of the effects of estrogen that are in common with conditions and agents known to produce CTAs have a role in producing endocrine based feeding pathology. Questions arise as to how estrogen functions as a CTA inducing agent. Would a masculinized female be more susceptible to estrogen induced food aversions as males are? Would an antihistamine, by blocking the effects of estrogen induced histamine release, prevent the formation of estrogen produced aversions?

Female and male rats were habituated to a daily drinking schedule of a 10 min measure plus supplemental water. On conditioning day animals received saccharin flavored water followed 30 min later by a s.c. injection of estradiol cypionate. Starting approximately 5 days later animals were again presented with saccharin daily for at least 10 test days. Female rats did not display aversions when doses of .00028 and .00034 mg/g of body weight were used while male rats showed strong aversions at these doses. Using doses of .0016, .0028, and .004 mg/g of body weight female rats did display strong aversions.

For the estrogen implant experiment female rats will be ovarectomized and then implanted with an osmotic minipump containing estradiol cypionate. For the next five days while the pump is implanted, the regular chow food will be taken away and either AIN or C21 diet will be made available during that time. After the fifth day the pump will be explanted and animals will be presented with the choice of both AIN and C21 diets for approximately four days constituting preference tests. Pilot animals using this procedure have already shown that consumption of the paired food is decreased by the third implant day, and that animals reject the paired diet in preference tests.

To test the hypothesis that histamine has a primary role in producing estrogen induced CTAs animals were injected with the antihistamine chlorpheniramine maleate 30 min before saccharin water was presented which was followed immediately by injection of estrogen. When tested a few days later female rats that did not receive the antihistamine showed strong estrogen induced saccharin aversions. Control animals that received only the antihistamine and oil vehicle, or only water and oil vehicles, on conditioning day displayed no aversion to saccharin. Importantly, the group of females that were injected with both the antihistamine and estrogen on conditioning day consumed equal amounts of saccharin as the control animals. This demonstrates that use of an antihistamine can block estrogen induced CTAs and implicates estrogen's effect upon histamine release as having a primary role in degrading the palatability of a paired food. A similar procedure using male rats is in progress.

153.15 GLUTAMATE DECARBOXYLASE ACTIVITY IS INCREASED IN THE VMN-AREA OF HYPERPHAGIC RATS. J. L. Beverly* and R. J. Martin. (SPON: J.M. Bowen). Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602

The transduction of energy metabolism into neural activity may be mediated by the formation of GABA and have a role in hypothalamic feeding regulatory mechanisms (Panksepp and Meeker, Brain Res. Bull. 5, Supp. 2:453, 1980). We recently reported an increase in glucose flux through the GABA shunt in the VMN-area in some, but not all, models of hyperphagic rats (Beverly and Martin, Fed. Proc. 46:1482, 1987). To further characterize GABA metabolism during hyperphagia the activity of the rate-limiting enzyme in GABA formation, glutamate decarboxylase (GAD), was measured in ventral hypothalami of four rat models: 1) Zucker lean and obese; in Sprague-Dawley rats (200-225 g); 2) after restriction for 7 days at 40% ad libitum (fed as a single meal at 1800 or 0700 hr); 3) 20 days following streptozotocin-induced diabetes; and 4) 35 min. following an injection of insulin (4.5 U/kg, i.p.). Immediately following decapitation a 1 mm slice directly anterior to the median eminence was placed in chilled saline and an 18 gauge (VLH) or 20 gauge (VMN-area) punch removed, homogenized and frozen on dry ice. GAD activity was assayed (Tappaz et al., Brain Res. 108:371, 1976) within 48 hr. Rates of GAD activity were elevated in the VMN-area of all models when compared to respective controls [1) obese = +18%, P=.05; 2) restricted = +35%, P=.001; 3) diabetic = +41%, P=.01; 4) +insulin = +25%, P=.05]. A decreased rate in GAD activity (-16%, P<.05) was noted in the VLH of rats treated with insulin. Attenuation of serum glucose levels by a gastric preload (2 ml of a 50% glucose soln.) blunted both the increases in food intake and GAD activity (+16%, P=.07) in rats treated with insulin. Increasing the amount of insulin injected increased GAD activity, relative to saline controls, in the VMN-area (3U/kg=+15%; 4.5U/kg=+25%; 6U/kg=+90%). Correlations of serum glucose to GAD activity in the VMN-area of r=-58(P=0.001) and VLH of r=.43 (P=.02) were noted. These data suggest an association between glucose availability to GABA-ergic neurons and GAD activity, at least in the VMN-area. The functional significance of increased GAD activity and the interaction of insulin and glucose on GAD activity require further investigation.

153.16 THE EFFECT OF HYPOTHALAMIC LESIONS ON HEPATIC THERMOGENESIS. A. P. Jones & J. Carrillo*, Pitzer College, Claremont, CA. 91711

Investigations into the role of the hypothalmus in the regulation of body weight have a long tradition in the neurosciences. The now classic demonstration by Hetherington and Ranson that electrolytic lesions of the ventromedial area of the hypothalamus produce massive obesity and hyperphagia in rats provided the impetus for much of this research. Early interpretations of this phenomenon tended to focus on the role of the ventromedial area as a "satiety center" with primary emphasis placed on its role in the control of food intake. More recent interpretations however have emphasized the role of the area in metabolic regulation. It has been noted that lesions of the ventromedial area lead to a variety of metabolic disturbances including hyperinsulinemia, increased lipoprotein lipase activity, decreased lipid mobilization and decreased thermogenisis from Brown Adipose Tissue. The role of decreased thermogenesis in the etiology of obesity has gained increased recognition in the last decade. The majority of these studies have focused on deficits in a protonconductance pathway in Brown Adipose Tissue mitochondria. Recently, significant deficits in heat production have also been reported to result from deficiencies in hepatic cytochrome P450 dependent monooxygenase activity. That such deficiencies may impact on body weight is suggested by the finding that P450 is depressed in genetically obese ob/ob mice and Fa/Fa rats and in ovariectomized rats. Given the extensive and metabolically relevant neural connections which have been demonstrated between the liver and the ventromedial area of the hypothalamus we decided to investigate whether lesions of this area affect P450 dependent metabolism.

Female Sprague-Dawley rats weighing 250g. were anesthetized with Nembutol (40 mg/kg) and then received either an electrolytic lesion to the Ventromedial area (exptl grp) or a sham procedure (control grp) which consisted of an identical surgical placement but no current was passed through the electrode. Food intake and body weight was measured for the next two weeks, following which, the animal was sacrificed by decapitation. Brains were placed in formalin for subsequent histological verification of lesion placement and livers were processed for the determination of P450. Animals in the experimental group ate significantly more food and gained significantly more weight than their control counterparts, however P450 levels were virtually identical in the two groups. These data suggest that although depressed P450 levels may be relevant to obesity seen in other animal models, it does not contribute to the obesity in rats with ventromedial hypothalamic lesions.

153.17 RAT SATIETIN (rSAT) INFUSED INTRACEREBROVENTRICULARLY (ICV) DOES NOT PRODUCE CONDITIONED TASTE AVERSION IN RATS. L.L. Bellinger and V.E. Mendel. Dept. Physiol., Baylor Coll. Dentistry, Dallas, TX, 75246 and Dept. Animal Physiol., Univ. of California, Davis, CA, 95616.

Satietin (Knoll, Physiol. Behav. 23:497.1979) is a putative satiety agent found in human serum and a variety of animal sera, including rat. When human (h) SAT was infused ICV in rats it was a strong anorexigenic agent. The mechanism of hSAT action is unknown but in a recent study (Bellinger and Mendel, Pharm. Biochem. Behav. 23:559.1985) hSAT produced conditioned taste aversion when infused ICV. Thus hSAT may be aversive in rats. On the other hand, the aversion may have been due to contaminants or a cross species reaction. In the present study rat plasma was extracted for rSAT. Male Sprague Dawley rats (275-315g) were fitted with chronic third ventricle cannulas. The rats (LD 12:12, lights out at 1330h) were given access to water, in calibrated bottles, for one hour/day (1230-1330h) and food ad libitum for ten days. At the end of this period the rats were divided into two groups (GRP) and infused ICV with 10 ul of saline (SAL), day 1. Thirty minutes later at 1230h the GRP were given either water flavored with (GRP 1) 0.5% banana (BFW) or (GRP 2) 0.5% almond (AFW) extract instead of tap water. Fluid consumption (FC) and food intake (FI) were recorded (1230-1330h and 24h for FI). The next day (day 2), GRP 1 was infused with saline and given AFW while GRP 2 was infused with 100 ug/rat of rSAT and given BFW. On day 3 the rats were given tap water and on day 4 they were given a choice between AFW and BFW. On day 1 FI and FC of the GRPs were similar. Infusion of rSAT on day 2 suppressed both one (2.6±0.4 1.2±0.5g, $P<0.02$) and 24 (13.8±1.2 vs 8.7±1.5g, $P<0.02$) hour FI. No FI differences were seen on day 3. Body weight decreased more after rSAT then after saline infusion on day 2 (-5.8±1.5 vs -12.2±1.5g, $P<0.01$). This difference remained the following day (+1.6±2.9 vs -10.1±2.9g, $P<0.01$) even though FI and FC did not differ between the groups. The GRPs showed neutral preference for BFW and AFW on day 1. The FC of GRP 2 was suppressed after rSAT on day 2 (6.4±1.0 vs 1.9±0.7 ml, $P<0.01$). On day 3 FC of both GRPs was similar. During two bottle testing similar amounts (GRP 1 vs GRP 2) of AWF (11.1±2.3 vs 10.9±3.0 ml) and BFW (7.0±2.0 vs 8.6±2.8 ml) were consumed. These data suggest that rSAT, unlike hSAT, does not produce taste aversion in rats and may not suppress ingestion by aversive means. Therefore, SAT may be a candidate for a physiological satiety agent.

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GENE STRUCTURE AND FUNCTION II

154.1 INTERACTIONS OF THE HYPOMYELINATION MUTATIONS shi and shi^{mld} with qk: ANOTHER PIECE OF THE PUZZLE. M.K. Wolf, J.-B. Gow*, M.E. Pomeroy*, and S. Billings-Gagliardi. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01655.

Double mutant mice which simultaneously express mutations at two of the loci which produce CNS hypomyelination, qk, shi, or jp, have been bred. Such mice may have phenotypes that suggest complex interactions between the loci. Interactions involving allelic mutations at the shi and jp loci are particularly surprising and unpredictable. Here we compare the white matter morphology of B6C3 hybrid-based $qk/qk*shi^{mld}/shi^{mld}$ ($qk*shi^{mld}$) mice to that of qk/qk*shi/shi (qk*shi) to learn more about these interactions of the myelin genes. Both $qk*shi^{mld}$ and qk*shi are produced by intercrossing the appropriate unaffected double heterozygotes resulting in 5 possible affected genotypes, including the double homozygote. Backcrossing the double heterozygote to the relevant single homozygotes yields 4 of the same possible affected genotypes, but not the double homozygote. In each case, one distinct morphological phenotype produced by the intercrosses is not produced by the backcrosses, confirming that this is the double homozygote (double mutant) phenotype.

At P-21 both qk*shi and $qk*shi^{mld}$ have approximately 20% of the number of myelinated axons found in comparable CNS regions of the single mutants. The vast majority of myelin sheaths present have fewer than 4 lamellae and only partially enclose axons; they are uncompacted, and show accentuated intraperiod lines. No evidence of major dense line has been seen in qk*shi white matter; however, in $qk*shi^{mld}$ a rare sheath has interrupted (abnormal) density at the correct location for the major dense line. This is far less frequent than in shi^{mld} homozygotes of comparable age, but otherwise has the same characteristics. In both double mutants the white matter is filled with parallel bundles of small oligodendrocyte processes. The exuberant whorls and tangles of myelin-like membranes and macrophages characteristic of qk are not present. Thus in both qk*shi and $qk*shi^{mld}$ double mutants the amount of myelin is drastically reduced compared to qk, shi, and shi^{mld}, suggesting intergenic synergism, but the morphology of myelin and oligodendrocytes is like shi or shi^{mld}, rather than qk, suggesting suppression of qk by both shi locus mutations. We conclude: 1. There is morphological evidence for two kinds of strong interaction between qk and both shi^{mld} and shi. 2. shi and shi^{mld}, which interact very differently with jp locus mutations, interact similarly with qk. Supported by NIH grant NS-11425 (Javits Award).

154.2 QUANTITATIVE DIFFERENCES BETWEEN USA AND SWISS shi^{mld}/shi^{mld} MICE. S. Billings-Gagliardi, J.-M. Matthieu*, D.A. Kirschner*, A.-L. Kerner*, and M.K. Wolf. Dept. of Anatomy, U. Mass. Med. School, Worcester, MA 01655; Lab. de Neurochimie, CHUV, U. de Lausanne, Lausanne, Switzerland; Dept. of Neuroscience, Children's Hospital, Boston, MA 02115.

Studies of the dysmyelinating mouse mutation shi^{mld} in our laboratories in the USA and Switzerland report different CNS myelin protein levels and white matter morphology. To resolve the apparent discrepancies, we exchanged breeding stocks and reinvestigated both sets of animals. Parallel developmental studies (15-80 days) confirm substantial quantitative differences between shi^{mld}/shi^{mld} mice (abbreviation: shi^{mld}) with Billings-Gagliardi and Wolf's "USA" versus Matthieu's "Swiss" genetic backgrounds. 1. Behavior: USA shi^{mld} have fewer and less severe convulsions, longer lifespan, and more chance of rearing offspring. 2. Biochemistry: In both Swiss and USA shi^{mld} CNP specific activities and MBP measured by RIA in total brain homogenates increase with age; however, at 50 days and older the levels of both proteins are twice as high in the Swiss. 3. Morphology: At a given age, the number of optic nerve axons myelinated is always greater in Swiss shi^{mld}. The proportion of myelin sheaths containing "MDL", i.e., any apposition of cytoplasmic membrane faces (usually partial and/or abnormal in shi^{mld} myelin) increases dramatically between 20 and 50 days in both Swiss and USA animals; however, twice as many sheaths have "MDL" in the Swiss, except at the youngest age.

These differences between Swiss and USA shi^{mld} could reflect either different mutant alleles at the shi locus or different regulatory genes influencing the expression of the same shi allele in Swiss and USA mice. Evidence for the regulatory gene hypothesis is provided by MBP measurements in homogenates of 30-day wild-type Swiss and USA brains. Swiss wild-type have significantly more MBP (23.11 ± 1.04 ng MBP/µg brain homogenate protein, $n = 8$) than USA (16.88 ± 0.37, $n = 13$). This hypothesis was further tested by crossing Swiss and USA shi^{mld} to obtain hybrid "international" mice. If Swiss and USA mice carry two different shi locus alleles, one might expect the "international" shi^{mld} double heterozygote to have intermediate protein and morphological measurements, as has been observed for the shi/shi^{mld} double heterozygote . However, "international" shi^{mld} have more MBP and CNP, and acquire significant amounts of "MDL" earlier than either Swiss or USA shi^{mld}, suggesting heterosis of multiple regulatory genes. Supported by NIH grants NS11425, 20824, 07264 and Swiss National Science Foundation grant 3.142.85.

154.3 A cDNA CODING FOR THE CARBOXY-TERMINAL REGION OF RABBIT NEUROFILAMENT PROTEIN H: A PROBE FOR THE ROLE OF H IN NEURODEGENERATIVE DISEASES. D. Soifer, K. Mack* and H.M. Wisniewski*. NY State Institute for Basic Research in Developmental Disabilities; and CSI/IBR Center for Developmental Neuroscience, Staten Island, NY, 10314.

Neurofibrillary tangles are only found in neurons which normally express the high-molecular weight neurofilament protein, NFP-H. Several antibodies raised against neurofilaments, especially the tail domain of NFP-H, have been reported to react with paired helical filaments (PHF) on tissue sections. We have prepared a cDNA library, in the expression vector, lambda gt11, from rabbit brain mRNA. A cDNA clone which directed the synthesis of a fusion protein which reacted with monoclonal antibodies against epitopes near the carboxy terminal of NFP-H, has been selected and characterized. This cDNA is 889 bases long and codes for the c-terminal-184 amino acids of NFP-H. The selected cDNA hybridizes to a 4.7 kB message on northern blots of rabbit brain RNA. The sequence of the cDNA includes highly repetitive regions, including one duplicated 60-base segment. The deduced amino acid sequence includes 9 serine residues, each surrounded by a similar group of amino acids: Ala.Lys.Ser.Pro.(Glu,Val).Lys. (5 have Glu., 4 have Val). These serines, adjacent to predicted beta-turns at each proline, are presumably sites for phosphorylation. Comparison of the sequence of the rabbit cDNA with the sequence of a similar region of a rat cDNA (Robinson et al, FEBS LETT 209:203 1986) indicates stretches of homology, especially in one 49-base segment. Although both cDNAs code for very hydrophilic polypeptides, there is considerable species diversity between the primary structures of the tail regions of the rat and rabbit NFP-H proteins. This diversity may account for the failure to induce tangles of neurofilaments in animals, such as rats, following treatment with doses of aluminum which are sufficient to induce such tangles in rabbits and to bring on seizures and behavioral pathology in both rats and rabbits. (Supported by the ALS Association and the NY State Office of Mental Retardation and Developmental Disabilities.)

154.4 DNA SEQUENCES INVOLVED IN MPTP NEUROTOXICITY. C. Mamalaki, R.C. Douglas*, S.G. Carlson*, C.M. Dersch* and M.M.S. Lo. National Institute on Drug Abuse, Addiction Research Center, Baltimore MD 21224.

Pheochromocytoma (PC12) cells infected with a recombinant retrovirus and selected on MPP+ (an active metabolite of MPTP) produced resistant mutants. The phenotypes and genotypes of 48 different mutants were analysed. The neurochemical analysis showed a distinct group of mutants which lacked catecholamine uptake, whereas two other groups have either completely normal or defective dopamine uptake. Other neurochemical markers appear to be either normal or reduced.

The chromosomal location of viral integrants showed in most cases only a single copy of the retroviral sequence. Restriction enzyme and Southern blot analysis of ten selected clones, using probes derived from the viral sequence, showed proviral sequences integrated into the same three chromosomal regions. These regions contain putative genetic sequences which normally code for proteins involved in MPTP neurotoxicity

DNA sequences from two of these gene targets were cloned from the MPP+ resistant mutants in EMBL3 or λgt11. These sequences have been subcloned into pBR and characterised by detailed restriction mapping. The presence of corresponding cellular mRNA in normal PC12, fibroblasts, and tissues was analysed by Nothern blot using probes derived from the rescued genomic flanking sequences. The expression and function of these genetic sequences will identify proteins, such as dopamine uptake and mitochondrial enzymes, which are involved in MPTP toxicity. These proteins may be also involved in the action of other neurotoxic and cytotoxic substances of abuse.

154.5 MOLECULAR CLONING OF cDNA TRANSCRIBED FROM MESSENGER RNA OF THE ALZHEIMER BRAIN. IDENTIFICATION OF cDNA FOR AMYLOID AND GLIAL FIBRILLARY ACIDIC PROTEIN. S.B. Zain*, M. Salim*, S. Rehman*, E.M. Sajdel-Sulkowska, W.G. Chou*, R.E. Majocha*, K. Moore* and C.A. Marotta. Harvard Med. Sch.; Massachusetts General Hosp., Boston, MA 02114; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178; and Univ. of Rochester, Rochester, NY 14642.

Recent reports have identified β-amyloid cDNA from recombinant cDNA libraries derived from non-Alzheimer tissues. However, progress in understanding the molecular basis of Alzheimer's disease (AD) may ultimately depend upon the direct analysis of gene products expressed in the AD brain itself. GFAP was the initial focus of interest in a recombinant cDNA library from Alzheimer mRNA since the glial-specific marker is a known protein that continues to be synthesized in lesioned areas. Previously, we demonstrated that high levels of GFAP are synthesized in vitro by mRNA of the AD brain (these Proceedings, 12:1400, 1986). Postmortem poly(A+) RNA was prepared from an AD brain frozen 2.5 hours after death and handled by routine autopsy procedures. The characteristics of the synthesized cDNA and of the identified GFAP-specific recombinants in the λgt11 expression vector system, the structure analysis of the inserts, and the size and levels of expression of the corresponding mRNA in control and AD tissues will be presented. Northern blots established that there was no apparent difference between the stability of the mRNA from the routinely obtained autopsy tissues when compared with mRNA from an oxygenated rapid autopsy case (0.5 hr. postmortem, courtesy A. Roses, Duke). Consequently, routine autopsy cases were used for cloning. The previous cDNA library was screened with monoclonal antibodies made to a synthetic β-amyloid peptide (Benes et al, these Proceedings, 1987) and with a corresponding synthetic oligonucleotide. A short cloned insert corresponding to a segment of the β-amyloid sequence was identified and characterized. A second Alzheimer cDNA library was generated from 5 hr. postmortem mRNA and screened with the β-amyloid insert and positive clones were obtained. Data on the sequence analysis, the Northern blot analysis and cellular localization of the corresponding AD mRNA will be presented. Structural comparisons between AD and non-AD sequences will be made. Supported by AG02126, CA11198, and McKnight Foundation.

154.6 **UNEXPECTED CNS TUMOR CYTOLOGY IN WILD TYPE AND MUTANT SV-40 T-ANTIGEN TRANSGENIC MICE.** Kevin A. Kelley* and Karl Herrup. Dept. of Human Genetics. Yale Medical School, New Haven, CT 06510

Transgenic mice were created by the injection of a few hundred copies of the early region of SV-40 into one-cell mouse embryos. The injected sequences came from either wild-type virus or a mutant that produces a modified form of T-antigen (Tag) that is transformation defective. A previous report from Brinster *et al.* (Cell 37:367, 1984), suggested that the injection of the wild type gene leads to the reproducible appearance of choroid papillomas.

Three transgenic animals were made from injection of the early region of the wild type virus. All of them died before 35 days of age. In addition, several stillbirths were found; one that we were able to assay proved to be DNA positive. Two animals were sacrificed at 28 postnatal days after becoming critically ill. One had a well developed choroid papilloma in the lateral and fourth ventricles, but the second showed no signs of choroid plexus involvement. Examination of the brain revealed an unusual persistence of the ventricular zone in the anterior horn of the lateral ventricle. Associated with this finding was an apparent continued migration of cells from this region anteriorly into the olfactory bulb.

Seven transgenotes were made from injection of the mutant Tag. Thus far, 3 have died -- one at P36 and two at 4.5 months; the brain of one older mouse has been examined histologically. Situated medially and beneath the cerebellum was a mass of tumor tissue containing two basic cell types: a small, undifferentiated and apparently mitotic type and a second type that resembled, in Nissl stain, a large motor or sensory neuron.

The appearance of these brains suggests several conclusions. First, there is a clear correlation between the age of death and Tag genotype (wild type/early death, mutant /later death). Second, Tag in the genome is associated with several pathologies besides choroid papilloma and the apparent neural nature of some of the unusual cells suggests that this may be an effective model system in which to develop new immortal CNS cell lines. Finally, the histological appearance of each transgenic mouse is seemingly unique. This suggests either that the site of chromosomal integration or a stochastic "second-hit" required for tumorigenesis is influencing the particular spectrum of cells that respond to the presence of the Tag gene.

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154.7 RAPID "EXON SCANNING" TO DETECT POINT MUTATIONS IN GAD AND OTHER CANDIDATE GENES FOR NEUROGENETIC DISEASES. D.L. Kaufman*[1], J.N. Lederman*[1], A.M. Wong*[1], J.H. Menkes*[2,3], and A.J. Tobin[1,4,5] (SPON: H. Pardes). Departments of [1]Biology, [2]Pediatrics, and [3]Neurology, [4]Brain Research Institute, and [5]Molecular Biology Institute, University of California, Los Angeles, CA 90024.

Glutamate decarboxylase (GAD) is a "candidate gene" for a number of hereditary diseases, including those leading to seizures, abnormal movements, and psychosis. Lesions within a candidate gene may be too subtle to be identified by Southern analysis. Moreover, cloning and sequencing a candidate gene such as GAD, which extends over 70 kb, may be too labor intensive to be useful to evaluate hypotheses of candidate genes in disorders that may be genetically heterogeneous. For GAD and other genes expressed principally in the brain, direct studies of proteins and mRNAs are generally limited to animals and to postmortem human tissue. In order to determine whether brain GAD is altered in neurogenetic diseases, we therefore have developed a technique to scan samples of genomic DNA for the presence of point mutations within the exons of candidate genes.

Our method is an extension of the method of Myers, et al. (Science 230, 1242, 1985), which employs ribonuclease to detect most (but not all) mismatched base pairs in hybrids between genomic DNA and RNA transcripts of cloned DNA fragments. Using transcripts of cDNA clones rather than genomic DNA clones, we find that -- for human beta-globin -- ribonuclease A cleaves RNA probes both at exon-intron boundaries and at mismatched bases within the exons of the DNA of patients with hemoglobinopathies. This result establishes the feasibility of exon scanning for mutations in the GAD gene in neurogenetic diseases. It also establishes the utility of this approach for determining exon-intron boundaries.

We have used exon scanning to search for point mutations in the GAD genes of patients with the following conditions: pyridoxine-dependent seizures (6 cases), petit mal (absence) epilepsy (4 cases), juvenile myoclonic epilepsy (4 cases), familial complex partial epilepsy (1 case), familial autism (1 case), febrile seizures (1 case), and Ramsey Hunt disease (2 cases). Our analysis has not revealed point mutations in GAD for any of these cases. Southern blotting analysis, however, has identified a small, highly polymorphic intron within the human GAD gene.

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154.8 MOLECULAR ANALYSIS OF tko: A BEHAVIORAL MUTATION IN DROSOPHILA. C. S. Royden and L. Y. Jan. Dept. of Physiol. and Howard Hughes Med. Inst., University of California, San Francisco, CA 94143.

The technical knockout mutation, tko, is one of a family of behavioral mutations in Drosophila melanogaster which cause flies to be paralyzed temporarily by a jolt of the culture vial, i.e. these flies become "bang sensitive". Two indirect arguments suggest that tko might affect neuronal excitability. First, another "bang-sensitive" mutation, bang senseless (bss), shows enhanced excitability at the larval neuromuscular junction (Jan and Jan, PNAS 75: 515-519, 1978). Second, a suppressor of tko, no-action-potential, temperature sensitive (nap^{ts}), also suppresses several mutations causing enhanced excitability such as bss and Sh (Shaker) (Ganetzky an Wu, Genetics 100: 597-614, 1982). We began to study the gene coding for tko in order to gain some insight into the defects which cause bang-sensitivity.

The tko gene has been well mapped genetically to a region of the X chromosome just distal to the well known zeste gene. This region has recently been cloned (Mariani et. al., EMBO J 4: 2045-2052, 1985). We used P-element mediated transformation to show that a 3.1 kb fragment of genomic DNA from this region complements two alleles of tko: l(1)tko$k11$, a lethal allele, and tko^{25t}, which causes the behavioral defect. This 3.1 kb fragment contains only one complete transcript, 0.68 kb in length. We have isolated cDNA clones corresponding to this transcript and have sequenced these clones as well as the corresponding genomic DNA.

The cDNA sequence contains an open reading frame coding for a protein with remarkable homology to the Euglena gracilis chloroplast ribosomal protein S12 and to the Escherichia coli ribosomal protein S12. This homology raises the possibility that the tko gene product may be a mitochondrial ribosomal protein. A defect in mitochondrial function could affect ion gradients and energetics in neurons and muscles, resulting in behavioral abnormalities. We can not exclude the possibility that the tko gene product could have evolved from S12 into a non-ribosomal protein of similar function, e.g. a protein that interacts with RNA. Experiments by which we hope to resolve these possibilities are in progress.

154.9 **TYPE II Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE IN DROSOPHILA.** D.S. Leonard*, J.B. Wall*, P.C. Pugh*, and M.B. Kennedy (SPON: T. Sejnowski). Div. of Biology, 216-76, California Institute of Technology, Pasadena, CA 91125.

The availability of cDNA clones for the subunits of rat brain Type II Ca^{2+}/calmodulin-dependent protein kinase (Type II CaM kinase) makes it possible to characterize genes for homologous kinases in an organism well suited for carrying out genetic analyses of their function. In preparation for such a study, we looked for an homologous kinase in D. melanogaster. A kinase activity with properties similar to the rat brain kinase was measured in Drosophila homogenates. This activity is more concentrated in fly heads than in bodies. Its specific activity in head homogenates is approx. 1/5 that of the kinase in rat brain homogenates. The Drosophila kinase is dependent on calcium and calmodulin, phosphorylates synapsin I at the proper site and is inhibited by a monoclonal antibody that specifically inhibits the rat brain enzyme.

We used this antibody to precipitate the subunits of the Drosophila enzyme. SDS-gel electrophoresis of the precipitated proteins revealed three major subunits of molecular weights 60, 58, and 52 kDal. These subunits can be autophosphorylated and are the major "endogenous substrates" in fly head homogenates. The set of three subunits was purified to approx. 50% homogeneity by methods used to purify the rat kinase. The partially purified Drosophila enzyme acquires a calcium-independent kinase activity following autophosphorylation, as does the rat enzyme. Thus, these regulatory properties of the rat kinase are conserved in the Drosophila kinase.

The conservation appears to extend to the nucleotide sequence level. Blots of restricted Drosophila genomic DNA were probed with a labeled cDNA containing the full coding region for the rat brain β-subunit (Bennett, M.K. and Kennedy, M.B. PNAS 84, 1794-1798, 1987). This probe hybridized strongly to a 6.5 kilobase restriction fragment of fly DNA under reduced stringency conditions (allowing about 33% mismatch). We are now screening Drosophila genomic and cDNA libraries for clones that encode the subunits of the Drosophila kinase.

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154.10 NEURONAL AND GLIAL EXPRESSION OF THE *DROSOPHILA MELANOGASTER* DOPA DECARBOXYLASE GENE. W.A. Johnson*, C.J. Beall*, S.J. Bray*, B.A. Morgan*, C.A. McCormick*, & J. Hirsh*. (SPON: D.D. Potter) Dept. of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

A cis-regulatory element selectively required for expression of the *Drosophila melanogaster* dopa decarboxylase gene *(Ddc)* in the central nervous system has previously been identified (Scholnick et al, 1986, Science **234**, 998-1002). Here we show that at least one additional regulatory element is required for normal neuronal expression of *Ddc* in the larval central nervous system. We find that *Ddc* is normally expressed in about 125 discrete neurons and in a diffuse network comprising a subset of cortical glial cells. The expression of in vitro altered *Ddc* genes was studied by immunohistochemical staining with anti-*Ddc* antibodies following germline reintegration with P element vectors. Normal neuron-specific *Ddc* gene expression requires both the initially identified element (element I) which is 60 bp upstream from the RNA startsite, and an additional regulatory element located 800 to 2,200 bp upstream. This latter element is required for neuronal expression, but is not necessary for glial expression of *Ddc*. Combinatorial interactions between multiple regulatory elements may serve to specify a precise pattern of cell-specific gene expression.

We have examined the consequences of selectively depleting *Ddc* expression in the CNS. The *Ddc* primary transcript is alternately spliced to yield mRNAs that encode distinct hypodermal and CNS *Ddc* protein isoforms (Morgan et al, 1986, EMBO J. **5**, 3335-3342). A mutation in the CNS-specific exon leads to a selective deficit of CNS *Ddc* enzyme activity and serotonin. Strains lacking immunocytochemically detectable CNS serotonin are fully viable. These strains are being assayed for more subtle physiological and behavioral abnormalities.

154.11 ISOLATION OF cDNA CLONES ENCODING RAT BRAIN CALMODULIN. M. E. Lewis, D. F. Tyrrell, Jr.*, J. M. Roberts-Lewis, B. Weiss, R W. Manning*, and L. G. Davis. Medical Products Department. E. I. du Pont de Nemours and Co., Wilmington, DE 19898 and Department of Pharmacology, Medical College of PA/EPPI, Philadelphia, PA 19129.

The calcium-binding protein, calmodulin (CaM), influences several key enzyme activities in brain, including calcium-dependent adenylate cyclase, phosphodiesterase, and protein kinase activities. CaM appears to modulate dopaminergic activity via adenylate cyclase, and it has recently been shown that chronic, behaviorally sensitizing treatments with amphetamine result in an increase in striatal CaM levels (Roberts-Lewis et al., Brain Res. 384, 383-6, 1986). In order to further study the regulation of CaM, we have isolated and sequenced several cDNA clones encoding rat brain calmodulin. To isolate these clones, we screened a lambda gtll rat brain cDNA library with radiolabeled synthetic oligonucleotide probes which were designed according to published cDNA sequences for eel and chicken CaM. Positive plaques were picked, grown, and the DNA insert was subcloned into pUC-18 for partial sequencing to confirm the identity of the clones. The appropriate inserts were restriction cut from pUC-18 and ligated into M13 mp19 for Sanger dideoxy sequencing. Three cDNAs were sequenced that corresponded to the known nucleotide sequence for CaM, which is strongly conserved across species. Nevertheless, there was considerable variation in codon usage for CaM mRNA between rat, chicken, and eel. We are presently using single-stranded, M13-derived probes for Northern and in situ hybridization analyses of CaM mRNA regulation in rat brain.

154.12 MOLECULAR CLONING OF THE cDNA FOR ARPP-21, A CYCLIC AMP-REGULATED PHOSPHOPROTEIN ENRICHED IN THE BASAL GANGLIA. T. Kurihara*[1,2], M. Ehrlich[1], J. Horiuchi[1], and P. Greengard[1]. (SPON: C.C. Ouimet) Laboratory of Molecular and Cellular Neuroscience, The 1. Rockefeller University, 1230 York Avenue, New York, NY 10021; 2. Suntory Institute for Biomedical Research, Wakayamadai, 1-1-1, Shimamotocho, Mishimagun, Osaka, 618, Japan.

ARPP-21 (cAMP-regulated phosphoprotein, Mr=21,000) is a cytosolic substrate for cAMP-dependent protein kinase, and is highly enriched in the basal ganglia (Walaas, S.I., Nairn, A.N., and Greengard, P., J. Neurosci. 3:302-311; Ouimet et al., Soc. Neurosci. Abstr. 12:1022, 1986). This neuron-specific phosphoprotein has been purified to homogeneity from bovine caudate nucleus and its amino acid sequence has been partially determined (Hemmings et al., Soc. Neurosci. Abstr. 12, 770, 1986).

Poly A^+RNA prepared from bovine caudate nucleus was used to construct a cDNA library in a modified Okayama-Berg plasmid vector. Partial amino acid sequences of bovine ARPP-21 were used to synthesize two non-overlapping oligonucleotide probes (32mer and 41mer) for screening of this library. The longest postively hybridizing clone, pTKA1, was chosen for further analysis. The identity of the clone was confirmed by comparison of the partial nucleotide sequence with the known peptide sequence of ARPP-21. Post-translational modification of specific amino acid residues was found to occur.

pTKA1 (2.5Kb) was subcloned into the pGEM4 vector to synthesize an antisense RNA to be used in Southern and Northern blot analyses. Following hydridization to bovine caudate poly A^+ RNA, a single band of 2.5 Kb was detected. Regional distribution of ARPP-21 mRNA was examined in the bovine central nervous system. Hybridization with total cellular RNA from multiple regons revealed that the mRNA was highly concentrated in the caudate nucleus, but could not be detected in the substantia nigra. The distribution pattern of ARPP-21 mRNA was therefore somewhat different from that of DARPP-32 mRNA, the product of which is also a neuronal phosphoprotein highly enriched in the basal ganglia. Developmental analysis of ARPP-21 in the mouse striatum indicated that its expression occurs postnatally, corresponding temporally with maturation of striatal neurons and their processes. Southern blot analysis of bovine and rat genomic DNA digested with several restriction enzymes indicated that the genomic structure is highly conserved between these two species.

154.13 **GENES AND MESSAGES ENCODING THE ALPHA, BETA, AND BETA-PRIME SUBUNITS OF BRAIN TYPE II CaM KINASE.** M.K. Bennett, R.F. Bulleit*, and M.B. Kennedy (SPON: K. Ocorr). Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

Brain Type II Ca^{2+}/calmodulin-dependent protein kinase is a dodecameric holoenzyme composed of varying proportions of two types of structurally-related subunits, 50kD α-subunits and 60kD β-subunits. The major forebrain holoenzyme is composed of ~9 α- and ~3 β-subunits, whereas the cerebellar holoenzyme is composed of ~2 α- and ~8 β-subunits. In addition, a minor subunit of 58kD, termed β', is often present in the forebrain holoenzyme and is more prominent in the cerebellar holoenzyme. Tryptic peptide maps of the β'-subunit reveal that it is closely related to the β-subunit, thus several labs have suggested that it is derived from the β-subunit by proteolysis.

We have examined whether these distinct but related subunits are coded for by separate genes or are generated by alternative processing of the transcript of a single gene. Rat genomic DNA was digested with four different restriction endonucleases, separated by agarose gel electrophoresis and blotted to nylon filters. The blots were probed with labeled cDNAs containing portions of the coding region for either the α- or β- subunit (see accompanying abstract). The α-subunit probe hybridized strongly to a single band in each of the four digests, suggesting that the α-subunit is encoded by a single gene. The pattern of bands recognized by the β-subunit probe was more complex, but also suggested the β-subunit is encoded by a single gene. Proof will await more detailed analysis of the gene structure. The bands labeled most strongly by the α-subunit probe were distinct from those recognized by the β-probe indicating clearly that the α- and β-subunits are encoded by different genes.

Among several cDNA clones that encode the β-subunit, we found two independent clones with an identical discrete deletion of 45 bases from the middle of the coding region. Messages complementary to these clones would encode a protein 15 amino acids shorter than the β-subunit, with a molecular weight of approximately 58 kD. Such messages could encode the β'-subunits. To see if such messages are present in the brain, S1 nuclease protection experiments were performed in which labeled probes including the 45 bases were hybridized to brain poly(A)$^+$ RNA, then digested with S1 nuclease. A small proportion of the labeled probes were digested to fragments of the size predicted if messages with the 45 base deletion were present. Thus, the β'-subunit may be synthesized on a distinct message. The most likely source of such a message is alternative splicing of a single β-subunit gene.

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154.14 **cDNA CLONES CODING FOR THE α-SUBUNIT OF BRAIN TYPE II Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE.** R.F. Bulleit*, M.K. Bennett, J.B. Hurley*, and M.B. Kennedy, Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

The Type II Ca^{2+}/Calmodulin-dependent protein kinase (Type II CaM kinase) is an abundant brain enzyme comprising ~1% of total brain protein. It is a large holoenzyme composed of 50k(α) and 60k(β) subunits. The major forebrain isozyme is an oligomer of ~9 α- and ~3 β-subunits, whereas the cerebellar isozyme contains ~2 α- and ~8 β-subunits. Within brain, about half of the kinase is soluble and distributed throughout the cytoplasm. Another portion is concentrated in postsynaptic densities where the α-subunit is indistinguishable from the "major postsynaptic density protein."

Recently our laboratory identified and sequenced cDNA clones containing the full coding region of the β-subunit (Bennett, M.K. and Kennedy, M.B. PNAS 84, 1794-1798, 1987). We now report the selection and sequencing of several cDNA clones coding for the α-subunit. A size selected rat brain cDNA library cloned into PBR322 was obtained from Gary Schull of the Univ. of Cincinnati. The library was screened with 2 probes, a 45 nucleotide "guessmer" (45mer) based on a partial amino acid sequence of the α-subunit and a restriction fragment from the β-subunit cDNA covering the N-terminal half of the coding domain. The β-subunit cDNA appears homologous to the α-subunit message since it will hybrid select mRNAs that code for both α and β-subunits and it hybridizes at low stringency to α-subunit mRNA on Northern blots.

From this library a single clone was selected (ck4-1α) that contains a 1.3kb insert that hybridized with both probes. A second size selected rat forebrain cDNA library cloned in λ-gt10 was screened with ck4-1α to identify clones covering the entire coding region. Several clones were selected. Those with the longest inserts were characterized further. They all hybridize on Northern blots to a 5.4kb band that is recognized by the 45 mer and is more abundant in forebrain than cerebellum. Restriction maps indicate that these cDNAs are all distinct from β-subunit cDNAs and that the entire region coding for the α-subunit is represented. The sequences of the α-clones contain regions of strong homology to the β-subunit as well as regions of divergence.

Northern analysis reveals that the 5.4 kb message for the α-subunit is far more abundant in the forebrain than in the cerebellum, while the 4.8 kb message for the β-subunit is expressed at roughly equal levels in the two brain regions. This presumably accounts for the difference in subunit composition of the holoenzymes from the two brain regions.

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154.15 MOLECULAR CLONING OF cDNA FOR SYNAPTIC GLYCOPROTEINS
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The potential structural diversity and resultant informational content of the carbohydrate moiety of glycoproteins make these molecules highly suitable for roles in the establishment and maintenance of synapses. While it is known that changes in glycoprotein composition accompany synapse formation and development, it has proven difficult to characterize these proteins in precise molecular detail. We report here an approach to the isolation of cDNA probes for novel members of this class of protein. Briefly, synaptic junctions were isolated from bovine brain and concanavalin A-binding glycoproteins selected by affinity chromatography. This glycoprotein fraction was injected into mice and the resultant antisera used to screen a rat brain cDNA library constructed in the expression vector lambda gt11. Of the approximately 200,000 plaques screened, 6 reacted positively with the antisera. The cDNA inserts from these plaques have been purified and the structure and expression of the corresponding genes analysed by Northern and Southern blots, in situ hybridization, and DNA sequencing.

Northern blot analysis indicated that 5 of the 6 clones recognized a single RNA species of 3.2 kb, while the sixth clone recognized a 1.3 kb species. Partial DNA sequencing of each clone showed no significant homology with any sequence registered in the GenBank DNA database. The clone designated SC1 detects the 3.2 kb message at abundant levels in the adult rat brain and much lower levels in heart. This message is absent in liver and kidney. The SC1 message is expressed at a relatively low level in brain at birth and increases substantially through postnatal day 20. It is found primarily on membrane-bound rather than free polysomes as is expected for a message encoding a glycoprotein. Clone SC2, which recognizes the 1.3 kb message, is present at high levels in brain but at much lower levels in all other tissues examined. SC2 is also found primarily on membrane-bound polysomes. In situ hybridization indicated that SC2 displays a predominantly neuronal pattern of expression which is most clearly seen in the cerebellum where binding is observed over granule cells and is especially intense over Purkinje cells. Results obtained from hippocampal regions are also consistent with SC1 expression in neurons although glial expression also seems likely. In order to establish the subcellular localization of proteins encoded by SC1 and SC2, antibody probes are required. To this end, fusion proteins have been isolated from bacterial lysogens carrying the SC1 and SC2 inserts in the lambda expression vector. (Supported by grants from NSERC to I.B. and from MRC to J.G.).

154.16 THE NCAM GENE OF THE MOUSE: SEQUENCE ANALYSIS OF LONG AND SHORT NCAM TRANSCRIPTS.
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The NCAM cell surface glycoproteins are the best described cell-cell adhesion molecules of the vertebrate nervous system. Their significance for the development of the CNS has been well established (Gennarini, G. *et al.*, J Neurosci. 6:1983, 1986). Three protein isoforms of NCAM with apparent mol. wts. of 180,000, 140,000 and 120,000 (NCAM-180, -140 and -120) are expressed in mouse tissues. However, the final number of different gene products of the NCAM gene is still unknown. There are indications that the different sizes of NCAM proteins are translated from different transcripts (Hansen, O.C. *et al.*, J. Neurochem. 44:712, 1985). In Northern blots, hybridizing NCAM-cDNA fragments to poly(A)$^+$RNA from neonatal mouse brain, at least 5 transcripts can be recognized, which are differently regulated during neuro-ontogenesis.

A single copy gene seems to encode all NCAM mRNAs which are generated by differential splicing. Using either short cDNA fragments (Goridis, C. *et al.*, EMBO J. 4:631, 1985) or synthetic oligonucleotides carrying sequences of the NCAM gene, we screened cDNA libraries. For this purpose we especially constructed a cDNA bank of neonatal (P2) mouse brain in gt11.

The complete sequences of short (Barthels *et al.*, EMBO J., 6:in press) and long NCAM transcripts from the cap site(s) to the polyadenylation signals have been determined. The analysis of the AA-sequence allows to suggest a likely model of relationship of the NCAM gene product to the immunoglobulin gene superfamily. The extracellular part of NCAM which is identical for the three protein isoforms, seems to consist of 5 S-S bonded globular domains. Alternative splicing has been mapped by S1 nuclease protection assay.

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PEPTIDES: RECEPTORS

155.1 **TRIAZOLOBENZODIAZEPINES INHIBIT TRH BINDING IN BOVINE BRAIN.**
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Binding sites for thyrotropin-releasing hormone (TRH) with the characteristics of receptors have been found in brain tissue by several investigators. Binding of TRH, or its more active analog MeTRH, to these sites has been shown in several studies to be inhibited by benzodiazepines from a number of classes, including the imidazobenzodiazepine, midazolam. The present report explores the effects of another class of benzodiazepines, the triazolobenzodiazepines, on 3H-MeTRH binding to brain tissue.

Binding of 3H-MeTRH to bovine hippocampal homogenate was measured using standard filtration binding assay technology. Inhibition experiments employed 1nM 3H-MeTRH as ligand, and 1uM TRH to define non-specific binding. Four triazolobenzodiazepines were examined: adinazolam, alprazolam, estazolam, and triazolam. Midazolam, chlordiazepoxide, diazepam, and flurazepam were also tested for comparison.

All 4 triazolobenzodiazepines tested inhibited 3H-MeTRH binding, as summarized in Table 1. The IC50 values were in the micromolar range, as found for the other benzodiazepines tested. These values showed a high degree of variability, probably owing to the poor solubility of these compounds at high concentrations. Hill coefficients were near 1 for alprazolam, estazolam, and triazolam; but less than 1 for adinazolam. The Hill coefficients for the other benzodiazepines tested were also equal to 1 in some cases, but less than 1 in others. Thus, the triazolobenzodiazepines inhibit TRH receptor binding in a fashion similar to that of benzodiazepines of other classes.

TABLE I.

Inhibition of 3H-MeTRH binding. Values are the mean ± S.E.M. from 3 determinations.

Compound	IC50 (μM)	Hill Coefficient
Adinazolam	**223.3 ± 78.4**	**0.53 ± 0.06**
Alprazolam	**46.0 ± 28.8**	**0.98 ± 0.25**
Estazolam	**467.7 ± 178.9**	**0.79 ± 0.13**
Triazolam	**19.3 ± 0.5**	**0.74 ± 0.28**
Chlordiazepoxide	**6.9 ± 1.8**	**0.62 ± 0.05**
Diazepam	**113.0 ± 58.4**	**1.01 ± 0.26**
Flurazepam	**67.3 ± 16.6**	**0.88 ± 0.07**
Midazolam	**3.3 ± 2.2**	**0.58 ± 0.22**

155.2 ONTOGENY OF CGRP BINDING SITES IN THE RAT CENTRAL NERVOUS SYSTEM.
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In the present study we studied ontogenical development of CGRP binding sites in the rat brain by in vitro macroautoradiography. Wistar albino rats at embryonic day 21 (E21) and postnatal days (P) 1, 7, 14, 28 and 56 were killed by decapitation. Cryostat sections 15μm thick were cut, thaw-mounted on gelatin coated slides. The procedures of autoradiographic study were done as described before (Inagaki et al., Brain Res., 374 (1986) 287). Briefly, slide-mounted sections were preincubated in 50 mM Tris-HCl buffer(pH 7.4) containing 0.02% bovine serum albumin, 2mM EGTA and 5mM MgCl , and then incubated for 60 min at room temperature in the preincubation buffer containing 0.2nM of I-human CGRP (hCGRP, Amersham) with or without unlabeled rCGRP. After rinsing, incubated slides were dried and juxtaposed to tritium-sensitive film and stored at 4°C for 1 week.

The distribution of CGRP binding sites undergoes major modifications during ontogeny. Medium to high densities of CGRP binding sites are evenly present soon after in most lower brainstem nuclei at early ontogenical stages, while uneven concentrations (very low to high) of sites are seen at adult. In the forebrain, densities of CGRP binding sites are generally very low at E21 and P1. At P7 moderate numbers of sites are already seen in the amygdala and certain small regions of the striatum, and these sites increase and reach high densities at P14 in the amygdala and at P28 in the striatum. At P14, CGRP binding sites are moderate in density in other forebrain nuclei such as the taenia tecta, olfactory tuberculum, nucleus accumbens, and piriform and insular cortex. These become to be dense at adult. On the other hand CGRP binding sites in the thalamus are already present at embryonic and early postnatal stages until P14 but decline at adult. Moderate to high densities of binding sites occur rather evenly throughout the lower brainstem nuclei during E21 to P14 except in the nucleus of spinal tract of trigeminal nerve, vestibular nuclei and inferior olive where sites are denser than in other regions at P7-14, while sites in certain nuclei in related to the auditory and visual systems such as inferior and superior colliculi and medial geniculate body are scarce until P7, increase after P14 and become to be dense at adult.

155.3 VASOACTIVE INTESTINAL PEPTIDE INDUCED PHOSPHORYLATION IN PRIMARY CULTURES OF CORTICAL ASTROCYTES. R.F. Alderson, D.A. Kniss and D.E. Brenneman, Lab. Dev. Neurobiology, NICHD, NIH, Bethesda, MD. 20892.

Astrocytes have been shown to express plasma membrane receptors for a variety of hormones and the binding of these ligands can elicit similar second messenger responses as seen in other cell types (McCarthy K.D. et. al.,J. Neurochem.,44:723,1985). The receptor for vasoactive intestinal peptide (VIP) has been partially characterized (Paul S. and Said, S.I., J. Biol. Chem. 262:158,1987) and the intracellular effects of VIP are thought to be mediated through the formation of cyclic AMP. We have identified a binding site for VIP on cortical astrocytes. In addition, the formation of cyclic AMP and the protein phosphorylation induced by VIP was examined.

Primary cultures of glial cells were prepared by the method of McCarthy K.D. and de Vellis, J.,J. Cell Biol.,85:890,1980) from rat cortex at post-natal day 2. GFAP positive cells were found to make-up 90% of the cell population. The presence of VIP receptors in these cultures was demonstrated in membrane preparations. The specific VIP binding site is composed of a high and low affinity component. The non-specific binding was found to be approximately 40% of the total binding. The ability of VIP to increase cyclic AMP levels in astrocytes was also tested. A 10 min. incubation with 10^{-6} M VIP resulted in a 2.2 fold increase in the levels of cyclic AMP as compared to the control (14.7 compared to 6.8 pmol/mg protein). However, a ten minute incubation with 10^{-10} M VIP failed to significantly increase cyclic AMP levels.

Experiments designed to examine VIP-induced protein phosphorylation were conducted on astrocytes plated at a density of 5.0 (10^5) cells/60 mm dish. The ATP pools of the cells were labeled by incubation in the presence of ^{32}P-orthosphosphate in DMEM for 4 hours. The effect of the VIP treatment on the extent of protein phosphorylation was evaluated by 2 dimensional gel electrophoresis. The second dimension gels were heated to 40^{o} C for 1 hour in 1M NaOH to hydrolyze the phosphoserine and phosphothreonine bonds enabling the phosphotyrosine residues to be visualized (Cheng Y. E. and Chen L. B., PNAS,78:2388,1981). A one minute incubation with 10^{-10} M VIP produced an increase in the ^{32}P content of two proteins; (I) 410 k Da\pI = 5.05 and (II) 82 k Da\pI = 5.2. After a 10 min. incubation in the presence of 10^{-10} M VIP, phosphoprotein I was no longer visible, protein II remained approximately unchanged and an additional protein was observed having the molecular weight of 50 k Da and a pI = 5.2 (III) . Moreover, a 50 k Da protein whose content of ^{32}P was not observed to be affected by VIP treatment in the alkalinated gels was shifted from a position with a pI of 4.8 to that of 4.5. When the astrocytes were treated with 10^{-6} M VIP for 1 min. only protein III appeared to have a higher ^{32}P content and the 50 k Da protein was found to be shifted to the 4.5 pI position. The identification of these proposed VIP-induced phosphoproteins and the analysis of their phosphoamino acid composition are underway.

155.4 EGG-LAYING HORMONE BINDS SPECIFICALLY IN SEVERAL TISSUES OF APLYSIA CALIFORNICA. J.V.A. Choate, G.T. Nagle, S.D Painter and J.E. Blankenship. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

Egg-laying activity in the marine mollusc Aplysia has become a model system for understanding how peptides regulate behavior. The neuroendocrine bag cells in this animal release a family of peptides, including the 36-amino acid egg-laying hormone (ELH), during a discharge of repetitive activity that normally precedes egg laying. ELH diffuses into the hemolymph and interstitial spaces to reach its target tissues, which include the gonad (ovotestis), certain identified neurons in the abdominal and buccal ganglia, as well as other reproductive and neural tissues. ELH specifically excites target neurons for prolonged periods. It also acts directly on the ovotestis to release ripe oocytes into the reproductive tract, but the specific target cell(s) in the ovotestis has not been identified. In order to better understand the mechanism of action of ELH on its targets, we have initiated binding studies as a first step toward characterizing the receptor for this neuroendocrine hormone.

The ligand used in these studies was purified bag-cell ELH labelled with ^{125}I by the Iodogen procedure. A mixture of three ELH-related peptides (A-ELH family) from the atrial gland, an exocrine organ in the oviduct of A. californica, was used as displacing ligand. These peptides are 78-83% homologous to ELH in primary structure, are equipotent in causing egg laying, and are available in large quantities. Three target tissues were examined for the presence of specific binding sites for ELH: pooled abdominal and buccal ganglia, the ovotestis, and the reproductive tract caudal to the atrial gland. Tissues were homogenized and a crude membrane preparation (P_2 pellet) was obtained by differential centrifugation. The membranes were incubated in nanomolar concentrations of ^{125}I-ELH, and a 1500-2000-fold excess of A-ELH was used as displacer. The incubations were terminated using vacuum filtration. Our preliminary studies indicate that ^{125}I-ELH binds in a saturable manner in all three tissues and is specifically displaced by an excess of A-ELH. The apparent density of binding sites was calculated for each tissue (pmol/mg protein): reproductive tract, 1.0-1.2; ganglia, 0.9-1.0; ovotestis, 0.1-0.2. Supported by NINCDS NS23169 (JEB) and NS22079 (SDP), and by NSF BNS85-17575 (GTN). We gratefully acknowledge the assistance of Drs. T.F. Murray and G. Mpitsos of Oregon State University.

155.5 GUANINE NUCLEOTIDE MODULATION OF ^{125}I-DESAMINOTYROSINYL-PHE-NORLEU-ARG-PHE-AMIDE BINDING TO FMRF-AMIDE RECEPTORS IN HELIX ASPERSA. K. Payza. Whitney Marine Lab, Univ. of Florida, St. Augustine, FL 32086.

Guanine nucleotides have been shown to modulate agonist binding to many neurotransmitter receptors coupled to second messenger systems. Since the actions of FMRFamide on various molluscan tissues have been associated with increases in cAMP, the effects of guanine nucleotides on FMRFamide receptor binding were investigated in the gastropod mollusc Helix aspersa. ^{125}I-desaminoTyr-Phe-norLeu-Arg-Phe-amide (daYFnLRFamide) binds reversibly, saturably, and specifically to Helix neural and cardiac membrane preparations, with a K_D of about 12 nM. A lower affinity site is also present. The SAR of peptides in inhibiting binding correlates with that of stimulating the isolated, perfused Helix heart, and is similar to the SAR of other molluscan bioassays.

GTP decreases the binding of ^{125}I-daYFnLRFamide to FMRFamide receptors in neural and cardiac membranes of Helix. Based on Scatchard analysis of radioligand binding, the GTP effect appears as a reduction in the number of high affinity sites. The non-hydrolyzable GppNHp is about as effective as GTP, whereas GDP is less potent. Furthermore, the nucleotides GMP and ATP have virtually no effect on the binding.

The specificity of the interaction suggests that Helix neural and cardiac FMRFamide receptors are coupled to a G-protein, and that GTP plays a role in modulating the binding and cellular actions of FMRFamide. (NIH grant HL-28440).

155.6 FUNCTIONAL RECEPTORS FOR ATRIAL NATRIURETIC FACTOR ON CEREBELLAR NEURONS. A. Novelli, D. Collado* and R.C. Henneberry*. Molecular Neurobiology Section, Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

Atrial Natriuretic Factor (ANF) was the term used by deBold in 1981 to describe the potent natriuretic, diuretic, and hypotensive peptides released by the mammalian heart.In peripheral tissues ANF has been shown to stimulate the synthesis of cGMP and is thought to exert its physiological effects by inhibiting effects of Angiotensin II. ANF and its receptors have been found widespread in the brain, and ANF receptors have recently been reported in the blood-brain barrier. We now report the presence of functional ANF receptors on mammalian neurons.

Cerebellar granule cells were cultured from 8 day old rat pups. ANF receptors were demonstrated by binding of iodinated ANF and by stimulation of guanylate cyclase by the various peptides of the ANF family. cGMP synthesis in response to ANF increased rapidly, peaking at about 3 min.; 50% of the total cGMP was released from the neurons by 20 min. Increased cGMP synthesis in response to ANF was present as early as day 2 after plating granule cells, and increased about 2-fold by day 6. ANF was effective in stimulating guanylate cyclase at low concentrations, with an EC_{50} of 10nM. The possible role of ANF in the regulation of the water content of neurons in normal and neuropathological conditions will be discussed.

155.7 TRITIATED D-ALA1-PEPTIDE T RECEPTOR BINDING TO MOUSE MACROPHAGES RAT HIPPOCAMPAL MEMBRANES AND HUMAN T-CELL LINE. C.C. Smith, P. Williams, P.L. Hallberg, E. Sternberg, C.B. Pert and M.R. Ruff. (SPON: P. Herscovitz). Section on Brain Biochemistry and Pre-Clinical Studies, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA, Department of Nuclear Medicine, Clinical Center, NIH, Bethesda, Maryland 20892.

We have recently deduced the portion of the AIDS viral envelope which attaches to the CD4 receptor in the first step of the process by which viruses gain entry into cells containing CD4, the HIV virus receptor. This attachment sequence occurring in the second variable region from the N-terminus in the California isolate where it was first identified, is the octapeptide peptide "peptide T", ASTTTNYT. It became apparent, that the core sequence is TTNYT, peptide T [4-8], since this sequence or similar CD4 receptor analogs, can be found in all 21 AIDS viral envelopes sequenced to date, and in the envelope sequence of HTLV-I, HTLV-II, simion AIDS and HIV-II, all thought to utilize CD4 as an entry protein.

To rapidly evaluate the relative potency of the pentapeptide analogs which we have been collecting so as to precisely define the conformational structural requirements for CD4 activity, we devised a receptor binding assay utilizing ^{3}H-D-Ala1-peptide T-NH_2. This compound binds with high affinity to membrane preparations, so that 0.1 mg wet weight of rat hippocampus in 2 ml buffer binds 2,000 to 3,000 cpm after 3 h at 30°C with 0.2 nM radiolabeled peptide added: binding is displaceable to 100 cpm. The AIDS viral envelope, gp120, at extremely low concentrations(10^{-10}-10^{-12}M), inhibits peptide T binding. The percent specific binding ranges between 95-99%.

Sensitivity to structural changes are readily detectable in this rapid filtration assay. It is generally observed that d-substitution of any amino acid in the core pentapeptide sequence, TTNYT, will decrease affinity by at least one order of magnitude, as will d-threonine substitution in the 8 position of the octapeptide T. As first demonstrated by monocyte chemotaxic bioassays, the pentapeptides are more potent than the octapeptide T and its analogs. Although differences are not as pronounced in the membrane binding assays, the rank order of potency of a large number of synthetic peptide analogs displace binding with an order of potency that is quite similar in all three tissues. This rank order of potency correlates very closely with that determined by chemotaxis.

Relative potency of these peptides as well as the sequences found in all CD4 trophic viruses, will be presented. Moreover, these radioreceptor assays appear to rapidly and accurately predict the potency of the various peptides in their ability to block viral infectivity in vitro.

155.8 RECEPTORS FOR SENSORY NEUROPEPTIDES MAY BE INVOLVED IN THE PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS P.W. Mantyh, C.R. Mantyh*, P. Popper, S.R. Vigna*, L. Kruger, A.I. Basbaum, J.D. Levine, and J.E. Maggio. (SPON:C.Clemente) Center for Ulcer Research and Education; Brain Research Institute, UCLA, Los Angeles, Ca 90024; Dept of Anatomy and Medicine, UCSF, San Francisco, Ca 94143 and Dept of Pharmacology, Harvard Medical School, Boston, Ma 02115

Previous reports have established that several neuropeptides are synthesized and released by subpopulations of primary afferent neurons. These neuropeptides include bombesin, calcitonin gene related peptide, somatostatin, and substance P (SP). It has also been hypothesized that several of these neuropeptides are involved in mediating the inflammatory and immune response peripherally and in conveying nociceptive information centrally. For example previous data has shown that SP immunoreactivity is increased in the arthritic joint and release of SP immunoreactivity from the spinal dorsal horn is enhanced in polyarthritic rat. In many systems when neurotransmitter release is increased, postsynaptic receptors undergo a corresponding downregulation. To investigate whether SP and the other sensory neuropeptide receptors exhibit a similar downregulation we used quantitative receptor autoradiography to explore if receptor binding sites for these neuropeptides change in the spinal cords of arthritic rats compared to normals. The experiments were performed on 250-350 g male Sprague-Dawley rats and the arthritis was induced by intradermal injection of 0.1 ml of a 10 mg/ml suspension of Mycobacterium butyricum in mineral oil. Twenty one days later the severity of arthritis was assessed, the animals were sacrificed, the lumbar and sacral spinal cords dissected out, sectioned on a cryostat and processed for quantitative receptor autoradiography. Littermates injected with vehicle served as controls. In laminae 1 & 2 of the spinal cord, receptor binding sites for bombesin were reduced by 53.3%, somatostatin 60.0%, substance P 35%, whereas CGRP receptor binding sites remained unchanged. These results indicate that receptors for several sensory neuropeptides are downregulated in the spinal cord in adjuvant-induced arthritis and especially in the areas known to receive the thin fiber nociceptive input (i.e. 1 & 2). These results suggest that certain neuropeptide systems are preferentially affected in an inflammatory pain state such as arthritis. In addition each receptor binding site appears to show a different degree of change. These results suggest that several neurotransmitter systems may be involved in the pathophysiology of rheumatoid arthritis and in signalling nociceptive information in the spinal cord. These results offer a rational approach to determining how sensory neuropeptides are involved in the pathophysiology of rheumatoid arthritis and in designing analgesic compounds to combat the pain and inflammation associated with arthritis. Supported by Southern California Arthritis Foundation, Sloan Foundation and NIH 23970.

155.9 DISTRIBUTION OF GONADOTROPIN RELEASING HORMONE ANALOG BINDING SITES IN THE RAT CENTRAL NERVOUS SYSTEM. L. Jennes, B. Dalati* and P. M. Conn*. Department of Anatomy, Wright State University Medical School, Dayton, OH 45435 and Department of Pharmacology, University of Iowa College of Medicine, Iowa City, IA 52242.

Gonadotropin releasing hormone (GnRH) has been implicated as a neurotransmitter in the central nervous system and may be involved in the facilitation or maintenance of certain reproductive behaviors. Accordingly, we conducted a study designed to use well-characterized GnRH agonists which can be labeled to high specific activity in order to identify the intracerebral binding sites of this releasing hormone. The distribution of GnRH binding sites in the central nervous system of the rat was studied with in vitro autoradiography. The GnRH agonists Buserelin (Hoechst, [D-Ser(tBu)^{6}-Pro^{9}-NHEt]-GnRH) and Leuprolide (Abbott, [D-Leu^{6}-Pro^{9}-NHEt]-GnRH) were iodinated with chloramine T to a specific activity of 0.8 mCi/μg as assessed by self-displacement in a receptor assay using a pituitary membrane preparation as source for GnRH receptors. About 50% of the ligand bound to the pituitary GnRH receptor in the above assay. Frozen sections were incubated for 2 hours in the presence or absence of 10 μM unlabeled GnRH in 100 pM iodinated agonist, rinsed, dried then placed against X-Omat AR Kodak diagnostic film. Specific, displaceable analog binding was measured in the lamina glomerulosa and plexiformis externa, the nucleus olfactorius anterior pars externa, and the frontal cortex at the sulcus rhinalis. In the septum, GnRH binding sites were evident only in the lateral and dorsal portions of the nucleus septi lateralis but neither in the nucleus medialis nor in the diagonal band. In the hypothalamus, only a small number of GnRH receptors were detectable in the mediobasal portion while further caudally, the entire interpeduncular nucleus was heavily labeled with radioactive GnRH agonists. Specific GnRH binding sites were also detected in the central gray and in the superior collicle. In the hippocampal formation, GnRH agonists bound to the dorsal and ventral subiculum as well as to receptors in the areas CA1 through CA4; in the amygdala only a few binding sites were measured. On a qualitative basis, the highest number of GnRH receptors in the central nervous system were found in the parasubiculum. The results suggest that specific receptors for GnRH exist in select areas of the central nervous system where the peptide may regulate sensory, behavioral and endocrine events. This project was supported by WSV Academic Challenge Grant and NIH HD 19899.

155.10 ELECTRON MICROSCOPIC LOCALIZATION OF NEUROTENSIN RECEPTORS IN THE SUBSTANTIA NIGRA OF THE RAT. A. Beaudet, K. Leonard,* M. Vial,* E. Moyse,* P. Kitabgi,* J.P. Vincent* and W. Rostène. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4, Centre de Biochimie du CNRS, Parc Valrose, 06034 Nice and INSERM U-55, Hôpital St. Antoine, 75012 Paris, France.

The pars compacta of the substantia nigra (SN) contains among the highest concentrations of neurotensin (NT) binding sites in rat brain (Moyse et al., 1987). Chemospecific lesion studies (Palacios and Kuhar, 1981) and combined autoradiographic/immunocytochemical experiments (Szigethy et al., 1986) have indicated that these binding sites are mainly associated with the cell bodies and dendrites of nigrostriatal dopamine (DA) neurons. In the present study, specifically labeled NT binding sites were localized in rat SN by electron microscopic (EM) autoradiography. Lightly pre-fixed, vibratome-cut slices of rat midbrain tegmentum were incubated with 0.1 nM ^{125}I-NT in the absence (total binding) or in the presence (non-specific binding) of an excess of non-radioactive NT. Following several buffer rinses, bound radioligand molecules were cross-linked to tissue proteins with glutaraldehyde. The slices were then post-fixed in OsO_4, dehydrated in ethanols, embedded in plastic and serially sectioned for light (LM) and EM autoradiography.

In LM autoradiographs of material incubated with ^{125}I-NT alone, the labeling was intense throughout the pars compacta of the SN. Silver grains predominated over the neuropil, but some were clearly visible over the endothelium of capillary blood vessels and along the plasma membrane of neuronal perikarya and proximal dendrites. The addition of non-radioactive NT to the incubation medium reduced the radioautographic labeling by 70-85% except over capillaries, which remained selectively labeled.

In EM autoradiographs from sections incubated with ^{125}I-NT alone, most of the silver grains were shared, i.e. overlaid more than one cellular profile. These shared grains were shown by line source analysis to originate from membrane bound radioactive sources, and their distribution was therefore assumed to reflect that of membrane-bound NT receptors. After subtraction of non-specific binding, 49% of shared grains were ascribed to axo-dendritic appositions. Most of these appositions were devoid of junctional specializations, but a few exhibited well differentiated asymmetrical synapses. Axo-somatic, dendro-somatic, dendro-dendritic and dendro-glial appositions, although less frequently encountered, were proportionally as densely labeled as axo-dendritic ones. In contrast, axo-axonic and axo-glial interfaces were only rarely labeled. These observations suggest that within the rat SN, NT receptors: (1) are predominantly associated with the plasma membranes of the perikarya and dendrites of presumptive DA neurons and (2) are rather uniformly dispersed along these plasma membranes rather than being concentrated exclusively opposite abutting axon terminals.

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156.1 STABILITY OF DOPAMINE AND DOPAC, BUT NOT HVA AND 3-MT IN ALZHEIMER'S DISEASE. CONTRAST WITH PARKINSON'S DISEASE. D.G. Morgan, J.O. Marcusson*, M.N. Gordon, L.Ljung*, E. Yang*, M. Gorgy*, S.P. Lerner*, B. Winblad*, C. Gottfries*, E.D. Bird, P. Riederer*, W. Tourtellotte*, and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA USA 90089-0191 and Dept. Of Pathology, University of Umea, Sweden S-90187.

Alzheimer's disease (AD) and age-matched control (CTL) brain specimens of caudate nucleus (CN) and putamen (PUT) were obtained from Umea Sweden, Gothenburg Sweden, Boston USA, and Vienna Austria (total n = 22-27 per group). Parkinson's disease (PD) and CTL (n = 13-15 per group) specimens were obtained from Vienna Austria, and Los Angeles USA (thus CTL for AD and PD are different specimens). Homogenates were a) acidified for analysis of dopamine (DA) and metabolites by HPLC, b) used to prepare membranes for D-1 and D-2 dopamine receptor assays, and c) assayed for choline acetyltransferase (CAT) activity.

HPLC analysis found small (nonsignificant) declines in DA and DOPAC concentrations in AD, but significant declines of homovanillic acid (HVA) (-35% CN; -45% PUT) and 3 methoxy tyramine (3-MT) (-30% in both CN and PUT). Serotonin declined by -35% in PUT, but was normal in CN. 5-HIAA levels did not change. CAT declined by -30% in CN and -25% in PUT, but these trends were not statistically significant. DA receptors were also stable in AD, with small (10-15%), nonsignificant declines in CN and PUT for D-1 and D-2 receptors.

In PD, DA and DOPAC were reduced by -90% in CN and PUT, much more than in AD. However, HVA was reduced by only -40% in CN, and -60% in PUT, while 3-MT was decreased by -55% in CN and -80% in PUT. This generally smaller reduction in HVA and 3-MT metabolites of DA implies enhanced DA release in those terminals which remain in PD. Serotonin was reduced in PD by -36% (CN) and -67% (PUT), with no significant change in 5-HIAA.

PD and AD present striking contrasts concerning striatal DA metabolism. In AD, DA and the "intraneuronal" metabolite, DOPAC, are stable, while both decline by approximately 90% in PD. The two "extraneuronal" metabolites which pass through catechol-O-methyl transferase, HVA and 3-MT, are reduced in both disorders. Assuming the "extraneuronal" metabolites are indicators of dopamine release, we conclude that in AD, the dopamine synaptic machinery is intact, but functioning at a reduced level compared to CTL. In PD, the DA synaptic machinery is severely compromised, but operating at an enhanced rate to compensate for the deficit, perhaps driven by exogenous L-DOPA. Supported by The John Douglass French Foundation (DGM) and AG-05142 (CEF).

156.2 AUTORADIOGRAPHIC STUDY ON NEUROTRANSMITTERS AND NEUROPEPTIDES RECEPTORS IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE. S.Katayama, S.Kito, R.Miyoshi 3rd Dept. of Int. Med., Hiroshima Univ. Sch. of Med, Kasumi, Hiroshima, 734 Japan (SPON: Y. Yamamura)

Recently the degeneration of cholinergic cell bodies in the nucleus basalis Meynert(NbM) has been detected in the brains of Alzheimer's disease(AD) (Whitehouse et al.,1981). Although AD and Parkinson's disease(PD) have distinct clinical and biological features, these two diseases share some common characteristics, that is , greater chances suffering from dementia in PD and higher incidence of extrapyramidal signs in AD than age matched controls. In this study, we examined receptor distributions of various neurotransmitters and neuropeptides, such as muscarinic, nicotinic acetylcholine, dopamine, noradrenaline, glutamate, aspartate, somatostatin, neuropeptide-Y and corticotropin releasing factor receptors in the human autopsied brains of AD and PD.

The employed methods were in vitro autoradiography on cryostat sections with use of ^{3}H- or ^{125}I-labeled radioactive ligands, including ^{3}H-QNB, ^{3}H-pirenzepine, ^{3}H-nicotine, ^{3}H-lisuride, ^{3}H-aspartate, ^{3}H-glutamate, ^{125}I-somatostatin--14(thy^{11}), ^{125}I-neuropeptide-Y and ^{125}I-tyrosyl-corticotropin releasing factor. The obtained autoradiograms were computer analysed by IBAS II of Zeiss Company and receptor densities were quantitatively calculated. This study was also to aim at differentiating cortical and subcortical dementias from view points of receptor densities and distributions.

In AD, ^{3}H-QNB binding sites in the NbM and ^{3}H-glutamate binding sites in the hippocampus were decreased, while in PD ^{3}H-QNB binding sites were increased in the putamen. As for somatostatin binding sites, two layer formation of somatostatin receptors were observed in the control cerebral cortex, that is, low and high densities in the superficial and deep layers, respectively. This layer formation disappeared in AD brains as the results of decreased receptor densities especially in the deep layer. Such changes of somatostatin receptors were not observed in PD brains. In PD brains, changes of the receptor distribution were more widespread than having been expected. ^{3}H-lisuride binding sites were increased in the globus pallidus(D2 receptor) and decreased in the parahippocampal gyrus and cerebellum (adrenergic receptor). It is considered that to compare neurotransmitter receptor changes between these two disorders will contribute to understanding of pathophysiology underlying these most important degenerating processes in the central nervous system.

156.3 SOMATOSTATIN CONCENTRATIONS AND BINDING SITES ARE DIFFERENTIALLY ALTERED IN DEMENTIA ASSOCIATED PARKINSON'S DISEASE AND PROGRESSIVE SUPRANUCLEAR PALSY. J. Epelbaum, F. Javoy-Agid*, C. Kordon and Y. Agid*, U. 159 INSERM, U. 289 INSERM, Paris, France.

Somatostatin (SRIF) concentrations are decreased post mortem in cortical regions of patients with senile dementia of the Alzheimer type (SDAT). In the present work, we tested wether somatostatinergic parameters were also modified in the brain of patients with other neurological diseases leading to dementia such as Parkinson's disease (PD) and progressive supra-nuclear palsy (PSP). The results indicate that SRIF concentrations, as measured by RIA in different cortical regions, hippocampus, caudate, putamen and hypothalamus, are decreased significantly, as compared to controls, only in the frontal (57 % decrease) and entorhinal cortex (55 % decrease) and hippocampus (59 % decrease) of demented parkinsonians while they are unchanged in PSP and non demented parkinsonians. SRIF receptor concentrations, as measured by 125I-Tyr0-DTrp8-SRIF binding in saturation experiments in 4 individual brains for each groups and binding at saturating concentration of ligand (n = 17, for controls, 11 for parkinsonians, 14 for demented parkinsonians and n = 9 for PSP), are not modified in the frontal cortex of the parkinsonians (Kd = 2.1 $\pm$ .4 nM ; Bmax = 464 $\pm$ 35 fmol/mg protein), demented parkinsonians (Kd = 3.0 $\pm$ .8 ; Bmax = 481 $\pm$ 140) or PSP patients (Kd = 2.8 $\pm$ 1.3 ; Bmax = 514 $\pm$ 100) as compared to controls (Kd = 2.1 $\pm$ .6 Bmax = 435 $\pm$ 55). The results demonstrate that somatostatinergic parameters in the cortex are differentially affected in SDAT, PD and PSP.

Both concentrations and binding are decreased in SDAT while only the levels of the peptide are reduced in demented parkinsonians ; in keeping with the occurrence of tangles and plaques in the cortex of these patients. In contrary, in PSP where the cortex is roughly spared somatostatin parameters are not modified as compared to controls.

156.4 **STABILITY OF L-GLUTAMATE RECEPTORS AND DECREASE IN A CaCl-DEPENDENT [^{3}H]-L-GLUTAMATE BINDING SITE IN ALZHEIMER'S DISEASE.** J.W. Geddes, D.T. Monaghan, R.J. Bridges, and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

Cognitive deficits in Alzheimer's disease (AD) may result, in part, from the loss of specific neurons in the hippocampal region. The vulnerable neurons, including stellate neurons in layer II of the entorhinal cortex and pyramidal neurons in CA1 and subiculum, are thought to utilize glutamic acid as a neurotransmitter. We have examined the status of the major classes of glutamate receptors in the hippocampus in AD and have previously reported that the disease is not associated with a large decrease in N-methyl-D-aspartate (NMDA) or in kainate (KA) receptors, except in cases with severe cell loss (*Science* 230: 1179, 1985; *Br. Res.* 399: 156, 1986). In addition, using the specific agonist ^{3}H-α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) to label the quisqualate (QA) receptors, we have found little change in QA receptor density or distribution in AD patients. The pharmacology of this site was identical to that described previously in the rodent brain.

In contrast to these data, Young and colleagues have reported a striking loss of NMDA and QA receptors in the hippocampus of AD patients (*J. Neurochem* 48: 543, 1987). Young and coworkers performed their binding assays in the presence of $CaCl_2$ which, from recent studies, induces [^{3}H]-L-glutamate binding to non-receptor sites. We have therefore examined glutamate binding using the protocol of Young and coworkers and compared the results to those obtained under our conditions in an effort to resolve these differing findings. We confirm the decrease in [^{3}H]-L-glutamate binding in AD when binding assays are done in the presence of Ca^{2+}(2.5 mM) and Cl^{-}(50mM). The same pattern of glutamate binding persisted in the presence of NMDA (200 μM), AMPA (100μM), QA (2.5 μM) and in the presence of both NMDA and AMPA or QA.

Thus, the pharmacology of Ca/Cl-dependent glutamate binding site which decreases in AD is not consistent with NMDA or QA receptors. The binding is, however, similar to the Ca/Cl-dependent glutamate binding site described previously in the rodent brain. This binding has been associated with a Cl^{-}-dependent, Ca^{2+}-stimulated glutamate transport site which we and others have recently characterized in both astrocytes and synaptic plasma membranes. A decrease in the ability to remove excitatory amino acids may contribute to the vulnerability of glutamate-using neurons in AD. Alternatively, the decreased uptake of glutamate may be secondary to the disease-induced loss of glutamate-using neurons.

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156.5 CHOLINERGIC RECEPTORS IN ALZHEIMER'S DISEASE: CHARACTERIZATION USING RECEPTOR BINDING ASSAYS. R. Quirion, D.M. Araujo, Y. Robitaille*, P.A Lapchak (Spon: N.P.V. Nair). Douglas Hospital Res. Ctr. and Dept. of Psychiatry, McGill University, Montreal, Québec, Canada H4H 1R3.

There is evidence to suggest that in human brain, the densities of cholinergic receptors may be altered by neurological disorders such as Alzheimer's disease (AD). However, it is not clear what subtype(s) of cholinergic receptor and what brain region(s) are most affected by the disease. The present study sought to clarify this point by characterizing the binding of radioligands selective for M_1 and M_2 muscarinic receptors and for the nicotinic receptor, to normal (n=4) and AD (n=6) human brain membrane preparations. [^{3}H]N-methylcarbamyl choline (MCC) was used to quantitate nicotinic receptor binding; [^{3}H]pirenzepine and [^{3}H]acetylcholine (ACh), under muscarinic conditions (see Quirion and Boksa, 1986), were used to study M_1 and M_2 muscarinic receptors, respectively. Human brain tissue was homogenized and suspended in buffer of the following composition (in mM), Tris.HCl 50, NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, (pH=7.4), for [^{3}H]MCC and [^{3}H]ACh binding, and in Krebs buffer for [^{3}H]pirenzepine binding. Then, supernatant was discarded and the membrane pellet was washed twice and resuspended in fresh buffer. The final membrane pellet was incubated with various concentrations of radioligand for 60 min at 4°C for [^{3}H]MCC and [^{3}H]ACh, and at 25°C for [^{3}H]pirenzepine. [^{3}H]MCC bound specifically, saturably, and with high affinity to a single class of nicotinic sites in both control and AD brains. However, there was a significant decrease in [^{3}H]MCC binding sites in AD compared to control brains. This decrease was most apparent in frontal (68%) and temporal (66%) cortex. [^{3}H]ACh (in the presence of 20 µM nicotine and 100 µM DFP) bound with high affinity to an apparently single class of sites in both cortical and sub-cortical structures, but the maximal number of sites labelled by [^{3}H]ACh was not markedly different for AD compared to control brains. The results obtained for [^{3}H]pirenzepine binding are not clear at present, but suggest that there may be a modest increase in M_1 muscarinic receptors in AD brains compared to controls. In summary, the results show that various cholinergic receptors may be differentially affected in AD. However, it appears that of the three receptor subtypes studied, M_1 and M_2 muscarinic and nicotinic, the latter is the most markedly altered in AD. The significance of this finding is not immediately apparent, but may be of therapeutic importance in the treatment of neurological disorders such as AD. (Supported by MRC, Canada and FRSQ, Québec).

156.6 DIFFERENTIAL EFFECTS OF DORSAL AND VENTRAL CHOLINERGIC STRIATAL LESIONS. B. Diamond, J. Johnson*, K. Sethi*, R. Borison Department of Psychiatry and Health Behavior, Medical College of Georgia, Augusta, GA 30912

The mediation of striatal dopamine (DA) effects via acetylcholine (ACH) has been characterized. It was the aim of this study to examine the role of ACH on DA mediated behaviors within the dorsal and ventral striatum. Three groups of male Sprague Dawley rats (250 g) were used. One group served as control while two other groups had bilateral injections with the cholinergic neurotoxin AF64A (3 nM/0.5 l) into either the dorsal or ventral caudate nucleus. Animals were given apomorphine (APO 0.5 mg/kg) or amphetamine (AMPH 3 mg/kg) once a week and stereotypy assessed. Animals with dorsal AF64A lesions exhibited spontaneous grooming, rearing and increased locomotion. Both APO and AMPH exacerbated these behaviors and produced intense chewing and gnawing on the cage grid whereas they produced only a slight increase in locomotion and grooming in controls. In contrast, animals with AF64A ventral lesions exhibited reserpine-like symptoms with spontaneous chewing and teeth clattering. DA agonist treatment did not produce a supersensitive response in these animals. These results demonstrate the selective nature of the ACH system within the striatum and provide evidence for the neuroanatomical involvement of ACH in movement disorders.

156.7 **HUMAN POSTMORTEM FRONTAL CORTICAL α_2 ADRENERGIC RECEPTORS.** G.N. Ko, B.J. Wilcox, M.A. Raskind*, M.M. Murburg*, and D.M. Dorsa. Univ. of Wash., and GRECC, Seattle VAMC, Seattle, WA 98108.

Improvement in memory in nonhuman primates has been reported to be associated with stimulation of post synaptic α_2 adrenergic receptors in the lateral frontal cortex (Arnsten, A.F. Science 230:1273, 1985). In light of this, information on the anatomy and pharmacology of human frontal cortical α_2 receptors may contribute to our understanding of disease associated cognitive impairment such as seen in Dementia of the Alzheimer's type (DAT), a condition in which central adrenergic abnormalities have been documented. The purpose of the present study was to demonstrate the existence of α_2 adrenergic receptors in the frontal cortex of human postmortem brain specimens using in vitro autoradiography.

Unfixed frontal cortex material from Brodmann's Area 8 and 9 was obtained, cut and mounted on brass microtome chucks. Coronal cryostat sections of 16μ thickness were thaw mounted on subbed microscope slides and allowed to air dry at room temperature before being stored. [^{3}H]para-aminoclonidine (PAC, 40 Ci/mmol) was used to label α_2 adrenergic binding sites in the mounted tissue sections (Unnerstall, J.R. Brain Res. Rev. 319:69,1984). Briefly, sections were preincubated for 30 minutes at room temperature in Krebs-phosphate buffer (pH 7.4 at 25°C) containing 100μM Na+ -GTP. Adjacent sections were then washed for 10 minutes at room temperature in the incubation buffer alone (0.17 M Tris HCl, pH 7.6 at 25°C plus 10 mM $MgCl_2$). Sections were then incubated for 60 minutes at room temperature in buffer containing a range of PAC concentrations between 0.05 to 25 nM. Nonspecific binding was assessed utilizing the above ligand in the presence of 10^{-5} M phentolamine. After a 10 minute wash at 4°C in incubation buffer and a rinse in cold deionized water, the sections were dried under a stream of cool dry air. Labeled tissue was then apposed to tritium sensitive film which after exposure and development was scanned by a computer based image analysis system (Dumas, Drexel University). Radioactive plastic standards calibrated to tissue containing known amounts of radioactivity were used to generate standard curves for quantitation.

PAC binding was displaceable by phentolamine in the dorsolateral frontal cortex. Saturation data and photomicrographs of autoradiograms will be presented.

The present results provide evidence for the existence of α_2 adrenergic receptors in human frontal cortex analogous to those described in nonhuman primate brain. These data suggest that a comparison of α_2 receptor density in the frontal cortex of normal and DAT brain tissue is feasible.

156.8 BILATERAL CHANGES IN CORTICAL M1 AND M2 RECEPTORS FOLLOWING UNILATERAL LESIONS OF THE RAT NUCLEUS BASALIS OF MEYNERT: AN AUTORADIOGRAPHIC STUDY. J.R. Atack*, G.L. Wenk, M.V. Wagster, K.J. Kellar, P.J. Whitehouse and S.I. Rapoport (spon. A. Noronha). Lab. of Neuroscience, NIA, NIH, Bethesda; Dept. of Neuropathology, Johns Hopkins School of Medicine, Baltimore and Dept. of Pharmacology, Georgetown University School of Medicine, Washington DC.

In Alzheimer's disease, cortical nicotinic receptors have been reported to be reduced, but changes in cortical cholinergic receptors are equivocal (Whitehouse & Au, Prog. Neuro-Psychopharm. Biol. Psych. 1986:10;665). However, it is not clear to what extent these changes are related to a degeneration of the cortical cholinergic input from the nucleus of Meynert (NbM) per se. We have therefore examined cortical cholinergic receptors in male albino rats with unilateral lesions of the NbM.

Animals received unilateral ibotenic acid lesions of the left NbM and were sacrificed after either 1 week (n=12) or 13 weeks (n=6). Sham operated rats were used as controls and were sacrificed after 1 week (n=7). 2-3 mm thick coronal brain slices were prepared immediately anterior to the hippocampus and were subsequently sectioned at 10 um intervals for autoradiography. Cortical tissue anterior to this tissue was retained for choline acetyltransferase (ChAT) and protein assays. Muscarinic M1 and M2 receptors were visualised directly using 3H-pirenzepine and 3H-oxotremorine, respectively. High- and low-affinity N-methyl scopolamine (NMS) binding sites were defined as carbachol-sensitive and carbachol-insensitive NMS sites, respectively. Nicotine receptors were identified using 3H-acetylcholine.

One week after lesioning, ipsilateral ChAT activity had fallen significantly ($p<0.05$) to 49% of control values (mean ± SD = 14.7 ± 3.5 and 30.0 ± 8.1 nmol/hr/mg protein, respectively). By 13 weeks, ChAT activity (23.9 ± 4.0 nmol/hr/mg protein) had increased significantly ($p<0.05$) by 63% relative to 1 week lesioned animals. Contralateral ChAT activity was unchanged at both 1 and 13 weeks. M1 receptor binding was unchanged at 1 week relative to controls, but at 13 weeks had decreased significantly ($p<0.05$) in both the left and right hemispheres by 31% and 39%, respectively. On the other hand, M2 receptor binding increased significantly ($p<0.05$) at 1 week postlesion in both the left and right hemispheres (33% and 54% increase, respectively), but returned to normal levels by 13 weeks. There were no significant differences between groups with respect to either nicotinic or high- or low-affinity NMS binding.

Since none of the receptors changed in parallel with ChAT activity, these results suggest that none of the receptors studied are specific for presynaptic cholinergic nerve terminals. Furthermore, bilateral changes in M1 and M2 receptors indicate that there is intrahemispheric regulation of these binding sites.

156.9 TETRAHYDROAMINOACRIDINE BLOCKS POTASSIUM CHANNELS AND INHIBITS SODIUM INACTIVATION IN MYXICOLA. Charles L. Schauf and Albert Sattin. Department of Biology, Indiana University-Purdue University, Indianapolis and Department of Psychiatry Richard L. Roudebush V.A. Medical Center and the Indiana University School of Medicine, Indianapolis, Indiana 46223

Tetrahydroaminoacridine (1,2,3,4 tetrahydro-9-aminoacridine; Tacrine; THA) is a potent, centrally-active anticholinesterase that appears to relieve some of the symptoms of moderate-to-severe senile dementia of the Alzheimer type (Summers et al., New Engl. J. Med. 315: 1241, 1986). In voltage-clamped Myxicola giant axons internally and externally applied THA blocked K^+ channels with a K_D of 100 uM and slowed K^+ activation. Whereas external THA had no effect on the Na^+ conductance, internal THA initially slowed the rate of inactivation of conducting Na^+ channels, and then rendered inactivation biphasic, the initial phase being 2-3 times faster than in control axons. Internal THA decreased maximum inward Na^+ currents by 35-50% and induced a significant steady-state non-inactivating inward current accompanied by an increase in the amplitude and time constant of Na^+ tail currents. At negative potentials the effect of THA was small, but for positive voltages Na^+ tail currents were prolonged and developed an initial plateau. It appears that THA may occlude Na^+ channels by entering them and rendering THA-occluded channels resistant to fast inactivation, and also that THA cannot easily dissociate from open channels. The K_D for the effects of internal THA on the Na^+ conductance was 10 uM. Internal concentrations of THA as high as 100 uM did not alter the time- or voltage-dependence of Na^+ activation, behavior expected if the initial rapid decline in inward current was not due to Na^+ inactivation, but rather reflected blockade by THA. THA had no effect on prepulse-induced fast or slow inactivation. In contrast to THA, doses of physostigmine greatly in excess of those needed for maximal inhibition of cholinesterases have no effects on ionic currents in axons. The muscarinic acetylcholine receptor agonist RS86 was also tested and found to be ineffective up to concentrations of 1 mg/ml. Effects of THA on ion channels may account for CNS hyperexcitability seen in some patients treated with THA. Since THA is a potent anticholinesterase, ion channel effects may contribute to its putative anti-dementia action in clinical states involving a CNS deficiency of ACh by selective augmentation of ACh release and/or the negation of autoreceptor effects of endogeneous ACh. (Supported by NSF Grant BNS 86-96095 and National Multiple Sclerosis Society Grant 1911A4, and by the Veterans Administration Research Service.)

156.10 MUSCIMOL BINDING IN THE SUBSTANTIA NIGRA OF DAY 16 AND ADULT RATS. J.N.D. Wurpel*, A. Tempel, E.F. Sperber and S.L. Moshe. Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Previous studies have shown that microinjections of muscimol (a GABA-A agonist) into the substantia nigra (SN) exert an anticonvulsant effect in adult rats, while similar injections are proconvulsant in 16 day old rat pups. We performed receptor binding studies in day 16 and adult rats in an effort to evaluate the receptor-dependent component of this difference of GABA pharmacology in the SN.

Adult (250-300g) and 16 day old (25-30g) rats were decapitated and the SN and cerebellum (CB) rapidly dissected and placed in ice cold saline. Crude membrane homogenates were prepared in 70 volumes of 50mM Tris-HCl then centrifuged twice at 48,000 x g, preincubated at 37 C for 45 minutes, and centrifuged again. Binding assays (^{3}H-muscimol; specific activity 17.5-30.8 Ci/mmol range 0.2-150 nM) were performed in triplicate. Protein concentrations were: 70-200 ug/tube for 16 day old pups; 100-200 ug/tube for adult rats. Results are from at least 3 separate experiments.

Computer assisted scatchard analysis revealed two affinity states present in SN and CB of day 16 and adult rats. In the cerebellum, there were no differences in the number of high ($B_{max}1$) and low ($B_{max}2$) affinity receptor sites (expressed as fmole/mg membrane protein).

In the SN, the B_{max} of the low affinity site was equivalent in both age groups. In contrast, the SN of 16 day old pups contained fewer high affinity receptor sites than the adult SN (13% adult values). The K_d's (nM) of the high (K_d1) and low (K_d2) affinity sites did not differ between 16 day old and adult rats.

(mean±SE)	Substantia nigra		Cerebellum	
	$B_{max}1$	$B_{max}2$	$B_{max}1$	$B_{max}2$
Pups	32.5±4.8	3168±511	247±45	2450±474
Adult	253±63	2433±120	300±76	3267±780

This SN specific difference in the number of high affinity receptors may be responsible for the age related effects of muscimol on seizures, accounting for the lack of an anticonvulsant effect in rat pups. (Supported by NIH Grant NS-20253 from NINCDS).

156.11 DIFFERENTIAL PATTERNS OF NEUROTRANSMITTER RECEPTOR MODIFICATIONS IN SENILE DEMENTIA AND PARKINSON'S DISEASE REVEALED BY AUTORADIOGRAPHY. R. Cortés[1,2], M.M. Dietl[2], M. Camps[2], E. Soriano[2], J. Zezula[2], A. Pazos[2], J.C. Reubi[1], A. Probst[1] and J.M. Palacios[2]. (SPON. E.S. Tolosa). [1]Dept. of Neuropathology, Inst. of Pathology, University of Basle, Switzerland, CH-4003. [2]Preclinical Research, Sandoz Ltd., Basle, Switzerland, CH-4002.

Receptor autoradiography was used to examine regional neurotransmitter receptor modifications in postmortem brain tissue from patients dying with senile dementia (SD) or Parkinson's disease (PD) in comparison with a control population. Receptors for amine (acetylcholine, dopamine, serotonin), aminoacid (GABA, glutamate) and peptide (neurotensin, substance P, somatostatin) neurotransmitters were examined. Losses of receptors were often associated with the loss of specific neuronal populations, e.g., neurotensin and somatostatin receptors decreased in the substantia nigra in PD and substance P receptors in the nucleus basalis in SD. However, dopamine (D_1 and D_2) and benzodiazepine receptors were unchanged in PD in the substantia nigra and basal ganglia, where animal experiments have localized these receptors to cell bodies and terminals of the nigrostriatal and striatonigral pathways. Several receptors were examined in the hippocampal formation in SD. Three patterns of receptor modification were observed: 1) Normal densities of receptors (i.e., muscarinic cholinergic, GABA/benzodiazepine and glutamate) in cases with numerous senile plaques and low or moderate neuronal loss in the CA1 field; 2) Marked losses of all neurotransmitter receptors in the CA1 accompanying a severe neuronal decrement (more than 50 %); 3) Generalized receptor losses (i.e. dopamine D_1, serotonin-1 and somatostatin) in the dentate gyrus, CA3 and CA1, apparently unrelated to the degree of neuronal loss. Receptor alterations were also found in regions distal to site of the neuropathological lesion, i.e. dopamine D_1 and somatostatin receptors were decreased in the putamen in SD, and benzodiazepine receptors were increased in the cerebellum in PD. These results illustrate that different patterns of receptor plasticity operate in neurodegenerative diseases in the human brain, and that animal models do not always reflect the changes occurring in human brain disease.

156.12 PRE- AND POSTSYNAPTIC STRIATAL DOPAMINE NEUROTRANSMISSION IN MPTP-TREATED PRIMATES. J.R. Barrio*, S.C. Huang*, J.S. Schneider*, J.M. Hoffman*, A. Luxen*, N. Satyamurthy*, M. Bahn*, R.E. Keen*, C. Nissenson*, J.C. Mazziotta, and M.E. Phelps. UCLA School of Medicine, Los Angeles, CA 90024.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces Parkinson-like symptoms in primates by damage to the nigro-striatal dopaminergic system. Nemistrina monkeys (n = 3) (3-5 Kg) injected with MPTP (IV, 0.25 mg/Kg, in 5 doses administered over a three week period) were subjected to serial PET scans after IV administration of 6-[F-18]fluoro-L-DOPA (FD) (IV, 5-10 mCi, specific activity: 5 Ci/mmol). Tomographic tissue-time activity curves of caudate (CA), cerebellum (CB) and temporal cortex (TC) and their ratios (CA/CB and CA/TC) were analyzed. No significant differences were observed in MPTP-pretreated, asymptomatic animals compared with controls (n = 6, CA/CB = 2.2 ± 0.6 and CA/TC = 1.6 ± 0.2, 3 h). However, animals with severe motor deficits showed no time-dependent accumulation of radioactivity in CA (CA/CB = 1.0). This is consistent with post-mortem striatal biochemical analysis (98%, 97% and 99% decrease in dopamine, DOPAC and HVA, respectively) and histological data (90% cell loss in the substantia nigra pars compacta). Postsynaptic function was evaluated in vivo with 3-(2'-[F-18]fluoroethyl) spiperone (FESP) and PET using a double-injection procedure. Controls (n = 6) and MPTP-treated symptomatic monkeys (n = 2) were injected with FESP, (bolus injection, IV, 4-6 mCi), first with a high specific activity (0.2 - 10 Ci/μmole) followed by a lower specific activity (20-40 Ci/mmole) 90 min later. The difference in the striatum kinetics between the two injections is dependent on the specific activity of the radioligand and on the dopamine-D2 receptor density (Bmax) in the striatum. Using this procedure no significant difference in the estimated Bmax (24.3 ± 12.0 pmole/g) was found between the MPTP-treated (symptomatic) and the normal control animals. Consistent with this observation in vivo, in one symptomatic animal, in vitro 3H-spiperone binding showed a receptor (dopamine-D2) density (Bmax) of 21.2 pmol/g tissue and a normal KD value of 35 pM. These results suggest that FD is useful for selectively assessing presynaptic neuronal degeneration caused by MPTP, also known to occur in Parkinson's disease. The preservation of dopamine postsynaptic receptor integrity in these studies is also in agreement with the symptomatic improvement observed after administration of L-DOPA in MPTP-injected primates and Parkinson's patients.

156.13 DOPAMINE D_1 AND D_2 RECEPTORS AND DYSKINESIA IN PARKINSONIAN MPTP-TREATED MONKEYS. P.J. Bédard, R. Boucher, P. Falardeau and T. DiPaolo. Lab. Neurobiologie, Hôp. Enfant-Jésus and Dept Molecular Endocrinology, Laval Univ Research Center and Laval University, Qué. Canada, G1J 1Z4.

Twelve monkeys were rendered severely parkinsonian by 1 methyl-4-phenyl, 1, 2, 3, 6 tetrahydropyridine 0.3-1.5 mg/kg administered intravenously. They were then divided into four groups. The first received no further treatment except supportive care. The other three groups were treated respectively with L-DOPA/carbidopa 50 mg/kg, (mixed D_1D_2 agonist) bromocriptine 5 mg/kg (D_2 agonist) and SKF 38393 5 mg/kg (D_1 agonist). The drugs were given once a day orally for one month. L-DOPA/carbidopa and bromocriptine treatment relieved the parkinsonian symptoms while SKF 38393 was ineffective. No dyskinesia was seen in monkeys on bromocriptine or SKF 38393 as opposed to the L-DOPA treated animals where the dyskinetic response appeared to increase with time. The animals were sacrificed three days after their last dose with an overdose of pentobarbital. MPTP alone induced a significant increase (25% p 0.01) in the number of [^{3}H]spiperone binding sites (Bmax) in the caudate nucleus and in the putamen. The Bmax of spiperone binding in the L-DOPA treated monkeys was on average 18% lower (p 0.01) than in the animals treated with MPTP alone. Bmax for the bromocriptine-treated group was 29% (p 0.01) less than in the MPTP-treated group or 11% (p 0.05) less than in the L-DOPA-treated monkeys. The SKF 38393 treatment induced a decrease of Bmax of 23% (p 0.01) as compared to animals treated with MPTP alone and no significant change compared to the L-DOPA or bromocriptine-treated animals. Thus D_1 receptor stimulation causes no behavioral effect but can affect the D_2 receptors. These changes could in turn be related to the incidence of L-DOPA induced dyskinesia. Supported by MRC of Canada, the Parkinson Foundation of Canada and Sandoz Canada.

156.14 ALTERATIONS IN REGIONAL NEUROPEPTIDE CONCENTRATION IN MPTP-TREATED MICE. B. Levant, G. Bissette, Y.-M. Parker* and C. B. Nemeroff. Laboratory of Psychoneuroendocrinology. Duke University Medical Center, Durham, NC 27710

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), causes destruction of nigrostriatal dopamine (DA) neurons and subsequent symptoms resembling Parkinson's disease (PD) in humans and subhuman primates. The MPTP-treated mouse has recently been proposed as an animal model of PD (Heikkila et al., 1984, Science, 224:1451-1453). Regional alterations in a variety of neuropeptides have been reported in human patients with idiopathic PD (Bissette et al., 1985, Ann. Neurol., 17:324-328; Agid and Javoy-Agid, 1985, TINS., 8:30-35). One goal of these studies was to examine neuropeptide alterations in this murine model of PD. Adult (8 week old), male, Swiss-Webster mice were injected daily with MPTP (30 mg/kg, IP) for 5 consecutive days. The mice were killed on day 10, the brains rapidly removed, frozen and dissected into 12 discrete regions. Brain regions were extracted and assayed for neuropeptide concentration by sensitive and specific radioimmunoassays previously described in detail. When compared to controls, the concentration of NT was decreased in the frontal cortex ($p<0.01$), caudate ($p<0.01$), and preoptic area ($p<0.02$) of the MPTP-treated animals. An increase in BOM concentration was found in the amygdala ($P<0.04$) whereas a decrease in the concentration of this peptide was observed in the preoptic area ($P<0.05$) of the MPTP-treated animals. Degeneration of nigrostriatal neurons was verified by reversed-phase HPLC assay of caudate•samples for DA, DOPAC, HVA, 5-HT and 5-HIAA. Caudate DA, DOPAC and HVA concentrations were decreased to 12% ($P<<0.01$), 28% ($P<<0.01$), and 42% ($P<0.01$) of control respectively. A decrease in 5-HT concentration to 69% of control ($P<0.02$) was found while 5-HIAA exhibited a nonsignificant increase to 118% of control, indicating a slight increase in 5-HT turnover. Although substantial depletion of striatal DA was observed in the MPTP-treated mice, the brain regions exhibiting changes in neuropeptide concentration differed substantially from those demonstrated in human IPD. The MPTP-treated mouse would therefore appear not to be a satisfactory model of human PD with respect to neuropeptide changes. The effects of MPTP on regional neuropeptide concentrations in aged mice should be investigated.

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158 SYMPOSIUM. THE ROLE OF NECTINS (CELL BINDING MOLECULES IN NEURAL DEVELOPMENT. L. Glaser, Univ. of Miami (Chairperson); E. Ruoslahti*, La Jolla Cancer Research Foundation; A.F. Horwitz*, Univ. of Pennsylvania School of Medicine; S.R. Hoffman, Rockefeller Univ.; R. Bunge, Washington Univ. School of Medicine; N. Ratner*, Washington Univ. School of Medicine.

The session will focus on the molecular bases by which cells recognize and respond to molecules in the extracellular matrix, with particular emphasis on cells in the nervous system. The symposium will start with a discussion of well characterized molecules involved in cell extracellular matrix interactions and proceed to the description of specific biological phenomena which require these interactions. The structural basis for cellular recognition of matrix proteins such as fibronectin and collagen will be described by Erkki Ruoslahti, (La Jolla Cancer Research Foundation) and the characterization of the cellular receptors for fibronectin and laminin will be summarized by A.F. Horowitz, (University of Pennsylvania School of Medicine). Cytotactin a recently discovered cell adhesion molecule involved in neuron glia interaction has been studied in detail by S.R. Hoffman, (Rockefeller University), who will summarize his observations in this system. One of the most complex and also best understood systems in which cells interact with the extracellular matrix as part of this cell differentiation process, is the interaction of Schwann cells and neurons with the basal lamina that they generate. The role of basal lamina in axon ensheathment and myelination will be discussed by R. Bunge, (Washington University School of Medicine), who has pioneered work in this field. The mitogenic response of Schwann cells to neurons depends on the presence of proteoglycans in the neuronal cell surface. The purification of a neuronally derived Schwann cell mitogen and its relationship to cell surface proteoglycans will be discussed by Nancy Ratner, (Washington University School of Medicine).

159 SYMPOSIUM. STRUCTURAL COMPUTER SIMULATIONS IN DEVELOPMENTAL AND SYSTEMS NEUROBIOLOGY. J. M. Bower, Caltech (chairperson); S. Fraser, Univ. Calif. Irvine; R. Linsker, IBM Watson Research Labs; L. Finkel*, Rockefeller University; C. Koch, Caltech; R. Traub, IBM Watson Research Labs.

This symposium will consist of a series of talks which demonstrate the use of structural simulations in studying the development and functional organization of complex neural networks. These computer models are based on the real anatomical and physiological properties of the part of the brain being studied. As such, modeling results are often directly related to experimentally addressable questions, a point which will be brought out in each of the presentations.

Scott Fraser will present his recently developed single axon-based model simulating the development of the retinotectal projections in frogs. Predictions of the model will be contrasted with recent experimental results. **Ralph Linsker** will describe his network-based hierarchical developmental model of the mammalian visual system (retina to cortex). The receptive field organizations of the simulated neurons that emerge from the model will be compared to known receptive field properties at different levels of visual processing. **Leif Finkel** has been exploring the dynamic interactions in topographic maps of cerebral cortex and the possible structural mechanisms underlying their reorganization following experimentally induced changes in peripheral stimulation. **Christof Koch** will describe his recent work modeling the electrical properties of single vertebrate neurons and modeling calcium dynamics in postsynaptic structures (spines and soma) following repetitive synaptic input. **Roger Traub** will present his model of hippocampus with which he has explored network mechanisms underlying epileptic and nonepileptic activity. **James Bower** will describe a structural simulation of piriform (olfactory) cortex which suggests the network properties underlying the rhythmic patterns of activity seen during olfactory discrimination and the possible consequences of this activity for olfactory processing.

160.1 RETINAL TRANSPLANTS IN PHOTOTOXIC RETINOPATHY. M. del Cerro, M. F. Notter, L. Qi Jiang, D. A. Grover, and C. del Cerro. Univ. of Rochester Sch. of Med., Rochester, NY 14642.

We have found (del Cerro et al., 84, 85, 86) that the developing retina can be successfully transplanted into normal adult eyes. Based on these results we examined the feasibility of transplanting neuroretinal cells into retinas that have late-stage phototoxic retinopathy. For this purpose adult albino Lewis rats that had been continuously exposed to fluorescent light illumination for three weeks served as hosts. The donors were 1-2 day old rats from either Lewis or Long-Evans strains. The transplants were placed in one eye, leaving the contralateral eye as a control. The trasnplanted material consisted of a strip of neural retina, or a 2 µl suspension of neural retinal cells. The transplants were labelled with 3H-thymidine, Fast Blue, or both. Survival time ranged from 10 to 30 days.

Fundus examination of control eyes showed palor caused by a considerable reduction of the retino-choroidal vascular bed after light irradiation. Host eyes showed the growth of the transplant and focal rearengement of vascularity. Histologically the irradiated eyes showed massive destruction of the outer retinal layers; no outer nuclear layer was present in the central or mid -peripheral retina, and only one or two discontinuous rows of rod cells was visible in the periphery. The transplants appeared as masses of retinal tissue, rich in photoreceptors cells, with their inner and outer segments growing on the host retina. Physical continuity, and common vascularization, existed between transplants and hosts. The results show that it is feasible to repopulate neuroretinal cells in the extensively damaged adult eye. The functional significance of this finding are being explored.

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160.2 SCHWANN CELL MYELINATION IN DAMAGED RETINA: ENHANCEMENT WITH PERIPHERAL NERVE IMPLANTS. M. R. Wells and U. Vaidya. Neurochemistry Research Laboratory, Veterans Administration Hospital and Dept. of Physiology, George Washington University, Washington, D.C. 20422.

Schwann cells have been shown to myelinate retinal axons after retinal lesions made through the sclera in neonatal animals and to a very limited exent in adults (Perry and Hayes, J. Neurocytol. 14: 297-307; 1985). The present study examined the potential of implanted Schwann cells to enhance the myelination retinal axons in damaged retina of juvenile and adult rats.

Either adult, male Wistar rats (approx. 250 g body wt.) or 5 day old animals of both sexes were used for experiments. In both groups, retinal lesions were made throurgh the sclera near the optic disc. In half of all animals, a small piece (1.5 mm) of sciatic nerve (autograft) was removed and inserted into the lesion. A 30 day survival was allowed for all groups. Retinas were examined either as whole mounts or in 1 u sections of plastic embedded material.

Adult animals receiving only retinal lesions had little or no myelination of retinal axons. As observed previously (Perry and Hayes 1985), young animals had much more myelination by Schwann cells after similar lesions. In implanted animals, myelination was variable, but much greater in adult animals compared to lesioned, unimplanted adults. Any increased myelination in young animals produced by the implant was obscured by a large variation. The myelination of retinal axons differed qualitatively between young and adult animals. In young animals, myelination was more diffuse and not limited to areas near the site of implantation. In adult animals, myelination of retinal axons appeared to occur adjacent to blood vessels and in areas disturbed by the process of lesioning and implantation.

The results suggest that implants of peripheral nerve into the the retina of adult rats enhances the myelination of retinal axons by Schwann cells in areas directly or indirectly injured by the lesion. In the developing retina of young animals, Schwann cell migration and myelination may be greater due to the incomplete formation of glial processes. As suggested in other studies (Blakemore et al., Acta Neuropathol. 71: 295-300; 1986), astrocytic processes may limit the extent of Schwann cell migration and myelination in retina. The migration and association of Schwann cells with unmyelinated or demyelinated axons in injured areas of CNS may be an important factor in the regeration of axons into peripheral nerve bridges.

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160.3 RECONSTRUCTION OF THE DAMAGED SEPTOHIPPOCAMPAL CIRCUITRY BY A COMBINATION OF FETAL GRAFTS AND TRANSIENT NGF INFUSION. G. Buzsáki, R.G. Bickford, S. Varon, D.M. Armstrong and F.H. Gage. Univ. of California, San Diego, Sch. of Med., Neuroscience Dept., M-024, La Jolla CA 92093.

The fimbria-fornix (FF) and the overlying neocortex were removed unilaterally by aspiration in 64 rats. Half of them received intraventricular infusion of nerve growth factor (NGF) for two weeks. In half of the NGF-treated rats and half of the untreated rats, fetal hippocampal grafts (TPL, E16) were placed in the lesion cavity. All rats were equipped with recording microelectrodes from 4 to 5 months after the initial surgery. EEG and unit activity were monitored during running in a wheel, drinking and immobility.

The number of surviving cells in the medial septum increased in the FF only < TPL < NGF < NGF+TPL groups order. Cell savings in the TPL group was observed only in rats in which the transplant fused with the host septum. Reinnervation of the host hippocampus, as assessed by the density of acetylcholinesterase (AChE)-staining, was most complete in group NGF+TPL extending from the dentate gyrus to CA1-CA3 fields, and the extent of reinnervation decreased from TPL > NGF > FF groups. AChE-positive reinnervation never exceeded the posterior curvature of the hippocampus, however. Depth profiles of EEG revealed a positive correlation between the power of rhythmic slow activity (theta) and the density of AChE- positive reinnervation. The best restoration of theta was observed in group NGF+TPL. Similar to normal rats, theta was present only during waking and absent during drinking and sitting still. Granule cells and interneurons discharged rhythmically during running, and the spikes were phase-locked to theta waves recorded from the contralateral (intact) hippocampus. Large amplitude theta waves were also present in rats with relatively few surviving neurons in the medial septum. Sharp waves (SPW, up to 4mV) were present in all groups during immobility and drinking. In groups FF, TPL and NGF and in some rats of group NGF+TPL SPWs and concurrent population bursts of neurons were observed also during walking and running. In TPL rats strong single pulse stimulation of the perforant path induced epileptic discharges in the host hippocampus.

These findings suggest that the combination of NGF treatment and fetal grafting saves the majority of septal area cells, results in a more complete reinnervation of the host hippocampus and consequently better physiological recovery. They also suggest that theta may be produced by a relatively small group of surviving "pacemaker" cells.

Supported by J.D. French Foundation and NIH AG06088.

160.4 **ALTERATIONS IN BLOOD-BRAIN BARRIER BY ADRENAL MEDULLARY TRANSPLANTS IN CNS PAIN MODULATORY REGIONS.** J. Sagen and G. D. Pappas, Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60612.

We have been concerned with the changes in pain sensitivity brought about by the transplantation of adrenal medullary tissue into the pain modulatory regions of the periaqueductal gray (PAG) and spinal cord subarachnoid space. It has been shown that the blood-brain barrier may be altered by the transplantation of peripheral neural tissue into the CNS. The intravascular injection of the protein tracer HRP indicates that such transplants retain their *in situ* permeability to macromolecules and allow some substances that normally do not pass the blood-brain barrier (BBB) to enter brain parenchyma. We were interested in the pharmacological relevance of these alterations in BBB. Chromaffin cells transplanted into the PAG or spinal cord produce analgesia when stimulated by nicotine, presumably by inducing release of catecholamines and opioid peptides from chromaffin cell granules. To assess pharmacological changes in BBB, rats with adrenal medullary implants were pretreated with either mecamylamine, a ganglionic antagonist with which normally passes the BBB, hexamethonium, a ganglionic blocker that normally does not pass the BBB, or saline vehicle. In saline pretreated animals, nicotine induced potent analgesia. This analgesia was completely blocked by mecamylamine, but was minimally affected by hexamethonium. Results of this study indicate that, although permeability to proteins may be altered by the transplantation of peripheral neural tissues to the CNS, the BBB to charged molecules may not be altered. Preliminary studies using D-ala-met-enkephalinamide (DALA), a long lasting opioid peptide derivative with limited ability to pass the BBB, indicate that adrenal medullary transplants improve penetration of peptides into the CNS, since systemic injections of low doses of DALA induced analgesia in implanted, but not control animals. Thus, transplants of peripheral neuronal tissue apparently allow some, but not all substances to bypass the BBB. Supported, in part, by NIH grant NS-07630.

160.5 ORIGINS OF VASCULATURE WITHIN FETAL CNS TISSUE TRANSPLANTED TO THE BRAIN. J.M. Krum and J.M. Rosenstein. Dept. of Anatomy, George Washington Univ. Sch. of Med., Wash. D.C. 20037

Pieces of fetal cerebral cortex transplanted to the brain grow rapidly and develop a vascular network throughout their substance. We examined the mechanism of vascular integration within these transplants (taken from E18-E21 donors) which were placed within the fourth ventricle of 10 - 15 day old Wistar rats.

DNA synthesis within endothelial cells served as an index of capillary growth. Tritiated thymidine was administered to recipient animals to determine angiogenic patterns at various postoperative time periods ranging from one day to one month. After processing for light microscopic autoradiography, host and graft endothelial labelling indices were determined in order to establish the temporal sequence and location of vascular proliferation. The vascular ultrastructure within each transplant was also examined. Some recipients were pre-labelled with ^{3}H thymidine prior to transplantation to determine if host vessels invaded the grafts; conversely, some transplants were pre-labelled in utero to determine if their vascular anlage survived.

After 24 hours, the majority of vessels present within the grafts appeared to be adjacent, mature host pial vessels with associated collagen and fibroblasts, which had been surrounded by the fetal cells. By 72 hours, small diameter vessels were proliferating rapidly within the transplants; ultrastructurally, these appeared to be a mixture of both pial and embryonic vasculature. The now completely engulfed host vessels were also heavily labelled at this time. One week old transplants contained a complex microvascular network which continued to proliferate, although at a lesser rate, while the incorporated host vasculature was quiescent. After one month, both vascular components had ceased to proliferate.

Pre-labelled transplants contained labelled vasculature even after one week, indicating that fetal CNS vessels survive grafting and presumably continue to grow. Transplants within pre-labelled recipients contained labelled, mature pial vessels at their margins as well as smaller labelled vessels located more deeply within the grafts. These results suggest that the vasculature which develops within intraventricular CNS transplants is chimeric, consisting of intrinsic fetal vessels which anastomose with capillary sprouts originating from passively incorporated pial vessels. Preliminary studies indicate that intraparenchymal CNS grafts develop a similar vascular composition, consisting of both fetal vascular anlage and host cerebral vessels. The nature of graft vascularization, particularly at brain surfaces where non-neural tissue can be incorporated, may play a role in barrier permeability at these transplantation sites.
(Supported by NS-17468).

160.6 BIODEGRADABLE POLY (DL-LACTIDE-CO-GLYCOLIDE) MICROCAPSULES FOR CONTROLLED RELEASE OF CATECHOLAMINES : A NOVEL DELIVERY SYSTEM TO THE CNS. A.McRae-Degueurce, D.L. Dillion*,R.M.Gilley*& T.R. Tice* U-259 Univ. of Goteborg Goteborg, Sweden & Southern Research Institute Birmingham, Al 35255

Injectable controlled release peptide,antibiotic and steroid delivery systems have been developed in which these substances were encapsulated within biodegradable Poly (DL-lactide-co-glycolide)s. The microcapsules serve 2 functions: first, they protect substances from degradation ;second, they release substances at a controlled rate at therapeutic doses in a target site for desired time periods _ up to months. In view of the success and advantages of this system it appeared of interest to apply this technology to the CNS as a means to deliver agents which do not cross the blood brain barrier or are rapidly degraded. In the present investigation L-DOPA or dopamine were encapsulated in biocompatible biodegradable Poly (DL-lactide-co-glycolide). The capsules range in size from 10-30µm in diameter.Rats received bilateral injections of 6-OHDA in the medial forebrain bundle. Following recuperation from the lesions the animals received a unilateral injection of the capsules (suspended in 2µl of saline) in the striatum. Rodents were perfused with 4% paraformaldehyde either 24 or 48 hours after receiving the capsules The brains were processed for immunocytochemistry with a tyrosine hydroxylase antibody. The results demonstrated intense fluorescence in the region where the capsules had been implanted. The contralateral striatum was devoid of fluorescence. Because, the injectable capsules appear to be ideal to deliver controlled release of drugs or other substances to specific brain regions this technology has considerable potential for both clinical and basic research.

160.7 INCREASED HYPOTHALAMIC CATECHOLAMINE CONCENTRATIONS AND DECREASED PROLACTIN LEVELS BY FETAL HYPOTHALAMIC TRANSPLANT IN IMPOTENT AGED MALE RATS. H.H. Huang*, J.Q. Kissane*, and E.J. Hawrylewicz. Department of Research, Mercy Hospital and Medical Center, Chicago, IL 60616.

We and others have reported that aged rats showed an elevated level of prolactin and a reduced level of gonadotropins, which in turn, caused a decrease in reproductive function. This can be due in part to the decreased catecholamine and GnRH activities in the hypothalamus of aged rats. Recently we have found that sexuality and fertility can be enhanced by fetal hypothalamic transplant in aged male rats. The present study was designed to investigate whether the restoration of reproductive function in impotent aged male rats by fetal hypothalamic transplant was through the increase of hypothalamic catecholamines and reduction of prolactin release. Eighteen to 20 month old impotent male rats received an anterior hypothalamus removed from a 17-19 day old male fetus and placed into the third cerebral ventricle, just anterior dorsal to the median eminence. Controls were either without surgery or grafted with cerebral cortical tissue. Just before and 3 months after transplantation, blood samples were collected from the aged males for prolactin measurement. Before and one to two months after transplantation, each grafted rat or control animal was put overnight into a cage which contained four young proestrous female rats. Vaginal smear of each female was monitored early the next morning. Sperm seen in the vaginal smear was regarded as copulation and ejaculation. The test was repeated twice. Finally, the animals were terminated and brains were removed and frozen with dry ice for catecholamine determination. Seven of 10 hypothalamus-grafted males (HG) restored their sexuality. None of 7 untreated controls (UC) changed their reproductive function and only one of 4 cerebral cortex grafted males (CG) copulated with one female. Serum prolactin in the HG rats which showed restoration of reproductive function were significantly lower than those of controls (112.7 ± 13.0 vs. 237.5 ± 40.9 (UC), 246.7 ± 25.5ng/ml (CG), $p < 0.02$). However, the concentrations of norepinephrine (NE) and dopamine (DA) in the median eminence (ME) and arcuate nuclei (ARC) of the HG rat's hypothalamus were significantly higher than those of UC and CG controls (91.3 ± 10.6 vs. 57.3 ± 9.7, 62.5 ± 12.0 ng NE/mg protein in the ME $p<0.05$; 71.3 ± 14.5 vs. 29.9 ± 4.5 ng NE/mg protein in ARC $p < 0.05$; 77.7 vs. 31.4 ± 9.4, 28.0 ± 5.2 ng DA/mg protein in the ME $p < 0.05$; 15.3 vs. 6.2 ± 0.9, 7.2 ± 0.3 ng DA/mg protein in ARC $p < 0.05$). Catecholamines and prolactin in HG rats which did not respond to the transplantation were not significantly different from the controls. These results indicate that the fetal hypothalamic grafts can survive and differentiate in the brain of impotent aged male rats and rejuvenate the neuroendocrine and reproductive function in senescent rats. (Supported by NIH Grant AG-04351-01).

160.8 MICROVASCULAR CORRELATES OF NEURAL GRAFTING IN THE ENDOCRINE HYPOTHALAMUS OF THE BRATTLEBORO RAT. D.E. Scott, Department of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501

This investigation has focused upon the neurovascular criteria that characterize neural grafting followed by changes in urine osmolarity and water consumption in Brattleboro host rats with Diabetes inspidus (DI). Male DI host rats were housed in metabolic cages for one week prior to surgery in order to determine water consumption and urine concentration values. Fragments of anterior hypothalamus were garnered from 17 day post-coitus fetal rats, minced in Eagle's Solution and stereotaxically introduced (with an 18 GA spinal needle) into the third cerebral ventricle of DI host rats. Rats were killed at various times post-surgery and prepared for routine microangiography, transmission electron microscopy and immunocytochemistry following techniques described previously. In all host rats that exhibited alterations in their physiological parameters of water consumption and urine concentration, several consistant morphological criteria were evident. Unfenestrated endothelia with distinct basal laminae were not restricted to the ventral regions of grafts but were commonly observed throughout all regions. Viable grafts commonly exhibited fenestrated capillary endothelia and distinct perivascular spaces. These were observed chiefly at the base of grafts in neuroanatomical continuity with the host median eminence. Magnocellular neurites with neurosecretory (peptidergic) inclusions were in contact with both fenestrated and unfenestrated capillaries of the graft. Viable grafts derived their blood supply from the entire periventricular stratum as well as the underlying host median eminence. It would appear that the distalward growth of vessels into graft parenchyma involves the fusion of endothelial primorida with different phenotypic characteristics. Magnocellular nerve cell bodies were observed on the surface of grafts and in the living state were in direct contact with host cerebrospinal fluid. Alternative routes for the neuroendocrine transduction and the central delivery of neuropeptide hormones into host vasculature are discussed. Supported by EVMF program grant.

160.9 **THE OUTGROWTH AND DISTRIBUTION OF PROCESSES FROM NEOCORTICAL GRAFTS TRANSPLANTED INTO THE CORTEX OF ADULT HOSTS.** J.A. Zaslow*, L.M. Smith and F.F. Ebner, Center for Neural Science, Brown University, Providence, R.I.

The purpose of these experiments was to study outgrowth from embryonic neocortical tissue transplanted into adult hosts using the cell surface antigen Thy-1.1 as a marker to identify donor tissue in the host brain. The Thy-1 antigen is a glycoprotein which exists in two distinct allelic forms known as Thy-1.1 and Thy-1.2.

Cortical cells were removed from E-14 mouse embryos that express the Thy-1.1 allele. The cells were prepared in two ways for implantation into adult Thy-1.2 host cortex: donor tissue was either left intact as rectangular solids of cortical neuroblasts and radial glia or they were dissociated into individual cell suspensions. The intact embryonic cortex was implanted without further manipulation. The cell suspensions were prepared by mechanical dissociation in 0.5% trypsin before being centrifuged, washed, and injected into the host cortex. The dissociation procedure removes any processes from the developing neurons and strips the radial glia of their long supportive processes leaving only round cell bodies in the injection suspension.

The OX-7 Thy-1.1 monoclonal antibody (Serotec) does not immunolabel any cells or processes in the Thy-1.2 host cortex. The transplanted cells and processes stand out clearly against the negative host cortex as a spherical dense area surrounded by a lighter haze of immunoreactivity. Examination of the host brain immediately surrounding the transplants shows numerous immunoreactive processes and some glial cells outside of the transplant. In addition, patches of immunoreactive processes are seen in the corpus callosum between the transplant and the midline, in the underlying white matter and, in a small number of cases, in the internal capsule. Many fewer processes were observed around the cell suspension transplants than around the chunk transplants. This stands in contrast to the apparently greater innervation of the cell suspensions by the host brain specific thalamic fibers (Erzurumlu and Ebner, Neurosci. Abs., 1986). We are examining the ultrastructure of the processes around the transplants to determine whether they make synaptic contacts with host neurons in the neuropil surrounding the transplants.

Clearly labeled Thy-1.1 positive astrocytes were found outside the transplant in the corpus callosum, in the underlying white matter, and in the internal capsule. Thy-1.1 labeled neurons are seen outside the transplant, but always near the host/transplant border. These results support the hypothesis that the intact glial cells of the chunk transplants remain functional and may provide an environment which enhances the outgrowth of graft cell neurites.

(Supported by NIH grant #13031).

160.10 **CAUDAL VERSUS ROSTRAL REGIONS OF EMBRYONIC RAT RHOMBENCEPHALON: FLOW CYTOMETRY, CELL CULTURE, IMMUNOCYTOCHEMISTRY, AND TRANSPLANTATION.** N. König*, H. Mansour*, MJ. Drian*, F. Sandillon*, F. Favier*, L. Marlier* and A. Privat* (SPON: European Neuroscience Association). Lab. de Neurophysiol., USTL, 34060 Montpellier Cedex, France (NK); INSERM 249 / CNRS 8402, Montpellier (HM, MJD, FS, LM and AP) and Service Comm. Cytométrie de Flux CNRS, Montpellier (FF)

The rhombencephalon is a major source of monoaminergic afferences to almost every region of the developing central nervous system. The present study aimed at comparing the cell populations situated between the pontine flexure and the onset of the spinal cord (caudal part) to those situated between the rhombencephalic isthmus and the pontine flexure (rostral part). The rationale for this subdivision was that descending monoaminergic fibre systems originate mainly in the caudal part, whereas ascending fibres originate in the rostral part. Embryos were obtained by laparotomy from anaesthetized rats (gestation days 13 and 14). Rhombencephala were dissected out and divided into caudal (C) and rostral (R) parts. Dissociated cell populations were fractionated by sedimentation in calcium and magnesium free (Puck's) saline. Then, they were either analyzed by flow cytometry (forward- and right-angle scatter) or plated for cell culture. Cells expressing serotonin, thyrosine hydroxylase, or dopamine, were detected by immunocytochemistry. Cell suspensions of C or R rhombencephalon were transplanted into the transected spinal cord of adult rats, and the survival and growth of serotonin expressing neurons were monitored by immunocytochemistry. The principal results were as follows: Compared with R cells, C cells had higher sedimentation velocities, and the C populations contained a higher percentage of cells with low right-angle and high forward-angle scatter values. These differences might be linked to the developmental advance of C cell populations. In cell culture, the C populations established a strikingly reticulated neurite pattern, in contrast with the more diffuse pattern of the R cells. Both populations contained serotonin, tyrosine hydroxylase, and dopamine immunoreactive cells; however, the relative percentages of these subpopulations were different and depended upon the age of the embryos. When transplanted into the transected spinal cord of adult rats, both C and R serotonin expressing cells survived and growed well; however, the innervation pattern was different (the C cells seemed to innervate preferentially the dorsal horns). Behavioural analyses will have to show if these different innervation patterns correspond to distinct functional properties.

GABA AND BENZODIAZEPINE: PHARMACOLOGY I

161.1 SPONTANEOUS BICUCULLINE SENSITIVE SINGLE CHANNEL CURRENTS IN OUTSIDE OUT MEMBRANE PATCHES OBTAINED FROM MOUSE SPINAL CORD NEURONS. D.A. Mathers* (SPON: J.A. Pearson). Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada.

The application of γ-aminobutyric acid (GABA) to outside-out patches obtained from cultured mouse spinal cord (SC) neurons triggers the opening of ionic channels permeable to chloride ions. However, even in the absence of exogenously applied GABA, spontaneous, chloride dependent single channel currents are seen in many of these patches (Hamill et al., Nature 305:805, 1983). The present experiments were aimed at clarifying the nature of these events.

Primary dissociated cultures of SC neurons were obtained from embryos of C57BL mice at day 13 of gestation. Cultures were maintained in Minimum Essential Medium with 10% horse serum for 2-3 weeks prior to use. Patch clamp recordings were made in the outside-out patch configuration using a List EPC-5 amplifier. The external membrane face was bathed in a solution of composition (mM): Tris Cl 137, KCl 4, $CaCl_2$ 1, $MgCl_2$ 1, Hepes 10, pH = 7.4. The inner membrane face was exposed to a solution containing Tris Cl 140, KCl 3, $MgCl_2$ 1, EGTA 11, Hepes 10, pH = 7.4. Replacement by $Tris^+$ of the bulk of Na^+ and K^+ ions blocked current flow in cation permeable channels in these patches. Drugs were applied to the external membrane face by bath perfusion. Membrane patches were voltage-clamped to a potential of -60 mV at 20-22°C.

The frequency of spontaneous single channel currents varied widely from patch to patch. In some cases no events were detected, while in others channel openings occurred at a frequency of up to 40 Hz. Open time distributions for these events were well fit by the sum of two exponential terms with mean parameters as follows: fast time constant, τ_f = 0.38 ± 0.04 ms (mean ± S.E.M.), slow time constant, τ_s = 2.8 ± 0.57 ms. The mean ratio of the number of events present in the fast and slow fit components was 46 ± 20.5. These spontaneous events were blocked by application of the specific GABA antagonist bicuculline methiodide (2-10 μM) to the external membrane face. In patches where a high frequency of spontaneous single channel current was seen, application of 5 μm GABA resulted in a wave-like response, while in patches with little spontaneous activity, a smoother, monophasic response to GABA was seen. These observations suggest that the spontaneous events seen in some SC cell membrane patches reflect the activity of GABA sensitive channels.

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161.2 POTENTIATION AND ANTAGONISM OF ADENOSINE-EVOKED DEPRESSION OF RAT CEREBRAL CORTICAL NEURONAL FIRING BY Ro 15-1788 AND Ro 5-4864. R.E. Stair*, M.H. O'Regan* and J.W. Phillis. Dept. of Physiol., Wayne State Univ. Sch. of Med., Detroit, MI 48201.

The mechanism of action of the benzodiazepines has long been a matter of interest to the scientific community. In addition to the general consensus that GABA plays a major role, there has been evidence suggesting that inhibition of adenosine uptake may be involved in benzodiazepine effects. Ro 15-1788 and Ro 5-4864 are benzodiazepine receptor ligands which have been reported to have mixed agonist/antagonist properties in various behavioral paradigms. They are also known to be adenosine uptake inhibitors. The effects of Ro 15-1788 and Ro 5-4864 on adenosine and adenosine 5'-N-ethylcarboxamide (NECA) -evoked depressions of rat cerebral cortical neuronal activity were studied. Iontophoretically applied Ro 15-1788 had both antagonistic and potentiative interactions with adenosine. Reductions in the magnitude of adenosine-evoked depressions of firing were evident during the period of Ro 15-1788 application, with a longer lasting potentiative effect becoming apparent upon termination of the Ro 15-1788 application. Iontophoretic application of Ro 5-4864 showed a similar pattern of antagonism and potentiation of adenosine-evoked depressions, during and immediately after application. Larger applications of Ro 15-1788 had a depressant action on neuronal firing which could be antagonized by caffeine (20 mg/kg, i/v), an adenosine receptor blocker. Applications of Ro 5-4864, in general, seemed to increase spontaneous neuronal activity. Depressions of cell firing evoked by NECA, an uptake resistant analog of adenosine, were consistently antagonized by both Ro 15-1788 and Ro 5-4864. These results indicate that Ro 15-1788 and Ro 5-4864 can act as adenosine antagonists and also have potentiative actions, which are likely to be due to their reported ability to inhibit adenosine uptake. These findings may account for some of the mixed agonist/antagonist actions of these drugs that have been described in the literature, and further support the adenosine hypothesis of benzodiazepine action.

161.3 MODULATION OF GABA-STIMULATED CHLORIDE INFLUX INTO MEMBRANE VESICLES FROM RAT CEREBRAL CORTEX BY TRIAZOLOBENZODIAZEPINES, NONBENZODIAZEPINES AND β-CARBOLINES. T. Obata* and H. I. Yamamura (P. Deshmukh). Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724

Benzodiazepines are widely used as anxiolytics, anticonvulsants, sedative-hypnotics and muscle relaxants. After the discovery of specific high affinity binding sites for benzodiazepines, the search of new benzodiazepine ligands resulted in the discovery of additional chemical classes of drugs. One is a group of nonbenzodiazepines such as CL218872, CGS9896, PK9084, buspirone and zopiclone, and the other β-carbolines such as β-CCM, β-CCE and DMCM. It has been found that the binding sites for benzodiazepines are closely associated with GABA receptors and chloride channels, suggesting the existence of GABA/benzodiazepine receptors chloride channel complex. To elucidate the biochemical functional coupling between GABA and benzodiazepine receptors and chloride channels, we examined the effect of triazolobenzodiazepines, nonbenzodiazepines and β-carbolines on GABA-stimulated $^{36}Cl^-$ uptake by membrane vesicles from rat cerebral cortex. Membrane vesicles (200 ul) were preincubated at 30°C for 10 min. Chloride influx was initiated by the addition of 200 ul solution containing $^{36}Cl^-$ (0.2 uCi/ml)and GABA. After incubation for 3 sec, the reaction was terminated by the addition of ice-cold buffer followed by rapid vacuum filtration through Whatman GF/C glass microfiber filters pretreated with 0.05% polyethylenimine. The content of $^{36}Cl^-$ on filters was determined by liquid scintillation counting. GABA stimulated $^{36}Cl^-$ uptake by membrane vesicles from rat cerebral cortex in a concentration-dependent manner at 3-300 uM. This concentration-response curve was shifted to the left by 1 uM alprazolam and a significant enhancement was observed with $^{36}Cl^-$ uptake stimulated by 30 uM GABA. Among triazolobenzodiazepines, alprazolam and triazolam significantly enhanced GABA (30 uM)-stimulated $^{36}Cl^-$ uptake at 0.1-10 uM. Adinazolam showed a small enhancement at 0.01-1 uM followed by the inhibition of GABA-stimulated $^{36}Cl^-$ uptake at 10-100 uM. β-CCM,β-CCE and DMCM inhibited GABA-stimulated $^{36}Cl^-$ uptake. Ro15-1788, a benzodiazepine antagonist, antagonized the enhancement by 1 uM of alprazolam and the inhibition by 1 uM DMCM of GABA-stimulated $^{36}Cl^-$ uptake. But the inhibition of GABA-stimulated 36Cl- uptake by 30 uM adinazolam was not antagonized by Ro15-1788. Nonbenzodiazepine agonists thus far examined except CL218872 had little effect on GABA-stimulated $^{36}Cl^-$ uptake. CL218872 showed a small enhancement at 1-10 uM followed by a significant inhibition of GABA-stimulated $^{36}Cl^-$ uptake at 100 uM. These results indicate that benzodiazepines allosterically modulate the GABA-gated chloride channel through benzodiazepine receptors and that high concentrations of adinazolam may directly block the GABA-gated chloride channel.

161.4 EFFECT OF FOOT SHOCK STRESS ON $^{36}Cl^-$ EFFLUX FROM RAT BRAIN SYNAPTONEUROSOMES. A. Concas*, S. Mele* and G. Biggio (Spon: P.L. Onali). Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.

Recently we have found that, similarly to β-carbolines and opposite to benzodiazepines (BZ), an emotional stress (handling or foot shock) decreases the density of low affinity GABA receptors in the rat cortex. Since GABA receptors appear to be associated to a chloride channel whose opening is triggered by the binding of GABA to the GABA receptors, we have studied the effect of foot shock stress on the efflux of $^{36}Cl^-$ from synaptoneurosomes of rat cortex. Foot shock stress delivered continuously for 20 min decreases $^{36}Cl^-$ efflux (25%) from cortical synaptoneurosomes of handling-habituated (unstressed) rats but failed to have the same effect in the synaptoneurosomes of naive (stressed) rats. The presence in the dilution buffer of pentobarbital (500 u M), GABA (100 uM) or muscimol (50 uM), reversed the stress-induced decrease of $^{36}Cl^-$ efflux from synaptoneurosomes of handling-habituated rats. On the contrary the GABA antagonists bicuculline (500 uM) e picrotoxin (1 uM) decrease $^{36}Cl^-$ efflux from synaptoneurosomes of stressed and unstressed rats. Moreover, since ^{35}S-t-butylbicyclophosphorotionate (^{35}S-TBPS) has been shown to specifically label a site on the chloride ionophore associated with the GABA/BZ receptor complex, in an attempt to further clarify the molecular events involved in the action of stress we have studied the effect of foot shock stress on ^{35}S-TBPS in the rat cortex. ^{35}S-TBPS binding to fresh unwashed cortical membranes was enhanced by foot shock stress an by bicuculline (1 uM) and inhibited by GABA mimetics (10 uM) and anxiolytic benzodiazepine, recognition site ligands (1 uM). These results indicate that stress decreases the function of Cl^- channels coupled to the GABA/BZ receptor complex.

161.5 DECREASE ON $^{36}Cl^-$ UPTAKE IN MEMBRANE VESICLES FROM BRAIN OF STRESSED RATS. M. SERRA*, S. MELE* AND G. BIGGIO. Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy

The chloride ionophore, a component of the GABA/benzodiazepine/barbiturate receptor complex, is regulated by GABA. Thus, we studied the effect of foot shock on $^{36}Cl^-$ uptake in membrane vesicles prepared from cerebral cortex of handling-habituated (unstressed) and naive (stressed) rats. Foot shock, delivered continuously for 20 min decreased by 20% ($p<0.05$) the basal chloride influx in brain vesicles of handling-habituated rats, but failed to change the basal $^{36}Cl^-$ uptake in brain vesicles of naive ones. GABA (1 uM) added to the membrane vesicles of handling-habituated foot-shocked rats increased the chloride influx to approximately the level of unstressed rats but failed to change the basal $^{36}Cl^-$ uptake in brain vesicles of unstressed rats. GABA (10-25 uM) produced a similar dose dependent increase in Cl uptake in both handling-habituated and naive rats. The GABA antagonist bicuculline (50 uM) decreased (25%) the basal $^{36}Cl^-$ uptake of unstressed (handling-habituated) rats but failed to further decrease the basal chloride influx of stressed (handling-habituated - foot shocked and naive) rats. The results indicate that foot shock stress produces a down regulation of the chloride channel function. This finding is consistent with the hypothesis that the emotional states related to stress decrease GABAergic transmission at the level of the GABA/benzodiazepine/barbiturate, receptor complex.

161.6 CHARACTERIZATION OF RAT BRAIN GLUTAMIC ACID DECARBOXYLASE (GAD). Y.-C. Chang and D.I. Gottlieb. Department of Anatomy and Neurobiology, Weashington University School of Medicine, St. Louis, MO 63110.

We previously described monoclonal antibodies (MAbs) specific for vertebrate glutamic acid decarboxylase (GAD) (Gottlieb et al., PNAS 83: 8808). Here we have purified GAD from the adult rat brain using a GAD-1 MAb immunoaffinity column. The purified enzyme was analyzed by SDS-PAGE and consists of a major band of 59 kDa, and bands of 63 and 55 kDa. This pattern is very similar to the enzyme purified from the chicken brain. The rat 59 kDa protein was purified further by preparative SDS-PAGE. Its N-terminus is blocked. Cyanogen bromide cleavage peptides were isolated and sequenced. Eight peptides yielded 103 sequenced amino acids. Dr. A. Tobin kindly supplied sequence information on a cDNA coding for feline GAD. (Kaufman et al., Science 256: 1138; Kobayashi et al., J.Neurosci., in press). The sequence from rat brain protein can be aligned with the feline cDNA coded sequence such that 63 of 103 amino acid residues are identical and 18 of 103 are conservative changes. We propose that the 59 kDa protein recognized by GAD-1 is homologous to the enzyme coded for by the cDNA described by Kaufman et al. and that differences between the two are caused by evolutionary variation.

The proteins purified by GAD-1 immunoaffinity chromatography are recognized by two extensively utilized antisera to GAD, that of Oertel et al. (Neuroscience 6: 2689) and E. Roberts and his colleagues (PNAS 71: 269). Each of these antisera react strongly on Western blots with all bands from the GAD-1 purified enzyme. Thus the enzyme recognized by GAD-1 is either identical to or highly homologous to that recognized by the two most widely used antisera.

Rat brain homogenates were separated into cytosolic and crude membrane fractions by centrifugation at 100,000xg. About 40% of the GAD activity was found in the cytosol and 60% in the membrane fraction. The latter could not be removed by extensive washing and homogenization in 2M NaCl but could be solubilized by 0.2% Triton X-100. This indicates a strong association with some type of membrane in the neuron. This solubilized enzyme was purified with GAD-1; its SDS-PAGE pattern was indistinguishable from the cytosolic enzyme.

A newly selected MAb, GAD6 stains the 59 kDa proteins but not the 63 kDa protein on Western blots. In homogenates of the brain quickly homogenized in SDS, GAD6 stains only the 59 kDa protein indicating that this protein is a major form of the enzyme in the brain and not an artefact of degradation. Because the 55 kDa form does not stain, this form may be generated during the immunoaffinity purification. (Supported by NIH grant NS12867.)

161.7 EFFECT OF RO 15-4513 ON NIGRAL-PARS RETICULATA NEURONS. G.P.Mereu+,N.Passino,M.Diana,F.Marrosu+,S.Aramo and G.L.Gessa. Depts.of Exp.Biology* and of Neurosciences; Inst.of Neurology+.University of Cagliari, Italy

Pars Reticulata (PR) neurons are inhibited by drugs that facilitate GABA transmission. They include benzodiazepines (BZs) and ethanol (ETH) among others. Vice versa,these cells are activated by GABA antagonists and by the so called BZ receptor "inverse agonists". We studied the effect of RO 15-4513, a newly synthesized azido analogue of the BZ antagonist RO 15-1788,on the spontaneous firing of PR cells and its interference with the action of ETH,diazepam (DIA) and RO 15-1788 upon these neurons.Indeed RO 15-4513 has recently been found to effectively antagonize the behavioural and biochemical effects of both ETH and BZs.

Extracellular single unit activity from identified PR neurons was recorded in male Sprague-Dawley rats prepared as d-tubocurarine-paralyzed, and locally anesthetized subjects. The intravenous (i.v.) administration of Ro 15-4513 at doses of 0.5-2.0 mg/kg caused a marked increase in the firing rate of most neurons tested (12 out 15).Maximal stimulation (about 130 % above baseline) was often elicited by a single dose of 0.5 mg/kg i.v.while higher or additional doses produced no further increase.The stimulatory effect of RO 15-4513 upon PR cells was counteracted and prevented by the i.v.injection of 1.0 mg/kg of Ro 15-1788.As expected,the i.v.administration of DIA (1.0 mg/kg) or ETH (1.0 g/kg) reduced the firing rate to 50-25 % of baseline.The inhibition produced by DIA and ETH was readily reversed by the subsequent administration of 0.5 mg/kg of Ro 15-4513.

The finding that Ro 15-4513 potently stimulated PR cells and antagonized the inhibition by DIA,and that these effects were eliminated by Ro 15-1788,indicates that this compound is a BZ receptor inverse agonist as suggested by Bonetti et al.(1984).Moreover our results have provided the electrophysiological evidence that RO 15-4513 is an ETH antagonist and support the idea that the CNS effects of ETH are mediated,at least in part, by an interaction with the GABA-BZ receptor complex. They also suggest that BZ receptor inverse agonist might result in a class of clinically effective ETH antagonists.(Supported by an Italian MPI grant to G.P.M.)

Bonetti E.P.et al (1984).Neurosci.Letts.Sup.18,S 30.

(Sponsor: M. Diana)

161.8 RO 15-4513, AN AZIDO ANALOGUE OF THE BENZODIAZEPINE ANTAGONIST RO 15-1788, INCREASES DOPAMINE TURNOVER IN THE PREFRONTAL CORTEX AND PRODUCES PROCONFLICT EFFECTS IN THE RAT. O. Giorgi, M.G. Corda, B. Longoni* and G. Biggio. Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.

The mesocortical dopaminergic (DAergic) neurons projecting to the prefrontal cortex (PFCx) are activated in response to stress. Thus, electric foot-shock increases DA metabolism in the PFCx, as reflected by the enhanced content of dihydroxyphenylacetic acid (DOPAC), and this effect can be reversed by benzodiazepines (BZDs). Moreover, the anxiogenic ß-carbolines FG 7142 (20 mg/kg, i.p.) and ß-CCM (8 mg/kg, s.c.) also increase the turnover rate of DA in the PFCx, indicating that BZD recognition sites play a modulatory role on the mesocortical DAergic system. Ro 15-4513 is an azide derivative of the BZD antagonist Ro 15-1788 that binds with high affinity to BZD recognition sites. In addition, Ro 15-4513 has proconvulsant effects in mice and antagonizes the effects of ethanol both in vitro and in vivo in rats. The present study was aimed at characterizing the pharmacological effects of Ro 15-4513 on the mesocortical DAergic system. Ro 15-4513 stimulated DA metabolism in the PFCx of the rat in a dose-dependent manner (5-20 mg/kg, i.p.). When given at a dose of 20 mg/kg, Ro 15-4513 increased DOPAC content in the PFCx by 52%. The BZD antagonist Ro 15-1788 failed to affect per se the DOPAC content in the PFCx, but at the dose of 40 mg/kg, i.p. reverted the increase in DA metabolism induced by Ro 15-4513 as well as by FG 7142 and ß-CCM. Taken toghether with previous reports, the above results indicate that Ro 15-4513 interacts with BZD recognition sites in the CNS as a partial inverse agonist. Behavioural and biochemical data support this view. Thus, in membrane preparations from the rat cerebral cortex, Ro 15-4513 concentration-dependently (0.1-10 μM) antagonized the increase in ^{3}H-GABA binding induced by 5 μM diazepam. Moreover, in the Vogel's conflict test, Ro 15-4513 (2-10 mg/kg, i.p.) enhanced the shock induced suppression of drinking in rats. Finally, at a dose of 10 mg/kg, i.p., Ro 15-4513 potentiated the convulsive effects induced by isoniazid (350 mg/kg, s.c.).

161.9 ACCELERATED DESENSITIZATION BY BENZODIAZEPINE OF $GABA_A$ RECEPTOR-MEDIATED $^{36}Cl^-$ INFLUX IN PRIMARY CULTURE OF RAT CEREBELLAR NEURONS. J. Kardos, A. Guidotti and E. Costa. FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Medical School, Washington, D.C. 20007.

Primary cultures of rat cerebellar neurons, 6-7 days in vitro, contain 95% granule cells, 3-4% GABAergic interneurons and express the GABA/benzodiazepine Cl^- ionophore receptor complex (J. Neurosci., 5, 2432, 1985). Preincubation (performed for 10 min at gradually decreasing temperature) preceeded the $^{36}Cl^-$ influx experiments usually conducted at 4°C in Earle's balanced salt solution (EBSS), pH 7.5. The $^{36}Cl^-$ influx was terminated 7 sec later by rinsing the dishes (less than 3 sec) with EBSS containing 1 mM furosemide and picrotoxin. The concentration response curve of GABA stimulated influx of $^{36}Cl^-$ was biphasic. Maximal stimulation (2 to 3 fold; 100 nmole/mg prot/20 sec) was reached between 1 to 10 uM GABA; higher concentrations of GABA (10 to 100 uM) led to a smaller stimulation of $^{36}Cl^-$ influx. Bicuculline methiodide (10^{-3} M) inhibited the effect of GABA. Bicuculline (0.1 to 1 uM), in a dose related fashion, reduced basal $^{36}Cl^-$ influx presumably by reducing the effect of small amounts of endogenous GABA released from the GABAergic cells in culture. Consistent with the reported relative potencies of enantiomeric dihydromuscimols, $^{36}Cl^-$ influx was stimulated by (S)-(+) dihydromuscimol. The $GABA_B$ receptor agonist (-)-baclofen (1 uM) failed to stimulate Cl^- influx supporting the concept that stimulation of $^{36}Cl^-$ influx by GABA involves $GABA_A$ receptors. The $GABA_A$ receptor-mediated increase of $^{36}Cl^-$ influx was shifted to the left by application of 1 uM diazepam, clonazepam, or oxazepam. This effect was stereospecific. Moreover, the effect of diazepam was blocked by 1 uM flumazenil (RO-151788) which per se failed to change GABA stimulated $^{36}Cl^-$ influx. In contrast, the anxiogenic beta-carboline-3-carboxylate derivative DMCM decreased the action of GABA. The mitochondrial type benzodiazepine ligand, RO5-4864 failed to enhance the GABA-stimulated increase of $^{36}Cl^-$ influx. These results indicate an involvement of central type benzodiazepine recognition sites. The addition of GABA (3 to 10 uM) or benzodiazepines (1 uM) to neurons, preincubated for 15 min with 10 to 100 uM GABA followed by extensive washing, failed to activate $^{36}Cl^-$ influx. The GABA response is decreased with time (from 7 to 30 sec) and this decrease is accelerated in the presence of diazepam. Taken together, these data suggest that GABA, in a time and concentration dependent-manner, desensitizes the receptor. In addition, benzodiazepines accelerate the desensitization of the $GABA_A$ receptor operated Cl^- channels.

161.10 DIFFERENTIAL DEVELOPMENT OF TOLERANCE TO THE ANTICONVULSANT AND BEHAVIORAL EFFECTS OF ZOLPIDEM AND MIDAZOLAM. B. Zivkovic, G. Perrault*, D.J. Sanger* and E. Morel*. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 31 Av. P.V. Couturier, 92220 Bagneux, France.

It is well documented that tolerance develops to the anticonvulsant and sedative effects of benzodiazepines. Zolpidem is a novel short-acting hypnotic of imidazopyridine structure which binds to a macromolecular element of brain membranes which also binds benzodiazepines (Arbilla et al., Eur. J. Pharmacol. 130: 257, 1986). Due to the similarities in the mechanism of action of zolpidem and benzodiazepines, the ability of zolpidem to induce tolerance to its anticonvulsant and sedative effects was compared to that of midazolam, a benzodiazepine which has a relative potency and duration of action similar to that of zolpidem.

Tolerance to the anticonvulsant effects was studied in mice. The animals received zolpidem (30 mg/kg, po, bid) or midazolam (30 mg/kg, po, bid) for 10 consecutive days; 36 hours after the last administration the ability of these drugs to antagonize convulsions induced by isoniazid (800 mg/kg, sc, ISO) or pentylenetetrazole (125 mg/kg, sc, PTZ) was studied. As shown in the table, the potency of zolpidem in antagonizing ISO- and PTZ-induced convulsions was similar in mice repeatedly treated with zolpidem and in mice repeatedly treated with saline. In contrast to zolpidem, midazolam was 3 times less active after its repeated adminstration.

Drug	ED_{50} (mg/kg)					
	ISO+		PTZ+		FR-10*	
	Acute	Repeated	Acute	Repeated	Acute	Repeated
Zolpidem	1.0	1.2	7.2	12	0.6	1.2
Midazolam	0.5	1.6	0.4	1.2	1.7	10

+ ip route, * sc route

Development of tolerance to the sedative effects was studied in rats trained to press a lever in a standard operant test chamber to obtain food pellets according to a fixed-ratio 10 schedule (FR10). Repeated administration of zolpidem produced only a small change in its potency in suppressing the rate of lever presses whereas repeated administration of midazolam decreased the potency of this benzodiazepine by a factor of 6 (see table). There was no evidence of cross-tolerance between the sedative effects of these two drugs.

These findings demonstrate that less tolerance develops to the anticonvulsant and sedative effects of zolpidem than to the effects of midazolam and suggest that the mechanisms of action of the two drugs are not identical.

161.11 MODULATION OF ^{36}CHLORIDE FLUX BY MIDAZOLAM IN INTACT CULTURED NEURONS FROM THE CHICK EMBRYO CEREBRUM. C.L. Thompson* and E.M. Barnes, Jr. Depts. of Biochem. and Physiol. and Mol. Biophys., Baylor Col. of Med., Houston, TX 77030.

A biochemical/pharmacological assay of GABA receptor function at the cellular level, by monitoring ^{36}Cl ion flux in cultured cerebral neurons, has previously been developed in our laboratory [Thampy, K.G., and Barnes, Jr., E.M. (1984) J. Biol. Chem. 259, 1753-1757]. This assay has been used to assess the modulatory interaction of a water-soluble benzodiazepine, midazolam, with GABA-gated Cl channels in this system. Neuronal monolayers on vinyl plastic coverslips were incubated in fresh DMEM at 37° for 15 min, rinsed, and then transferred to HEPES buffered saline at 22° containing 40 mM K, ^{36}Cl (5 µCi/ml), and effector ligands. The 10 sec uptake assay was terminated by rapid transfer to ice-cold saline.

Midazolam had no effect on ^{36}Cl uptake by neurons when added simultaneously with ^{36}Cl. A 5 min incubation of neurons with midazolam prior to addition of ^{36}Cl produced the maximal stimulation. Longer intervals of preincubation resulted in a progressive loss of the ability of midazolam to stimulate ^{36}Cl uptake. This fading of the response was even more pronounced when GABA was present and is therefore likely to reflect desensitization. Under optimal conditions, 0.1 nM to 1 µM concentrations of midazolam stimulated the basal Cl flux, observed in the absence of exogenous GABA, from 50% to 270%, respectively. For this response, the EC_{50} of 6 nM correlated well with the Ki value (8 nM) for displacement of [^{3}H]flunitrazepam from cortical membranes. Picrotoxin (100 µM) or bicuculline (100 µM) completely abolished the midazolam stimulation. These antagonists, however, had no effect themselves on the basal Cl uptake. This suggests that midazolam potentiated the effect of endogenous GABA, whose release was promoted by high K medium. Indeed, when assayed at 5.4 mM K, the midazolam effect was lost. Midazolam was also unable to further stimulate ^{36}Cl flux in the presence of a saturating (50 µM) dose of GABA. In order to remove endogenous GABA, neurons were preincubated in depolarizing medium containing 131 mM K and 5.4 mM Na. Under these conditions, midazolam was ineffective unless GABA was added. A kinetic analysis of ^{36}Cl flux suggested that 10 nM midazolam reduced the apparent $K_{0.5}$ for GABA from 5 µM to less than 2 µM without affecting the Vmax. In addition to providing a method for studying the effects of benzodiazepines on endogenous neurotransmission, our findings are consistent with electrophysiological experiments demonstrating that these compounds potentiate GABA responses.

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161.12 PRESENCE AND DISTRIBUTION OF A PUTATIVE PRODUCT OF PROCESSING OF DIAZEPAM BINDING INHIBITOR IN HUMAN BRAIN. C. Ferrarese, M. Frigo*, R. Piolti*, F. Tamma*, A. Cappellini *° and L. Frattola*. Dept. of Neurology, University of Milan and °Dept. of Pathology, Ospedale San Gerardo, via Donizetti 106, 20052 Monza, Italy.

Diazepam Binding Inhibitor (DBI), a 104 aminoacid polypeptide which coexists with GABA and elicits proconflict responses in the rat, has been purified and characterized also in human brain. Tryptic digestion of rat and human DBI yelds 8 peptidic fragments; the fifth one in both species maintains the same pharmacological properties of the DBI molecule. The rat fragment, which is 18 aminoacids long and is called octadecaneuropeptide (ODN), has been found in rat brain by radioimmunoassay (RIA) and is co-released with DBI and GABA from neurons in primary culture. We investigated the presence of the corresponding human fragment, 20 aminoacids long and called eicosapeptide (EP) (table), in various autoptic and bioptic human brain areas. Using antibodies raised in rabbits against synthetic EP, we performed a specific RIA in acetic acid extracts of normal autoptic brain areas (cerebral cortex, cerebellum, dentate gyrus, nucleus caudatus, thalamic nuclei -MD and VPL-, hypothalamus, mamillary bodies, substantia nigra, periaqueductal grey, choroid plexus) of fresh bioptic samples and of bioptic samples kept at room temperature for different times. Significant differences of EP-like immunoreactivity (EP-L.I.) content were found in the different brain areas, with the highest amount in cerebellum (550 ± 45 pmol/mg prot), followed by dentate gyrus (500 ± 37 pmol/mg prot) and cerebral cortex (350 ± 48 pmol/mg prot); lower amounts were detected in the other brain areas). Reverse-phase HPLC analysis of the extracts from different areas revealed one major peak of EP-L.I., with the same retention time in fresh and autoptic samples, corresponding to authentic E.P.

The regional distribution of EP-L.I. in the different brain areas is similar to that of DBI: these data support the hypothesis of a physiological role of this peptide as a product of processing of DBI, with similar pharmacological profile. Modifications of DBI and of its product of processing in pathological brain areas and in cerebro-spinal fluid of subjects with neuropsychiatric disorders can now be studied.

TABLE: Aminoacid sequence of tryptic fragment #5 of human and rat DBI

Human	QATVGDIMTERPGMLDFTGK	(EP)
Rat	QATVGDVNTDRPGLLDLK	(ODN)

TROPHIC INTERACTIONS I

162.1 THE REGENERATION BLASTEMAS OF ANEUROGENIC LIMBS ARE ANTIGENICALLY DISTINCT FROM THOSE OF NORMALLY-INNERVATED LIMBS IN NEWT LARVAE. D. M. Fekete & J. P. Brockes. MRC Cell Biophysics Unit, 26-29 Drury Lane, London, England WC2B 5RL.

The limbs of urodele (tailed) amphibians regenerate after amputation by forming a localized growth zone, called the blastema, at the distal tip of the limb stump. Under normal circumstances, division of the blastemal (progenitor) cells requires nerves at the amputation plane during the first several weeks of regeneration. No such requirement for nerves exists during early development as amputated limbs can regenerate at stages prior to the arrival of axons. Furthermore, the nerve requirement can be circumvented by allowing a limb to develop in the near absence of nerves (the 'aneurogenic limb'). The mechanism of aneurogenic regeneration is unknown, but may involve a system of nerve-independent growth control similar to that occuring during normal development.

We have used monoclonal antibodies to identify the blastemal cells that arise after amputation in these different circumstances. One antibody, called 22/18, was previously shown in adult newts to react with a subset of blastemal cells whose division is dependent on the nerve, but not with normal, uninjured tissue (Kintner and Brockes, J.E.E.M. 89: 37, 1985). We have recently shown that 22/18 does not react with developing limb buds nor does it stain the blastemas of such buds unless amputation is performed after the nerves reach the amputation plane (Fekete and Brockes, Development, 99: 589, 1987). We now report that 22/18-staining is much reduced in the blastemas of sparsely-innervated limbs compared with normal controls.

Aneurogenic limbs were created by joining two newt embryos (*Pleurodeles waltl*, head process stage) at the flank by parabiosis. At the tail process stage the epibranchial placodes and most of the neural tube and neural crest were removed from one member from the level of the hindbrain to the 10-14th somite. 3-4 weeks later, the forelimbs had developed to the 2-digit stage and were amputated through the proximal humerus. After 5-10 days, when early bud-stage blastemas were evident, the parabiotic pairs were fixed, frozen-sectioned and immunostained as described by Fekete and Brockes (1987). The unoperated host animal served as an important internal positive control in these experiments. Nerves were detected using a rabbit antiserum directed against the 70kD neurofilament protein (gift of P. Hollenbeck). In all cases, a few axons were detected in the 'aneurogenic limbs' but these were drastically reduced in number compared with host limbs. Eight such sparsely-innervated limbs (n=6 animals) were found to have reduced levels of 22/18 immunoreactivity compared with host blastemas. Five of these aneurogenic limbs were completely 22/18-negative.

In summary, 22/18 appears to be specific for regeneration versus development, and specific for blastemal cells that arise after amputation in the presence of the nervous system versus its absence (in either development or the aneurogenic limb). The antibody reactivity seems to mark a cell transition involved in the imposition of nerve-dependent growth control.

D.M.F. is a Smith Kline and French Fellow of the Life Sciences Research Foundation.

162.2 EFFECT OF MUSCLE FIBER DEGENERATION ON THE MAINTENANCE OF IDENTIFIED NERVE TERMINALS IN THE LIVING MOUSE. M.M. Rich and J.W. Lichtman, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

Evidence from a number of laboratories argues for two seemingly different ways a postsynaptic cell may maintain synaptic terminals. Either the continuous release of diffusible factors by target cells is responsible for stable synaptic connections or the association of presynaptic terminals with membrane bound, or extracellular molecules is sufficient. With regard to the latter idea, McMahan, Sanes and colleagues have demonstrated that motor nerve terminals can reside for long periods on synaptic basal lamina when the underlying muscle fibers have degenerated (e.g., J. Cell Biol. 78: 176).

To directly assess the consequences (or lack thereof) of muscle fiber death on motor nerve terminal maintenance, we viewed the same neuromuscular junction in living mice before and various times after muscle fiber degeneration. Muscle fibers in the adult mouse sternomastoid muscle were caused to degenerate by cutting or freezing them on either side of the endplate band. Care was taken to avoid any damage to the innervating nerves. Before, and various times (1 day to 3 months) after damaging the muscle, the endplates were visualized by staining the nerve terminals with 4-Di-2-ASP (a mitochondrial probe; J. Neurosci. 7: 1207) and the muscle cell's acetylcholine receptors were stained with fluorescently tagged α-bungarotoxin. Light and electron microscopical sections of the muscle were used to confirm muscle fiber loss and the presence of basal lamina ghosts.

Within several days of muscle damage, changes in the staining of individual motor terminals were obvious. Nerve terminals that previously contained continuous strips of contacts became spotty in appearance, suggesting that many of the synaptic contacts had detatched. At about 1 week following muscle damage, the muscle fibers regenerated in the basal lamina ghosts and new acetylcholine receptors were visible. These new receptors were inserted only under the maintained sites of contact. Thus the new acetylcholine receptor distribution was quite spotty and corresponded to the maintained distribution of nerve terminal contact. By labeling the initial receptor sites with rhodaminated bungarotoxin and the new receptors with fluoresceinated bungarotoxin we found that only at the spots where nerve terminal still could be stained were new acetylcholine receptors present. Our results suggest that muscle fiber degeneration rapidly eliminates nerve terminal contacts and thus that the presence of the postsynaptic cell may be important for the day to day maintenance of nerve terminals.

162.3 DENDRITIC GEOMETRY OF SYMPATHETIC GANGLION CELLS IS REGULATED BY POSTGANGLIONIC TARGET SIZE. James T. Voyvodic, Washington University Medical School, 660 S. Euclid, St. Louis, Mo. 63110

Dendrites of sympathetic neurons in the rat superior cervical ganglion (SCG) continue to grow in adulthood; furthermore, the growth of these dendrites is largely independent of the presence of preganglionic innervation (Voyvodic, 1987 J. Neurosci. 7:904-912). Since dendritic length increases in proportion to the overall size of the animal (op. cit.), the question arises whether dendritic geometry is regulated by the size of the peripheral target tissues that these cells innervate. In the present study I have assessed the retrograde regulation of dendrites by measuring ganglion cell geometry after experimental alteration of the relative size of a peripheral target (the submandibular salivary gland). The approach I have adopted takes advantage of the fact that ganglion cells axotomized early in life do not survive. Thus, partial denervation of the submandibular gland at birth effectively changes the ratio between the size of the target and the number of SCG cells that innervate it.

In newborn male Holtzman rats a portion of the postganglionic sympathetic nerve innervating the right submandibular gland was exposed and one of the branches of the nerve was transected. Care was taken not to disturb the other branches. Animals were evaluated at 12 weeks of age. The partial denervation had no effect on the weight of the submandibular gland; however, in operated animals the number of SCG cells innervating the gland was only 5 to 30% of the number in control animals. Therefore, the ratio of target size to the number of innervating ganglion cells was significantly increased compared to normal animals.

Ganglion cell geometry was assayed by intracellular injection of 6-carboxyfluoroscein and HRP into cells projecting to the submandibular gland; such cells were identified by retrograde labelling with another fluorescent label (Di-I). In control animals, cells projecting to the submandibular gland had a mean dendritic length of 3411 um, $\pm$ 359 um (SEM) and bore an average of 5.8 primary dendrites ($\pm$ 0.6 SEM). In experimental animals, the mean dendritic length was 5667 um, $\pm$ 586 (SEM) with an average of 16.8 primary dendrites ($\pm$ 1.4 SEM). Thus, cells innervating relatively larger targets elaborate more complex dendritic arbors.

These results suggest that the growth of ganglion cell dendrites is regulated by retrograde signals from the peripheral target.

This work was supported by a NSF Graduate Fellowship, and by NIH grants NS 11699 and NS 18629 to Dale Purves.

162.4 DEVELOPMENT OF FUNCTIONAL Na-CHANNELS IN CULTURED SYMPATHETIC NEURONS IS DEPENDENT ON THE PRESENCE OF TARGET CELLS - A NEW TYPE OF TROPHISM. J.C. Prat*,Taruna D. Wakade* and Arun R. Wakade (SPON: J. Ranck) Dept. of Pharmacology, SUNY Health Science Center at Brooklyn, New York 11203.

Neurotrophic molecules produced by effector organs are essential for the survival of various types of neurons. Nerve growth factor, supports the surival of sympathetic neurons in culture, where they develop a dense neurite network and acquire noradrenergic characteristics. However, it is not known whether the cultured sympathetic neurons are functionally identical to their counterparts developing in the body. In the present study we have compared the functional behavior by inducing release of ^{3}H-norepinephrine (^{3}H-NE) by electrical stimulation of sympathetic neurons of almost identical age but growing in the chick embryonic heart and in culture, with and without heart cells. We demonstrate here a very close correspondence between the functional behavior of neurons developing with the heart cells, either in vitro or in vivo. For example, electrically-evoked (10 Hz for 60 sec) release of tritium from ^{3}H-NE-loaded heart was blocked by 10 nM tetrodotoxin (TTX), 0.25 mM Ca and potentiated by 3 mM tetraethylammonium (TEA). In sharp contrast, the release evoked by stimulation of ^{3}H-NE-loaded cultured neurons (1 Hz for 15 sec) was not blocked by 300 nM TTX, low Ca, or potentiated by TEA. Veratrine, although produced a large increase in tritium release from the heart, had very little effect on the release from cultured neurons. However, when neurons were co-cultured with dissociated heart cells, the evoked release was blocked by TTX, low Ca, potentiated by TEA, and veratrine induced a massive release. Neurons grown in a medium conditioned by the heart cells, or co-cultures of heart and neuronal cells, did not respond to TTX and veratrine. A dramatic alteration in the functional behavior of neurons by co-culturing with heart cells indicates that the effector organ has an important role in the development of normal ionic conductances of sympathetic neurons growing in the body and culture.

162.5 REGULATION OF MOLECULAR COMPONENTS OF THE SYNAPSE IN THE DEVELOPING AND ADULT RAT SUPERIOR CERVICAL GANGLION. K. Wu* and I.B. Black. Dept. of Neurology, Division of Developmental Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

The rat superior cervical sympathetic ganglion (SCG) was used to begin studying regulation of molecular components of the synapse. Ganglion postsynaptic densities (PSD's) exhibited a thin, disc-shaped profile electron microscopically, comparable to that previously described for brain. Moreover, the presumptive major ganglion PSD protein (mPSDp) was phosphorylated in the presence of Ca^{2+} and calmodulin, bound ^{125}I-calmodulin, and exhibited an apparent Mr of 51 KD, all characteristic of the brain mPSDp. These initial studies indicated that ganglion and brain mPSDp's are comparable, allowing us to study synaptic regulation in the well-defined SCG. To obtain sufficient quantities of the ganglion mPSDp, we used synaptic membrane fractions. During postnatal development, calmodulin binding to the mPSDp increased 411-fold per ganglion from birth to 60 days, whereas synaptic membrane protein rose only 4.5-fold. Consequently, different synaptic components apparently develop differently. Moreover, denervation of the SCG in adult rats resulted in an 85% decrease in mPSDp calmodulin binding, but no change in synaptic membrane protein two weeks postoperatively. Our observations suggest that the presynaptic innervation regulates specific molecular components of the postsynaptic membrane structure in a selective fashion. (Supported by NIH Grants NS 10259 and HD 12108. I.B.B. is the recipient of a McKnight Research Project Award).

162.6 INSULIN GROWTH FACTORS REGULATE MITOSIS OF PRESUMPTIVE SYMPATHETIC NEUROBLASTS. E. DiCicco-Bloom and I.B. Black. Div. of Developmental Neurology, Cornell Univ. Med. Coll., New York.

Since mature mammalian neurons do not divide, initial developmental generation is critical for adult brain organization. Although the process of neurogenesis has been studied descriptively, governing mechanisms are unknown. We have developed a new system for defining regulation of the mitotic cycle in presumptive neuroblasts to analyze this problem.

Dissociated embryonic rat superior cervical ganglia were cultured in fully defined medium; after exposure to ^{3}H-thymidine from hours 24 to 48, incorporation (Inc) was assessed by scintillation spectroscopy or immunocytochemistry (IC) and autoradiography (AR).

To identify cultured cells, we performed IC for tyrosine hydroxylase (TH): virtually all cells expressed this important neurotransmitter phenotypic trait. Subsequent AR revealed that 25 to 35% of TH cells simultaneously exhibited nuclear labeling (labeling index, LI), suggesting that DNA synthesis occurred in culture. Identification of single cells, containing two labeled nuclei at different stages of the cell cycle, suggested that mitosis was ongoing. Our observations indicated that a virtually pure population of TH ganglion cells, cultured in fully defined medium, synthesized DNA and underwent mitosis in vitro.

To define factors controlling Inc, we assayed cultures incubated in media lacking individual components. Insulin (Ins) deletion produced the most marked effect, reducing Inc 4-fold. The ED50 for the hormone was 500ng/ml. Conversely, Ins more than doubled the LI over non-Ins controls while not altering cell numbers, indicating that increased Inc reflected increased cells entering the mitotic cycle. Ins did not non-selectively stimulate multiple metabolic processes, since protein synthesis was not affected.

Since Ins is but one of a family of growth promoting factors, we also examined effects of insulin-like growth factor (IGF) I and II. IGF I replicated the Ins effect with an ED50 of 1 ng/ml, exhibiting a 500-fold greater potency than Ins. In contrast, IGF II barely doubled Inc at 100 ng/ml. Thus, all Ins family factors stimulated mitosis, probably acting via the IGF I receptor. Further, nerve and epidermal growth factors and interleukin 2 had no effect on the LI, suggesting Ins factors are highly specific mitogens for presumptive ganglion neuroblasts. (Supported by NIH Grants HD 00676, BRSG S07 RR 05396, a Teacher-Scientist Award from the Mellon Fdn., and a McKnight Research Project Award).

162.7 ENDOGENOUS OPIOID SYSTEMS REGULATE GROWTH OF NEUROTUMOR CELLS IN CULTURE. P.J. McLaughlin and I.S. Zagon. Dept. Anatomy, The M.S. Hershey Med. Ctr., The Pennsylvania State University, Hershey, PA 17033.

Exogenous and endogenous opioid agonists are known to inhibit human and animal tumor growth in vivo in a stereospecific and naloxone reversible manner. Paradigms using opioid antagonists to perturb interaction of endogenous opioids and opioid receptors (= endogenous opioid systems) have demonstrated that tumorigenic events are regulated by a delicate equilibrium between opioids and receptors, and suggest that endogenous opioids exert a tonic and inhibitory influence on neoplasia. In order to ascertain if these mechanisms act at the cellular level, the role of endogenous opioid systems on growth was examined in cultures of neuroblastoma cells (= NB). Cultures of S20Y NB were seeded and, beginning 24 hr later, various concentrations (10^{-6}M to 10^{-10}M) of methionine-enkephalin (= met-enk), or sterile water (= CO), were added to the media; drug and media were changed daily. Cells were counted (trypan blue exclusion test) 12, 24, 48, 72, and 96 hr after initial drug exposure. Growth curves demonstrated a dose-dependent inhibition of NB, with 10^{-6}M met-enk reducing cell number by over 60%. Growth inhibition was not observed in NB cells cultured in media containing both met-enk and naloxone. The mitotic index of NB cells grown in 10^{-6}M met-enk for 48 hr was 72% less than CO. To further assess the role of endogenous opioid systems in NB growth in vitro, the opioid antagonist naltrexone (= NTX) was added daily to cultures in dosages of 10^{-4}M to 10^{-8}M. A dose-dependent stimulation of growth was recorded. A dosage of 10^{-6}M NTX, for example, increased cell number at 24, 48, and 72 hr by 1.7-2.4 fold from CO; the mitotic index at 48 hr was 60% greater than CO. To determine if S20Y NB cells in culture expressed opioid binding sites, assays of 72 hr CO cultures were conducted with ^{3}H-DADLE and ^{3}H-met-enk. Specific and saturable binding to both ligands, with Kd values of 0.30 and 2.13 nM, respectively, and Bmax values of 10.2 and 19.6 fmol/mg protein, respectively, were noted. Radioimmunoassays revealed that both β-endorphin and met-enk were detected in media from log phase cells cultured for 3 days at levels notably above those found in media prior to culturing. S20Y NB cells also contained measurable levels of both endorphin and enkephalin. Our studies show that endogenous opioids directly regulate cell proliferation, and may do so through an autocrine-like mechanism.

Supported by NIH grants NS-20623 and NS-20500.

162.8 **BRAIN ASTROCYTE POPULATIONS ARE CONTROLLED BY A MITOGEN INHIBITOR WITH EGF RECEPTOR IMMUNOREACTIVITY.** M. Nieto-Sampedro, J.T. Broderick* and F. Gómez-Pinilla Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

Brain injury causes a time-dependent increase in *in vitro* astrocyte mitogenic activity both adjacent to the lesion and in deafferented areas which is presumably responsible for the astrocyte proliferation observed after the lesion. A comparison of the shape of the dose-response curves of mitogenic activity in extracts of normal brain tissue and tissue adjacent to an injury suggests that the increase in activity is achieved to a large extent through a decrease in the inhibition of the mitogen/s.

Epidermal Growth Factor (EGF) is a well characterized mitogen the sequence of which occurs in a variety of membrane-bound or secreted proteins, including plasminogen activator, low density lipoprotein receptor and tumor growth factor. Its action appears to be mediated by a transmembrane glycoprotein with tyrosine kinase activity, the.EGF receptor (EGFR). While investigating the identity of the rat brain cells that bear EGFR immunoreactive material (EGFR-IR) we found that EGF receptor immunoreactivity first appeared in astroglia at about day 16 postnatal,was maximal at 19 days postnatal and then became weaker as the animals reached adulthood. Adult brain astrocytes stained weakly if at all for EGFR. This situation changed dramatically after injury. One to three days after ablation of the entorhinal cortex of an adult rat, GFAP-positive astrocytes adjacent to the injury site and in the deafferented hippocampus became intensely EGFR positive. Both GFAP and EGFR immunostaining continued to increase over the next 15 days as a thick layer of fibrous astrocytes covered the injury boundary. In the deafferented dentate gyrus EGFR/GFAP positive astrocytes become very prominent and migrated towards the outer molecular layer.

When tested for their effect on the *in vitro* incorporation of ^{3}H-Thymidine into purified astrocytes promoted by brain extracts, two anti-EGFR antibodies (one mouse monoclonal and another rabbit polyclonal) caused a remarkable enhancement. A similar effect was achieved by pre-treating the brain extracts with the antibodies immobilized on Protein A-Sepharose beads. The antibodies themselves did not have any direct effect on the glial cells, indicating that they acted by removing a mitogen inhibitor from the brain extracts. This inhibitor seems to share epitope/s with the EGFR. Injection of the antibody into the rat cortex caused the rapid appearance of numerous EGFR/GFA-positive astrocytes at the injection site.

The localization of EGFR immunoreactivity and the action of anti-EGFR both *in vitro* and *in vivo* suggests that a protein immunologically similar to EGFR may be involved in controlling the conversion of resting astrocytes into reactive astrocytes.as well as in astrocyte proliferation.

Supported by grant AG 00538-09 A from the National Institutes of Aging.

162.9 LOCALIZATION OF CELLS IMMUNOREACTIVE FOR EPIDERMAL GROWTH FACTOR (EGF) RECEPTOR IN THE ADULT AND DEVELOPING RAT FOREBRAIN. R.Loy, J.E.Springer and S.Koh. Dept. Neurobiology and Anatomy, Univ. Rochester Med. Center, Rochester, N.Y. 14642.

EGF is likely to be an important trophic factor in the CNS. Immunocytochemically EGF has been localized to a variety of cells in the basal forebrain. In CNS cultures, EGF stimulates cellular differentiation and myelination, secretion of extracellular proteins, and glial enzymatic activity, presumably through specific receptors for EGF (EGF-R). We investigated the localization of these receptors immunocytochemically using a monoclonal antibody to rat EGF-R, IgG-151, produced and characterized by C. Chandler and E. Shooter, and purified and generously provided by E. Johnson. Eight female and two male young adult Long Evans rats were anesthetized and perfused with 1-4% paraformaldehyde in PBS. Five rat fetuses, aged 14, 15 and 18 days of gestation, and one rat pup one day old, were decapitated and the brains or whole heads immersion-fixed for 24-48 hours. Coronal or horizontal frozen sections were processed by the avidin-biotin immunoperoxidase method, using an affinity-purified secondary antibody. In the adult brains, large, multipolar EGF-R immunoreactive cell bodies are found in the nucleus basalis, vertical and horizontal limbs of the nucleus of the diagonal band of Broca, and the medial septal nucleus. Immunoreactive neurons are also scattered throughout the caudate/putamen and in the lateral septal nucleus, bed nucleus of the stria terminalis, the olfactory tubercle, the basolateral, basomedial and lateral nuclei of the amygdala. Additional immunoreactive neurons occur in layers II-VI of the cingulate and neocortex and within the hippocampal formation. These latter are most likely interneurons, based on their morphology and distribution within the molecular and infracellular layers. Immunoreactivity associated with fiber tracts and terminal fields occurs in stratum oriens and stratum lacunosum of Ammon's horn and in the molecular layer of the dentate gyrus, in the fornix, caudate/putamen, olfactory tubercle, piriform cortex and neocortex. Immunoreactive cells appear by as early as embryonic day 14 in the nucleus of the diagonal band and nucleus basalis. These observations suggest that sensitivity to EGF develops very shortly in these cells following neurogensis. It is interesting to note that some neurons in these basal forebrain regions show a similar early development of receptors to nerve growth factor (NGF). These multipolar neurons have widespread projections to the neocortex and hippocampus, some of which are cholinergic. In contrast to the localization of cells which express NGF receptors in the adult rat brain, cells which express EGF-R are more widespread, and presumably represent a more divergent population of transmitter-specific systems. Supported by NSF grant BNS-8511564.

162.10 **NEUROTROPHIC EFFECTS OF HIPPOCAMPAL MEMBRANES ON PRIMARY CULTURES OF RAT SEPTAL NEURONS.** M.B. Emerit*, J. Segovia*, and B.C. Wise. FIDIA-Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007.

Primary cultures of rat septal neurons prepared from rat fetuses 17-days in gestation and maintained under serum-free conditions are used to study the survival, growth and maturation of the CNS cholinergic neuron. Under our culture conditions, greater than 98% of the cells are neurons while the remaining cells are identified as astrocytes. Immunocytochemical staining for choline acetyltransferase (ChAT) indicates that 3-5% of the cell population are cholinergic neurons. Cultured septal neurons express ChAT activity (maximal activity seen at 6 days in vitro (DIV)) and develop the ability to synthesize acetylcholine (Ach) (maximal in situ synthesis seen at 10 DIV). Treatment of septal neurons with hippocampal membranes (20 ug protein/ml) prepared from 21 day old rats increases the rate of expression (maximal activity seen at 4 DIV) and the maximum levels of ChAT activity (1.5- to 2-fold increase). In contrast, soluble extracts of the hippocampus are 10-fold less potent than membranes in stimulating the expression of ChAT activity. Membranes prepared from cerebellum and other brain regions, with the exception of striatum, have negligible or low activities toward stimulating ChAT activity in septal neurons. The activity present in hippocampal membranes is developmentally regulated with highest levels observed in membranes prepared from 14-21 day old rats. Anti-NGF antibody while inhibiting the effects of NGF treatment does not block the stimulatory effect of membranes on ChAT activity. Furthermore, treatment of septal neurons with maximal amounts of NGF (100 ng/ml, 1.6-fold increase in ChAT) and hippocampal membranes (20 ug protein/ml, 1.75-fold increase in ChAT) results in an additive increase in ChAT activity indicating that the membrane effect is not due to NGF and that different mechanisms may be involved in the two trophic activities. Trophic activity is released from membranes by trypsin treatment, 0.5 M NaCl, incubation at 37°C or detergent solubilization. Trypsin digestion of detergent solubilized membrane extracts, in contrast to heat denaturation, does not result in diminished trophic activity of the extract. Sephadex G-75 chromatography of a 0.5 M NaCl extract of membranes indicates a molecular weight greater than 66,000 daltons for the active factor. However, since activity in solubilized membrane extracts is not inactivated by trypsin, it is possible that a peptide fragment is the active component. These results indicate that hippocampal membranes contain neurotrophic activity which may be important for the terminal differentiation and synaptogenesis of cholinergic neurons.

162.11 **A MUSCLE-DERIVED FACTOR(S) INDUCES DIFFERENTIATION OF CATECHOLAMINE TRAITS IN CULTURED RAT CORTEX.** *L. Iacovitti, T. Shirao, D.J. Reis.* Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

We have previously reported a) that a population of embryonic rat cortical neurons can express the catecholaminergic (CA) enzyme tyrosine hydroxylase (TH) *in vitro* but not *in vivo* (J. Neurosci. *7* : 1264, 1987) and b) that their number can be dramatically increased by co-culture with muscle. In this study we sought to a) further characterize the muscle factor(s) mediating this effect and b) determine whether this factor(s) regulates TH expression by increasing the survival of TH neurons or by inducing CA differentiation in a greater number of cortical neurons.

Confluent cultures of skeletal muscle cells from the L6 cell line were harvested, homogenized in phosphate buffered saline (PBS, 60×10^6 cells/ml) and centrifuged at $40,000 \times g$ for 1 hr to separate soluble and insoluble fractions. Cerebral cortex from embryonic day 13 rat was dissociated and grown in culture in media containing either a) 80% PBS (control), b) 80% soluble muscle fraction (0.06-9.12 mg total protein/ml) or c) 80% insoluble muscle fraction (resuspended in PBS to a final concentration of 1.0-10.0 mg protein/ml). We found that the soluble but not the insoluble fraction of muscle produced a dose-dependent increment in the number of TH immunoreactive cortical neurons, increasing their number 12-fold at the highest concentration of factor tested (control: 1472 ± 162; soluble: $18,662 \pm 960$; insoluble: 1196 ± 261 TH cells/culture). The soluble agent(s) will diffuse into the media as evidenced by a 4-6 fold increase in TH cell number in cultures grown on 100% conditioned media (CM) generated from muscle. The active factor(s) is stable to freeze-thawing (3x), to incubation at 4°C for 48 hrs, to heating at 90°C for 30 min, and to trypsin treatment (100 µg/ml, 37 °C, 2 hrs).

To establish whether this factor(s) was a survival or a differentiation factor, total cell survival was determined by measuring DNA content in cultures fed either control media or 100% CM. All cultures contained the same amount of DNA (3.07 ± 0.22 ng/culture) despite a dramatic difference in the number of TH neurons present in the different media (control: 1417 ± 178; CM: 6580 ± 359 TH cells/culture), indicating that CM does not affect cell survival but rather contains a factor(s) promoting CA differentiation in cortex. The increase in TH cell number in CM-fed cultures was paralleled by a comparable increase in TH catalytic activity (control: 202 ± 67 cpm in 1021 ± 129 TH neurons vs. CM: 976 ± 121 cpm in 4906 ± 359 TH neurons). However, other CA traits were not induced by CM: the CA enzymes aromatic L-amino acid decarboxylase, dopamine β-hydroxylase and phenylethanolamine N-methyltransferase were not detected immunocytochemically nor were endogenous CA's or the uptake of exogenous CA's seen with histofluorescence. Finally, since the number of TH neurons declined to near 0 by 14 days *in vitro* in both control and CM fed cultures, it suggests that this factor(s) does not alter the phenotypic fate of these neurons.

The results of these studies indicate that muscle contains a thermally-stable, trypsin-resistant diffusable factor(s) which will initiate but not perpetuate expression of the CA enzyme TH in cultured rat cortex. Expression of other CA traits does not appear to be similarly regulated. These findings suggest a possible role for muscle factors in the normal signaling of CA differentiation in central neurons.

162.12 EXTRINSIC INFLUENCES ON THE GROWTH OF HYPOTHALAMIC DOPAMINERGIC NEURONS *IN VITRO* J.P. Grierson*, R. Ventimiglia & H.M. Geller. (SPON: M.F. MacDonnell) UMDNJ-Robert Wood Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854.

We have designed a series of experiments to investigate the role of the extracellular environment in the control of growth of dopamine neurons of the hypothalamus. In particular, these studies have addressed the influence of glial cells and serum constituents on the growth and morphology of these neurons.

Dissociated neuronal cultures were prepared from fetal rat (E17) hypothalami and plated onto an astrocyte monolayer. Control cultures were maintained on cortical astrocytes and in Dulbecco's-modified Eagle's medium (DMEM) plus Bottenstein and Sato's N2 (defined) medium with added thyroxine. Indirect immunocytochemistry using an anti-tyrosine hydroxylase (TH) antiserum (A. W. Tank, S.U.N.Y.) was performed on cultures at various ages from 1 to 12 days in culture and TH-immunoreactive neurons were drawn onto 1 mm^2 graph paper (final magnification 400x) using a camera lucida. Using these figures the area of the cell body was calculated, along with the number and length of processes, number of branches and total neurite length.

TH immunoreactivity appears to be constitutively expressed in dopaminergic neurons of the hypothalamus and can be used as reliable marker for dopaminergic neurons. TH positive neurons accounted for about 0.2% of the total neuronal population and could be identified as early as 2 days in culture. TH-positive neurons maintained in defined medium, displayed a variety of morphological forms (as has been noted *in vivo*). Neuritic extension continues rapidly until day 6 *in vitro*, at which point it stabilizes. Neurons grown in DMEM plus fetal calf serum had significantly shorter neurites, while there was no difference in somatic area, number of processes or number of neuritic branches. The effect of gonadal steroids added to the defined medium is currently under investigation.

To test the hypothesis that astrocytes of the corresponding brain area might provide a better substrate for growth than dissimilar astrocytes, hypothalamic neurons were plated onto monolayers of hypothalamic astrocytes. Initial results indicate that the two glial populations are equally able to support the wide variety of TH-positive neurons and there is no difference between the various morphological parameters. The presence of fibroblasts in the glial monolayers seems to inhibit neurite extension of TH-positive neurons, in contrast to what has been observed for other types of neurons in dissociated culture. Ongoing work is evaluating the effect of dissociated pituitary cells on these neurons. (Supported by grants from N.I.H. and N.S.F. (H.G.) and a Merck Fellowship (R.V.).

ACTION POTENTIALS AND ION CHANNELS VIII

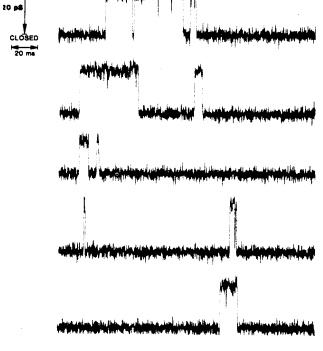
163.1 **CONTROL OF SODIUM CHANNEL MOBILITY IN NERVE: BRAIN ANKYRIN AND FODRIN ASSOCIATE WITH VOLTAGE-DEPENDENT SODIUM CHANNELS** Y. Srinivasan*, L. Elmer†, J. Davis†, V. Bennett†, and K.J. Angelides† (SPON: H. Epstein). Dept. Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030 and Dept. Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

The voltage-dependent Na^+ channel lends unique excitbility characteristics to nerve and muscle cells. Since certain neurologic disorders may be associated with an anomalous distribution of these molecules on cell surfaces we have examined their localization, lateral mobility, and elements that may segregate Na^+ channels in developing and mature neurons using fluorescently labeled channel toxins, channel specific antibodies, and cytoskeletal protein specific antibodies. Fluorescence recovery after photobleaching (FRAP) indicates that channels may be restricted by the cytoskeleton. By fluorescence immunocytochemistry and FRAP we have found that on mature neurons Na^+ channels are segregated and immobilized to the axon hillock and presynaptic terminal while on developing neurons they are freely mobile and diffusely distributed. The immobilization of the Na^+ channel on mature neurons appears to parallel the appearance of specific cytoskeletal elements and the development of the cytoplasmic matrix within the hillock imposes an additional barrier to the diffusion of Na^+ channels.

Biochemical studies have shown that brain ankyrin and fodrin bind to and co-purify with Na^+ channels from rat brain through sequential DEAE-, hydroxylapatite, wheat germ agglutinin-Sepharose chromatography, and through sucrose gradient centrifugation. Quantitative immunoblots show that enrichment of the Na^+ channel protein is paralled by enrichment of brain ankryin and approaches a 1:1 stoichiometry at the hydroxylapatite step. Moreover, the [^{3}H]-saxitoxin binding activity associated with the channel can be quantitatively precipitated from the solubilized and purified preparation with anti-ankyrin antibodies in the presence of increasing concentrations of added brain ankyrin or with fodrin linked to Sepharose 6. Preliminary direct binding studies with [^{125}I]-ankyrin show a K_d of 20 nM for the Na^+ channel. Together with the photobleach data a model emerges where Na^+ channels are distributed and maintained at specific regions of the axon by their interaction with ankyrin isoforms. (Supported by grants from the NIH and the Muscular Dystrophy Association).

163.2 SYNTHESIS OF AN ION-CHANNEL FORMING PEPTIDE PREDICTED FROM THE PRIMARY STRUCTURE OF THE VOLTAGE SENSITIVE SODIUM CHANNEL. S. Oiki*, W. Danho*† and M. Montal, Department of Neurosciences, Roche Institute of Molecular Biology, and †Peptide Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

We proposed a model for the arrangement and folding of the polypeptide chain of the voltage sensitive sodium channel within the bilayer (*FEBS Lett.* 193:125-134, 1985). The model consists of 4 homologous regions, each containing 8 membrane-spanning structures, probably α-helical. Four amphipathic helices, one from each homologous region, are postulated to form a negatively charged channel lining. The tetrameric array provides a conductive polar pathway traversing the non-polar membrane interior. We propose here a synthetic approach to study channel proteins inspired by the testability of the model. In order to locate the ion channel forming domain 4 peptides 21-22 amino acids long, encompassing the sequence encoding the putative amphipathic helices of each of the 4 homologous domains, were synthesized by solid-phase peptide synthesis. We focus here on the results obtained with 1 peptide identified as Sc_1, (*FEBS Lett.* 193:125-134, 1985) with the sequence (from the rat brain channel I (Noda *et al. Nature* 320:188-192, 1986): DPWNWLDFTVITF-AYVTEFVDL. The peptide lipid solution in hexane is spread into monolayers at an air-water interface (0.5 M NaCl, 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2) and, subsequently, bilayers are formed by the apposition of 2 monolayers at the tip of patch pipettes. As shown in the figure, at an applied constant voltage of 100 mV, the current flowing through individual channels is clearly discerned. Conductance histograms show that the most frequent event has a single channel conductance, γ, of 20 pS. However, smaller (γ = 10 pS) and larger (γ = 40-50 pS) events are detected with significantly lower frequency of occurrence, under these conditions. The single channel open and closed lifetimes for the 20 pS events are in the ms time range. The synthetic 22mer channel forming peptide provides opportunities to study by protein engineering the mechanisms of ion permeation through sodium channels.



163.3 **ARE GLIAL AND NEURONAL SODIUM CHANNELS THE SAME?**
B.A.Barres, L.L.Y.Chun*, and D.P.Corey. Program in Neuroscience, Harvard Medical School; Department of Neurology and Neuroscience Group, Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA

Type 1 astrocytes in the rat optic nerve express functional voltage-sensitive sodium channels (Barres *et al.*, 1986). We used the patch-clamp technique to compare directly the properties of the glial sodium channels with those in neurons. Acutely dissociated, immunohistochemically identified postnatal retinal ganglion cells and optic nerve type 1 astrocytes were studied under identical experimental conditions. Glial whole-cell currents activated at more hyperpolarized potentials: -41 ± 1.7 mV compared to -31 ± 2.9 mV for neurons. Similarly, the voltage at which half of the channels were inactivated occurred at a more hyperpolarized potential: glia -80 ± 5.8 mV, neurons -55 ± 4.0 mV. The kinetics of the whole-cell glial current were also significantly slower, taking longer to peak and longer to decay. The current decay time constants at -30 and 0 mV test steps were: glia 3.0 and 1.3 msec; neurons 1.8 and 0.5 msec. The amount of TTX required to block the current by half was identical: glia 2.8 ± 1.6 nM; neurons 2.6 ± 1.1 nM.

The properties of single sodium-channel currents were studied using cell-attached recording. Averages of single channel currents were significantly different, and reproduced the whole-cell kinetic differences. The glial first latencies were strongly voltage-dependent, as are those of neuroblastoma cells (Aldrich, Corey and Stevens (1983)). However, the glial first latencies were significantly longer than those found in retinal ganglion cells. In addition, average glial open-times were longer. The observed single-channel differences account for the slower whole-cell glial current.

So far, the slow channels have not been observed in neurons and their expression appears confined to glia: retinal ganglion cells and cortical motor neurons express only the fast channel, while type 1 astrocytes and ependymal cells express only the slow form. But other glial cells express the fast sodium channel: O2A progenitor cells in the optic nerve, which differentiate into oligodendrocytes and type 2 astrocytes, express a channel with voltage-, time-, and TTX-dependence identical to that in neurons. Interestingly, the type 2 astrocyte expresses a mixed phenotype: 85-90% fast form and 10-15% slow form. Thus far, the slow form of the sodium channel seems to be co-expressed with the RAN-2 surface antigen. Type 2 astrocytes begin to express RAN-2 as they mature, and astrocytes from *cortex*, which lack RAN-2, appear (in preliminary experiments) to have the *fast* form of the channel. In other experiments, the cortical astrocyte channels have been found to have low TTX sensitivity (Bevan *et al.*, 1985) suggesting that CNS sodium channels do not fall into just two groups.

The differences between the fast and slow channel types may occur because of a different amino acid or subunit composition, posttranslational modification or local environment. Both types can occur together in cell-attached patches on type 2 astrocytes, arguing against a difference in local cytoplasmic or membrane environment.

163.4 THE DIFFERENTIAL EFFECTS OF MYELIN AND AXONAL DEGENERATION ON THE EXPRESSION OF EXCITABLE ION CHANNELS ON SCHWANN CELLS.
S.Y. Chiu* (SPON: W. Welker). Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

A recent study (Chiu 1987, J. Physiol.,386,181-203) has shown that myelination led to an apparent alteration of ion channel properties on Schwann cells. In a normal adult rabbit sciatic nerve, whole-cell patch-clamp recordings at the cell body region of Schwann cells showed a voltage-gated sodium current in cells which lacked myelin, but not in those cells which produced myelin.

This report further studies the changes in the TTX-sensitive voltage-gated sodium (I_{Na}) and TEA-sensitive potassium current (I_K) in these Schwann cells when the axon was transected, and to correlate these changes with myelin and axonal degeneration. Whole-cell recordings were performed on Schwann cells associated with myelinated and non-myelinated axons in the distal nerve stump of transected 10-week-old rabbit sciatic nerves at 0-13 days after in vivo Wallerian degeneration. Axonal degeneration was determined from cross-sectional electron-microscopy of whole nerve bundles. The degree of myelin degeneration was assumed to be proportional to the measured whole-cell membrane capacity which should decrease when myelin was detached from a Schwann cell. Indeed, in the myelin-forming Schwann cells, but not in the non-myelin-forming ones, the measured capacity decreased 9-fold at 13 days.

In the non-myelin forming Schwann cells in which only axonal, but not myelin, degeneration occurred, I_{Na} decreased slowly when axons degenerated. Both declined to about 50% by day-3, and further to 17-27% by day-6.

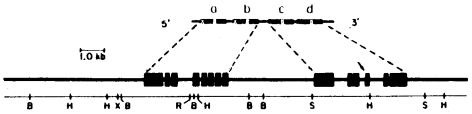
In myelin-forming Schwann cells in which both myelin and axonal degeneration occurred, I_{Na} and I_K increased progressively as Wallerian degeneration persisted. Furthermore, an inverse relation existed between whole-cell capacity and current density, suggesting that the appearance of these currents might be related to the loss of myelin.

An hypothesis is suggested that the expression of excitable Na and K channels on a Schwann cell is under opposite regulations by degenerating myelin and axons. The post-transectional decrease in I_{Na} in the non-myelin forming cells suggests that axons normally exerted a positive influence on Schwann cells to express Na channels, and that when the axons died, the cell ceased to express these channels. On the other hand, the post-transectional increase in Na and K currents in the myelin-forming cells is probably a result of the degeneration of myelin, and not of axons.

Supported in part by grants NS-23375 from U.S.P.H.S. and RG-1839 from the U.S. National MS Society.

163.5 **Genomic Organization of a Drosophila Gene with Homology to Vertebrate Sodium Channels.**
A. Wei, A. Butler*, N. Scavarda*, D. Pauron*, and L. Salkoff. Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

A Drosophila gene was isolated which encodes a large polypeptide with an organization virtually identical to vertebrate sodium channels. Four homologous domains, containing all putative membrane spanning regions, are conserved, and repeated in tandem with connecting sequences of usually lower conservation. All regions of the protein presumed to be critical for channel function are highly conserved. These include regions proposed to function in voltage-sensitive gating, inactivation and ion selectivity. Most strikingly conserved are the 24 putative gating charges of the vertebrate protein, all of which are found in identical positions in the Drosophila sequence. Ten introns interrupt the coding regions of the four homologous domains. Two conserved intron positions flank a region proposed to function as an ion selectivity filter. The coding region of the gene is contained within 12Kb of genomic sequence. Northern blots reveal a developmentally regulated message of about 10-14Kb. This transcript is most abundant during embryonic and pupal stages, and far less abundant in adults. At very early embryonic stages (0-8hrs), a second, shorter transcript is seen. This may represent an alternate form of the message, differentially spliced from the same gene. The gene cytologically maps to the tip of the right arm of the the second chromosome, in region 60D-E. This position is close to, but not coincident with the seizure locus (60A-B), which has been proposed to code for structural components of the sodium channel. Through cross-homology, two independent, non-overlapping genomic clones were isolated which also map to this region. The sequenced gene may be one member of an extended gene family elaborated through gene duplication.



Genomic map showing all exons (filled boxes) of the four homologous domains (a,b,c,d) of the channel gene. A diagram of the deduced polypeptide subdividing each homologous domain into six transmembrane segments is presented at the top. The single exon in the conserved linker c-d is indicated by an arrow. Exons of the linker regions a-b and b-c are not indicated. Restriction enzyme recognition sites for BamHI, EcoRl, HindIII, Sall, and XhoI are shown.

163.6 EXPRESSION OF SODIUM CURRENTS IN EMBRYONIC *DROSOPHILA* NEURONS: DIFFERENTIAL REDUCTION BY ALLELES OF THE *PARA* LOCUS.
D.K. O'Dowd, S. Germeraad and R.W. Aldrich. Department of Neurobiology, Stanford University, Stanford, CA 94305.

The $para^{ts}$ mutants, like several other temperature-sensitive paralytic mutations in *Drosophila*, have been proposed to affect sodium channels based on physiological and pharmacological evidence. The mutant adults paralyze at 29° (as opposed to wildtype above 40°) (Suzuki et al., PNAS 68, 890, 1971), show altered membrane excitability manifest as a change in excitation threshold (Siddiqui and Benzer, PNAS 73, 3253, 1976; Wu and Ganetzky, Nature 286, 814, 1980) and exhibit reduced sensitivity to veratridine in cultured larval neurons (Suzuki and Wu, J. Neurogenet. 1, 225, 1984). However, the binding of a high affinity ligand for the sodium channel, TTX, appears normal (Kauvar, Mol. Gen. Genet. 187, 172, 1982). We have investigated the hypothesis that *para* affects sodium channels by direct examination of sodium currents in embryonic neurons.

As described previously, sodium currents can be recorded from voltage-clamped *Drosophila* neurons differentiating in gastrula-stage embryo cultures (O'Dowd and Aldrich, Soc. Neurosci. 12, 43, 1986). Our initial examination of the whole cell sodium currents in $para^{ts2}$ neurons revealed a striking reduction in the percentage of cells expressing sodium currents after 24 hours of culture. Under these culture conditions 65% of wildtype neurons express sodium currents. $para^{ts2}$ cells exhibited a 34% reduction in the percentage of cells with measurable sodium currents. Extending our observations to other alleles has shown that the magnitude of the reduction of sodium channel expression in the population is allele dependent, $para^{ts1}$ and $para^{ST76}$ exhibiting 22% and 95% reductions, respectively. In contrast, the percentage of neurons with outward potassium currents in cultures from each of the three *para* alleles is not different from wildtype.

The mechanism of action of *para* in these cultured neurons could be through a direct effect on sodium channel structure, biosynthesis or membrane insertion or through an indirect mechanism which reduces the viability of a class of cells normally expressing sodium currents. The currents observed in *para* neurons showed voltage-dependent activation and inactivation similar to wildtype, although at this time we cannot rule out the possibility of subtle changes in gating. Detailed examination of the voltage- and time-dependent properties of the sodium currents and the survival of mutant neurons under various culture conditions will be useful in differentiating between the possible mechanisms. [Supported by American Cancer Society postdoctoral fellowship to D.O'D. and NS23294 and Searle Scholar's Program/Chicago Community Trust to R.W.A.]

163.7 CLONING AND SEQUENCING OF cDNA FOR A PROBABLE POTASSIUM-CHANNEL COMPONENT FROM THE SHAKER LOCUS OF DROSOPHILA. T.L.Schwarz*, B.L.Tempel, D.M.Papazian*, Y.N.Jan, and L.Y.Jan. Howard Hughes Medical Institute and Dept. of Physiology, UCSF San Francisco, CA 94143.

The Shaker locus of Drosophila melanogaster is thought to code for a structural component of an A-channel, a type of K^+-channel (for review see Tanouye et al., Ann. Rev. Neurosci. 9,255.) We have cloned 200kb of genomic DNA from this region and used it to isolate several cDNA. These cDNA appear to represent a family of transcripts produced by alternative splicing of a large transcription unit whose exons are spread over 60kb of the genome and span the locations of many Shaker mutations.

The cDNA whose sequence we report here is 2.9kb long and is probably not full length since a preliminary analysis of wild type and mutant RNA reveals larger transcripts. The cDNA contains a single, large, open reading frame that is flanked on either side by stop codons, and therefore might contain the entire coding sequence. If the first methionine within the open reading frame is taken as the translational start site, a protein of 616 amino acids is predicted. This protein appears to be an integral membrane protein; it contains a highly hydrophobic central region with hydrophilic regions at either end. Within the hydrophobic region we have identified 6 hydrophobic stretches of 19 or more amino acids that are likely to cross the membrane.

A computer search for homology with sequences in the Dayhoff protein database detected significant homology of the Shaker product to the vertebrate Na^+-channel. The homology consists of a stretch of 120 amino acids that are 27% identical. This region includes, in the Na^+-channel, a sequence called S4 that may be involved in the voltage-dependent gating of the channel. The S4 has been hypothesized to form a transmembrane alpha helix along which positive residues are regularly arrayed (Noda et al.,Nature 312,121; Catterall, Ann. Rev. Biochem. 55,953; Guy and Seetheramulu, PNAS 83,508; Greenblatt et al., FEBS Lett. 193,125). The Shaker sequence preserves the essential features of the S4 region: there are 7 positively charged residues each of which is separated from the next by 2 hydrophobic amino acids. This sequence is a seventh potential membrane-crossing.

Although proof that this cDNA is a structural component of a K^+-channel must await physiological tests, the hypothesis is strengthened by the structural properties described above. The existence of homology between the Shaker product and Na^+-channels suggests that these proteins are descended from a single, voltage-sensitive ancestor, and that the homologous region may constitute a voltage-gating domain that is present in other channels as well.

163.8 A NEW BEHAVIORAL MUTANT IN DROSOPHILA MELANOGASTER, WITH A PHENOTYPE OF NEURONAL HYPEREXCITABILITY. L.C. Timpe, W. Moats*, Y.N. Jan and L.Y. Jan. Dept. of Physiol. and Howard Hughes Med. Inst., Univ. of Calif. San Francisco, San Francisco, Calif. 94143.

We are interested in identifying new mutations in genes whose products are ionic channels, or whose products are necessary for the normal expression and function of ionic channels. Since the autosomal chromosomes of Drosophila have been searched for behavioral mutants much less thoroughly than has the X chromosome, we looked for new mutations on an autosome, the 3rd chromosome. Flies were fed the chemical mutagen ethyl methanesulfonate, and balanced lines were made for individual mutagenized chromosomes. About 2000 lines were screened for temperature-sensitive (ts) paralysis, and for leg shaking under ether anesthesia. Roughly 12 new ts paralytics were found; one of these, $groggy^{ts}$ (grg^{ts}), has been characterized by intracellular recordings from the larval neuromuscular junction preparation.

Recordings from muscle fibers in grg^{ts} larvae reveal spontaneous depolarizations of the membrane at room temperature. For several reasons we believe that these depolarizations are the result of spontaneous firing of the motoneurons: 1.) the spontaneous depolarizations resemble components of the evoked junctional potentials in amplitude and time course, 2.) both kainic acid (5mM) and glutamate (5mM), which block evoked junctional potentials, block the spontaneous depolarizations as well, 3.) tetrodotoxin (3uM) also blocks the spontaneous depolarizations in muscle. Tetrodotoxin is known to block action potentials in motoneurons in this preparation. The neurophysiological phenotype of grg^{ts} has been mapped to the right arm of the 3rd chromosome, between curled (50.0) and stripe (62.0).

Of the many potential explanations for a phenotype of neuronal hyperexcitability, we have tested the possibility that it is due to a defect in a voltage-sensitive potassium current. Using the two microelectrode voltage clamp technique, we measured the amplitude of the transient outward current, the "A" current, in pupal indirect flight muscle. The A current has the same size and rate of inactivation in grg^{ts} pupae as in controls. We then measured the amplitude of the delayed rectifier, or "K" current, in larval muscle of Shaker/Y ; grg^{ts}/grg^{ts} double mutants. The Shaker mutation in these flies abolishes the A current, allowing a better separation of the K current. Again the K current in grg^{ts} larval muscle has the same amplitude as in controls. Thus the defect does not appear to be in the A or K channels which are expressed in muscle. If the mutant phenotype is due to a defective potassium channel, the grg^{ts} mutation may affect a Ca^{++}-dependent channel, or perhaps a potassium channel which is found only in nerve.

163.9 FOUR DISTINCT POTASSIUM CHANNELS IN CULTURED *DROSOPHILA* MYOTUBES. M.S. Brainard* W.N. Zagotta and R.W. Aldrich. Department of Neurobiology, Stanford University, Stanford, CA 94305

A number of mutations have been shown to affect potassium channels in *Drosophila* muscle (see Salkoff and Tanouye Physiol. Rev. 66:301-329 1986). Single channel analysis of the effects of such mutations will prove a powerful approach for studying the molecular mechanisms of ion channel gating. As an initial step towards studying the effects of mutants at the single channel level we have characterized wildtype potassium channels in cultured embryonic myotubes using whole-cell, inside-out and outside-out configurations of the patch clamp technique.

The myotubes differentiate *in vitro* from primary cultures of mid-gastrula stage embryos of *Drosophila*. The cultures were incubated at 26°C for 8 to 36 hours prior to recording. Recording solutions contained physiological concentrations of K^+ with internal Ca^{++} buffered to 10 nM with 11 mM EGTA. The whole-cell outward currents exhibited a characteristic developmental appearance, beginning with an A-type current between 8 and 12 hours followed at later developmental times by slowly inactivating and delayed K currents.

At least four different types of potassium channels contribute to these whole-cell currents: A fast transient 13 to 16 pS A-type potassium channel (A1) (see abstract by Zagotta and Aldrich), a slow transient 16 to 20 pS channel, a 40 pS channel that does not inactivate during voltage pulses up to 240 ms and a 50 to 70 pS channel with little voltage dependence between -50 and 80 mV. Other channel types, including one with a conductance of 9 pS, are occasionally but rarely seen.

The 16 to 20 pS channel ensemble averages inactivate with a time course slower than that of the macroscopic A-current. Channel openings with mean duration of 5 to 10 ms occur in well defined bursts. The latency to first opening is strongly voltage dependent, but there is little voltage dependence occurring in the open durations. The channel shows significant inactivation by 500 ms prepulses over a range of voltages from -80 to 0 mV.

The 35 to 40 pS channel exhibits an increasing open probability with depolarization to voltages between -10 and 70 mV. This voltage dependence is due to a decrease in closed durations at more positive voltages. The open durations are voltage independent with a mean of 1 ms.

Gating of the 50 to 70 pS channel is relatively voltage-independent, with mean open durations of 1 to 3 ms. between -50 to 50 mV. Open probability is independent of internal calcium concentration between 10 nM and 500 uM. The channel has complex gating, with occasional abrupt changes between high and low probability modes. [Supported by Training grants NIMH MH17047 to M.S.B. and NIH NS07158 to W.N.Z. and NIH NS23294 and a Searle Scholars Program Fellowship to R.W.A.]

163.10 SINGLE A-TYPE POTASSIUM CHANNELS IN WILD-TYPE AND *SHAKER DROSOPHILA* MYOTUBES. W.N. Zagotta* and R.W. Aldrich. (SPON: R. Numan). Department of Neurobiology, Stanford University, Stanford, CA 94305

The ability to combine single-channel analysis with genetic and molecular manipulations makes *Drosophila* an ideal system for the study of ion channel gating mechanisms. Mutants at the *Shaker* locus have been shown to alter or eliminate a fast transient potassium current, known as A-current, in muscles of pupae and larvae (Salkoff and Wyman, Nature 293:228-230, 1981; Wu and Haugland, J. Neurosci. 10:2626-2640, 1985). In one *Sh* allele, Sh^5, the voltage-dependent gating of the A-current was altered. We have used the patch-clamp technique on cultured embryonic myotubes to study the gating behavior of single A-current channels and the alteration of their gating by the Sh^5 mutation.

A-currents can be recorded from myotubes which differentiate *in vitro* from primary cultures of *Drosophila* embryos at mid-gastrula stage. This A-current channel (type A_1) is distinct from another A-current channel found in cultured larval neurons which is not altered in *Shaker* mutants. It is expressed early in the myotube differentiation in relative isolation from other potassium channels. The density of other channels in the membrane increases later in development. The current through the A_1 channels possesses many similarities to the A currents recorded in pupal and larval muscle. It is blocked by 5mM 4-aminopyridine and is half inactivated after 500 ms prepulses to voltages between -30 mV and -35 mV. The A_1 channel current is absent in myotubes homozygous for the Sh^{KS133} or Sh^{P} alleles or deficient between the B55 and W32 chromosomal breakpoints.

The single A_1 channels have a conductance between 13 and 16 pS and a single exponential open duration distribution with a mean of 1.5 to 3.0 ms independent of membrane voltage. The voltage dependence of the macroscopic inactivation arises primarily from an increase in opening rates at higher voltages. While the channel does not open in obvious bursts, reopenings contribute to a second, slower inactivation time constant. A five-state sequential kinetic model can account for the kinetics of the single channels and ensemble averages over the activation voltage range. In myotubes from Sh^5 mutants, the macroscopic current-voltage relation and prepulse inactivation curve are shifted 10 to 20 mV in the depolarized direction. Detailed examination of the single A1 channels in Sh^5 mutants have begun to elucidate which rate constants in the model are altered by this mutation. [Supported by NIMH Training grant NS07158 to W.N.Z. and NIH NS23294 and a Searle Scholars Program Fellowship to R.W.A.]

163.11 HIGH EXTERNAL POTASSIUM ALTERS THE VOLTAGE-DEPENDENT GATING OF TWO DISTINCT K^+ CHANNELS IN *DROSOPHILA* LARVAL CNS NEURONS. C. K. Solc and R. W. Aldrich. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

The gating of ion channels was originally thought to be independent of the concentration and species of the permeant ions. Recent work from several laboratories, including studies done by Armstrong and co-workers, shows that elevating the concentration of external K^+ slows the closing of voltage-dependent K^+ channels. We report here the effects of elevating external $[K^+]$ on the gating of A-channels and single sustained K^+ channels in larval Drosophila CNS neurons.

Neurons in primary cultures from larval *Drosophila* brain express several distinct voltage-dependent K^+ channels (Solc and Aldrich, Soc. Neurosci. Abst. 12:44, 1986.), including a transient A-type channel and a sustained channel (K_1). The gating of both channels is altered when the external solution is changed from a low $[K^+]$ solution (2 mM KCl, 140 mM NaCl) to a high (symmetrical) $[K^+]$ solution (140 mM KCl). Whole-cell A-current recorded in low $[K^+]$ activates within 5 msec, inactivates along a double exponential time course with time constants of ~30 msec and ~300 msec, and reverses at ~-80 mV. In high $[K^+]$ the current reverses, as expected, at 0 mV but the macroscopic inactivation rate increases by ~50%. The faster inactivation in high $[K^+]$ is seen in both inward (V_C <0 mV) and outward (V_C >0 mV) A-currents when compared to (outward) A-currents in low $[K^+]$ at the same voltages. Thus it cannot be due to a series resistance error or ion accumulation, nor can it be accounted for by a shift in voltage, as the macroscopic inactivation rate of the A-current is relatively voltage-independent.

Single sustained K_1 channels in low $[K^+]$ solution begin to open at voltages positive to -20 mV. The single-channel current-voltage relation reverses at ~-65 mV, is linear between 0 and +50 mV, and has a slope conductance in this range between 20 and 60 pS. In high $[K^+]$ the reversal potential shifts to 0 mV and the slope conductance increases by 50 to 100%. In addition, the probability of being open (P_o) in high $[K^+]$ increases to 0.6 to 0.8 between -100 mV and +100 mV. The increase in P_o is due to a large decrease in the mean closed durations. In low $[K^+]$ the channel rarely opens at voltages below -40 mV, but in high $[K^+]$ the frequency of opening in this voltage range increases to 300 to 500 sec^{-1}. At higher voltages (V_C >-20 mV), closed duration distributions recorded in both solutions can be fitted by a sum of three exponentials. In low $[K^+]$, 30 to 40% of the closed durations are shorter than 0.8 msec, while in high $[K^+]$, 80 to 90% of the closed durations are shorter than 0.8 msec. High $[K^+]$ also has small effects on the open durations at higher voltages. These results are consistent with the idea that high external $[K^+]$ alters gating either by direct interactions with the channel, or through the release of modulatory substances from other cells in the chamber. [Supported by NS23294 and a Searle Scholars Program fellowship to R.W.A. and by an NSF graduate fellowship to C.K.S.]

163.12 GENETIC AND PHARMACOLOGICAL SEPARATION OF FOUR POTASSIUM CURRENTS IN *DROSOPHILA* LARVAE. Satpal Singh and C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Muscle fibers in *Drosophila* larvae have been reported to exhibit four K^+ currents; a fast and a slow voltage-activated K^+ current, and a fast and a slow Ca^{++}-activated K^+ current. A clear separation of all the four K^+ currents by treatments which selectively eliminate single currents will be helpful in understanding the mechanisms underlying these currents. A distinction among various K^+ currents will also have a bearing on the question as to whether the voltage-activated and the Ca^{++}-activated currents pass through separate sets of channels or are manifestations of different activation mechanisms for the same type of channels. The two voltage-activated K^+ currents have been already distinguished from each other by using *Shaker* mutations which affect only the fast current between them (Salkoff and Wyman, Nature 293: 228, 1981; Wu and Haugland, J. Neurosci. 5: 2626, 1985). We now report a clear genetic and pharmacological distinction among all the four K^+ currents in the muscle fibers of *Drosophila* larvae.

Two-microelectrode voltage-clamp experiments were performed on muscle fibers of normal and mutant larvae. The *slowpoke* mutation (Elkins et al., PNAS 83: 8415, 1986) provided a distinction between the two Ca^{++}-activated K^+ currents. Although this mutation eliminated the fast Ca^{++}-activated K^+ current, we found that it did not affect the slow Ca^{++}-activated K^+ current. The two fast currents could be separated from each other by either *Shaker* or *slowpoke* which eliminated respectively the voltage-activated and the Ca^{++}-activated current. At present no mutation is known to remove completely either the voltage-activated or the Ca^{++}-activated slow K^+ current. However, it was possible to make a pharmacological distinction between these two currents by the use of quinidine. Quinidine at a concentration of 1 mM completely eliminated the voltage-activated slow K^+ current without any apparent effect on the Ca^{++}-activated slow K^+ current. All of the four K^+ currents in the larval muscle fibers of *Drosophila* can therefore be distinguished completely from one another. A combined use of mutations and pharmacological agents in this system allows us to extract individual currents to analyze their properties without contamination from other currents.

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63.13 MOLECULAR ANALYSIS OF EAG: A GENE AFFECTING POTASSIUM CHANNELS IN DROSOPHILA. R. Drysdale* and B. Ganetzky. Lab. of Genetics, Univ. of Wisconsin, Madison, WI 53706

Mutations of the *eag* locus in *Drosophila* cause spontaneous repetetive firing of action potentials in motor axons and abnormal release of neurotransmitter at the larval neuromuscular junction (Ganetzky and Wu, J. Neurogenet. 1: 17, 1983). Voltage clamp studies of larval body wall muscles (Wu *et. al.*, Science 220: 1076, 1983) as well as single channel recordings from dissociated neurons (Sun and Wu, Abstr. Soc. Neurosci. 11: 787, 1985) indicate that *eag* $^+$ is intimately involved in the function of potassium channels. To elucidate the function of the *eag* product we undertook a molecular analysis of the gene. We cloned over 60kb of genomic DNA, beginning by taking advantage of a chromosomal inversion broken both at *eag* and in a previously cloned gene (Drysdale and Ganetzky, Abstr. Soc. Neurosci. 11: 788, 1985).

We have now analyzed the cloned region in a collection of *eag* mutant alleles to define the extent of the *eag* gene and to identify the coding regions. By southern blotting experiments five mutations, including a deletion, insertions and the original inversion breakpoint were located on the molecular map of the region. These mutational sites encompass a region of 40kb. At least one of the transposable element insertions is capable of back mutation to *eag* $^+$. These revertants have lost the transposon from its previous site of insertion. Several mRNA transcripts derived from the *eag* region have been identified and corresponding cDNA clones have been isolated from wild type cDNA libraries. To identify which of the transcription units corresponds to the *eag* coding region we are now comparing transcription in this region in *eag* mutant, revertant and wild type strains.

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164.1 RESPONSE OF SI CORTICAL NOCICEPTIVE NEURONS TO SMALL INCREASES IN NOXIOUS THERMAL STIMULI APPLIED TO THE FACE OF TRAINED MONKEYS. D.R. Kenshalo, Jr., E.H. Chudler, F. Anton* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies have reported neurons in the primary somatosensory cortex of monkeys that respond to noxious thermal stimuli applied to the face of anesthetized monkeys. However, the response characteristics of these neurons have not been determined in the awake monkey. In the present study, we examined the response properties of SI cortical nociceptive neurons in behaving monkeys trained to detect small temperature changes in the noxious range.

Monkeys initiated a trial by pressing an illuminated button. A contact thermode, positioned on the upper lip, subsequently increased in temperature from a baseline of 38°C to temperatures of 44° to 48°C (T1). After a random foreperiod of 3 to 9 sec, the thermode increased in temperature an additional amount (T2). The monkey received a fruit juice reward for releasing the button within 2 sec of the onset of T2. If the monkey released the button prior to the onset of T2, no reward was delivered and the temperature returned to baseline. Neural activity was recorded while the animal performed the thermal detection task. In addition, mean detection speed to the onset of T2 was determined.

To date, 22 SI neurons have been isolated that increased their discharge frequency to noxious thermal stimuli applied to the face. Twenty-one of these neurons were classified as wide-dynamic-range and one as nociceptive-specific. All of the neurons had receptive fields comprising 1 or 2 divisions of the trigeminal system.

Thirteen neurons increased their discharge frequency in a graded fashion to increasing heat intensities. The remainder of the neurons (9) responded to noxious thermal stimulation, but did not grade their response in a systematic fashion. In 8 cortical nociceptive neurons, when T2 ranged between 0.2° to 1.0°C from a preceding T1 of 46°C, the peak discharge frequency was positively correlated with the intensity of the temperature change and the mean detection speed.

These data demonstrate that SI neurons respond to noxious thermal stimuli applied to the face of unanesthetized, trained monkeys. It appears that at least two populations of nociceptive neurons exist. One type of neuron encodes small changes in noxious thermal stimulation and may participate in the monkey's ability to detect temperature changes in the noxious range. The other type of neuron does not encode the intensity of noxious thermal stimulation, but may play a role in arousal or alerting responses to noxious stimulation.

164.2 DIRECT PROJECTION OF THE CORTICOSPINAL TRACT TO THE SUPERFICIAL LAMINAE IN THE RAT USING THE ANTEROGRADE TRANSPORT OF *PHASEOLUS VULGARIS*-LEUCOAGGLUTININ. E.J. Casale and A.R. Light. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514.

We have previously reported that the corticospinal tract (CST) arising from the sensorimotor cortex (SMC) in the rat projects to medial portions of laminae I and II within the cervical enlargement. These studies utilized the TMB method of visualizing anterogradely transported WGA-HRP. Although this method is sensitive, preterminal axons cannot be distinguished from synaptic terminals. In order to verify whether the CST terminates within these laminae, *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was used as an anterograde tracer allowing direct visualization of axons and boutons.

PHA-L (10%) was pressure-injected via multiple penetrations throughout the extent of the SMC on one side in Sprague-Dawley rats. Following 4 weeks survival, rats were perfused transcardially with buffered 4% paraformaldehyde/10% saturated picric acid. Cervical and lumbar enlargements and cerebral cortices were cut on a Vibratome, and sections processed using the avidin-biotin complex, immunoperoxidase method (Vector Labs).

Within the SMC, many cells were labeled in a Golgi-like manner. Diffusion of PHA-L was very restricted, and large areas of SMC remained free of label. Within the spinal cord labeled axons were found throughout all segments. In the white matter, most labeled axons were found within the contralateral dorsal column; a few axons were found within the ipsilateral dorsal column and ventral funiculus. In the contralateral gray matter, labeled axons were found in all laminae. Within the dorsal horn, the majority of labeled axons were found within laminae III-V, and had a predominantly rostrocaudal orientation. Swellings which appeared to be boutons *en passant* were observed at regular intervals along most axons; preliminary electron microscopic observation showed that all swellings contained vesicles. The presence of synaptic boutons within laminae I and II was confirmed. Most labeled boutons were found in the most medial 1/3 of these laminae, and many arose from collaterals of axons terminating in deeper dorsal horn laminae as well.

The presence of terminal labeling within the medial portions of laminae I and II suggests that the SMC is capable of modulating the activity of spinal neurons which have receptive fields on the extremities, and which may be nociceptive-specific.

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164.3 DIRECT SOMATOSENSORY PROJECTIONS FROM THE SPINAL CORD TO THE HYPOTHALAMUS AND TELENCEPHALON. R. Burstein, K. D. Cliffer and G. J. Giesler, Jr., Dept. of Cell Biol. and Neuroanat., University of Minnesota, Minneapolis, MN 55455.

A variety of somatosensory stimuli can cause marked changes in the firing of neurons in the hypothalamus and telencephalon. The pathway for somatosensory information from the spinal cord to these areas has been generally believed to be multisynaptic. In the present study, we have used anatomical and physiological techniques to demonstrate the existence of a direct somatosensory projection from the spinal cord to the lateral and medial hypothalamus (LH, MH), the basal forebrain (BF), the septal nuclei (SN), and the nucleus accumbens (NA) in rats.

Retrograde tracers (Fluoro-gold, WGA-HRP and rhodamine-labeled microspheres) were injected into the above-mentioned areas and, after appropriate processing, the spinal cord was examined for retrogradely labeled cells. An anterograde tracer, PHA-L, was injected iontophoretically into cervical or lumbar spinal cord. The hypothalamus and telencephalon were then processed for immunohistochemical visualization of fibers and varicosities labeled for PHA-L. In physiological experiments, neurons in the dorsal horn of the spinal cord were antidromically activated from the hypothalamus and physiologically characterized.

Retrograde tracers injected into the LH labeled approximately 2500 neurons distributed bilaterally (60% contralateral, 40% ipsilateral) throughout the length of the spinal cord. Approximately 50% were found in the nucleus proprius, 25% in the lateral spinal nucleus, 10% in the marginal zone, and 10% in the area around the central canal. Injections into the MH labeled a comparable number of neurons bilaterally (50% contralateral, 50% ipsilateral) in a similar distribution. Injections restricted to the SN, the BF, or NA each labeled fewer cells in the spinal cord than did injections into the hypothalamus. The distributions of neurons labeled by these telencephalic injections were similar to those produced by hypothalamic injections.

PHA-L injected into the cervical and lumbar enlargements labeled fibers and varicosities in LH, MH, BF, medial SN, nucleus of the diagonal band of Broca, NA and in the supraoptic decussation, including the caudal portion of the optic chiasm.

Ten dorsal horn neurons were antidromically activated from the contralateral LH. These neurons responded to innocuous mechanical stimuli but were more strongly activated by noxious mechanical or thermal stimuli.

These findings, obtained by three independent techniques, demonstrate that neurons in the spinal cord project further rostrally than had been previously shown, carrying somatosensory information directly to the hypothalamus and telencephalon. Since the hypothalamus, septal nuclei and nucleus accumbens are all believed to participate in the expression of emotional behavior, it will be important to determine whether direct spinal projections to these areas play a role in the production of emotional responses to somatosensory stimulation.

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164.4 THE PRIMATE DORSOLATERAL SPINOTHALAMIC PATHWAY. A.V. Apkarian, R.T. Stevens, and C.J. Hodge, Department of Neurosugery, SUNY Health Science Center, Syracuse, NY 13210, USA.

There are two distinct spinothalamic pathways in cat, a dorsolateral spinothalamic tract (DSTT) comprised of lamina 1 cell axons and a ventral spinothalamic tract (VSTT) comprised of laminae 4-10 cell axons. The DSTT and the VSTT cross segmentally and ascend to the thalamus in the dorsolateral funiculus (DLF) and the ventral quadrant (VLQ) respectively. Both of these pathways can transmit nociceptive information to the lateral thalamus in the cat. The purpose of this study was to determine whether there is a similar DSTT in primates.

Injections of WGA-HRP were made into the somatosensory thalamus of macaque and squirrel monkeys. Some animals had thoracic spinal cord VQ or DLF lesions made ipsilateral to the injection site. After a 5 day survival time, the animals were sacrificed and the tissue processed for HRP histochemistry using the tetramethyl benzidine technique. Laminar locations of retrogradely labeled neurons were collected from all levels of the spinal cord.

The results for both types of primates studied were similar with minor variations. The average numbers of spinothalamic cells per section in the cervical and lumbar enlargements were 2 to 10. Spinothalamic neurons were found in the cervical and lumbar enlargements in laminae 1 through 10 with particular concentrations in laminae 1,4,5,7 and 8. In the cervical enlargement 25-55% of spinothalamic neurons were found in contralateral lamina 1. Between 15 and 25% of lumbar spinothalamic neurons were found in contralateral lamina 1. Lesion of the thoracic VQ ipsilateral to the thalamic WGA-HRP injection resulted in nearly complete loss of labeled cells in contralateral lumbar laminae 7-10. However, there was still a large number of labeled neurons found in contralateral lumbar lamina 1, and there was only a partial loss of the contralateral lumbar lamina 5 label. Lesion of the thoracic DLF resulted in a large decrease in the number of contralateral lumbar lamina 1 cells labeled. In the DLF lesion animals only 1-3% of the contralateral lumbar spinothalamic population was found in lamina 1. The spinothalamic cell distributions in the cervical enlargement of the lesioned animals were similar to those found in the control animals.

These results indicate that there is a prominent DSTT in primates, which, as in cats, is the primary ascending route for lamina 1 cell axons, the most prominent nociceptive specific input to the thalamus. It is reasonable then to assume that a nociception signaling DSTT exists in humans.

164.5 COLLATERAL SPINOTHALAMIC TRACT PROJECTIONS TO MEDIAL AND/OR LATERAL THALAMUS EXAMINED WITH MULTIPLE RETROGRADE FLUORESCENT LABELING IN THE CAT. A. D. Craig, Jr., A. J. Linington* & K.-D. Kniffki. Divisions of Neurobiology and Neurosurgery, Barrow Neurological Institute, Phoenix AZ 85013 and Physiologisches Institut der Universität, D-8700 Würzburg, FRG.

Previous physiological data indicate that spinothalamic tract (STT) cells that project to medial and/or lateral thalamus in the cat, in particular lamina I STT cells, have significantly different response characteristics that correlate with their axonal projections. These functional and structural differences could be reflected in the differential distribution within the spinal gray matter of STT cells afferent to medial and/or lateral thalamus. The present study tested this hypothesis with the multiple retrograde fluorescent labeling method in the cat.

Multiple injections of 0.5-1.0 µl of 2% Diamidino Yellow (DY) and 2% Fast Blue (FB) were placed in medial and lateral thalamus, respectively (or vice versa), at sites which were extrapolated from recordings in each case in lateral thalamus and which were designed to include all major STT projection regions. Survival times were successively extended (up to 5 wks) to maximize labeling in both the cervical and lumbar spinal cord. Single- and double-labeled cells were located and counted in sagittal, transverse and horizontal sections from segments C5-7 and L5-S2.

Labeling observed with fluorescent tracers was quantitatively similar to labeling in control cases injected with horseradish peroxidase. On average, 2088 STT cells were counted in the segments examined, of which 46% were in lamina I, 8% in lamina V, 5% in lamina VI, 20% in lamina VII and 21% in lamina VIII. Of the entire population, 54% projected only to medial thalamus and 30% only to lateral thalamus, while 16% projected to both. This same overall projection pattern was found for STT cells in laminae I, VII and VIII, but STT cells in laminae V and VI were labeled predominantly from lateral thalamus. In three cases labeling in lamina I from medial thalamus was reduced (to less than that from lateral thalamus in L7) because the injections had missed part of the nucleus submedius. Lastly, STT cells were differentially distributed within lamina I; cells in its ventrolateral aspect generally projected only to medial thalamus, while cells in its dorsomedial aspect projected predominantly to lateral thalamus.

The projection pattern of lamina I STT cells presently observed corroborates the previous physiological findings. It is most striking that about half of the STT originates in lamina I, and that half of these cells project only to medial thalamus. The probable existence of further anatomical correlates of functionally distinct classes of STT cells is indicated.

Support provided by the Hermann-und-Lilly-Schilling Stiftung and the Deutsche Forschungsgemeinschaft.

164.6 SINGLE-UNIT RESPONSES IN MONKEY MEDIAL THALAMUS DURING NOCICEPTIVE AND NONNOCICEPTIVE DISCRIMINATION TASKS. G.H. Duncan, M.C. Bushnell and J.S. Feine*. Faculté de médecine dentaire, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Medial thalamic nuclei have been implicated in the processing of nociceptive and other somatosensory information, although their specific role is not known. Changes in arousal modify the responsiveness of many of these neurons (Casey 1966), suggesting a role in the affective aspects of perception. The current study evaluates the contribution of medial thalamus to discriminative processes and the importance of behavioral factors to neuronal responsiveness in monkeys performing discrimination tasks involving noxious heat, innocuous cold, and light stimuli.

A rhesus monkey was trained to detect small changes in the temperature of a thermode on the face. Following a light cue the monkey began a trial by pressing a lever, after which the thermode changed from a temperature of 37°C to one between 29°C and 48°C. After a variable time, the temperature changed again, and the monkey released the lever to receive a juice reward. A similar task using visual stimuli was also employed. Single unit responses were recorded while the monkey performed the tasks.

Of the 34 medial thalamic cells examined, 19 responded during the behavioral tasks. The most common response occurred at trial initiation (18 of 19 cells), and was usually related to both the sensory (signal light onset) and motor (lever press) events; however, sometimes the response was synchronized more closely to one event than the other. These cells did not respond to irrelevant lights in the task, indicating a lack of simple visual inputs. Six of the cells that responded in the task discharged after the first temperature change; the firing frequency usually continued to increase throughout the stimulus period of 3-9s, suggesting an anticipatory role. Three of these 6 cells responded during all levels of temperature and light, while the others responded differentially to heating and cooling. Eight of the 19 responsive cells discharged after the final stimulus which cued the monkey to release the lever. Usually both thermal and visual relevant cues evoked firing, but some cells responded preferentially to noxious heat cues, including small temperature changes that the monkey did not detect. When the cells that responded in the task were examined outside the task, 3 cells responded maximally to noxious stimuli and 3 to tap, while the others were not driven by innocuous or noxious tactile stimuli.

These data indicate that the responsiveness of medial thalamic neurons to sensory stimuli is highly dependent on the behavioral significance of those stimuli. However, some of these cells appear to respond to noxious thermal stimuli independently of the animal's behavioral response.

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164.7 'INHIBITORY' RESPONSES TO NOXIOUS STIMULI OF NEURONS LOCATED IN THE PERIPHERY OF THE VENTROPOSTEROMEDIAL NUCLEUS (VPM_P) OF THE CAT. C. Vahle-Hinz, J. Brüggemann* and K.-D. Kniffki. Physiologisches Institut, Universität Würzburg, D-8700 Würzburg, FRG.

Studies from various laboratories have recently demonstrated that the ventral periphery of the ventroposterolateral nucleus (VPL_{vp}) is remarkably distinct from VPL proper: it receives among other inputs those from the spinothalamic tract and holds nociceptive neurons.

In a combined electrophysiological and histological study on the neuronal representation of noxious stimuli applied to the cat's face, mouth and canine teeth in the region of the thalamic ventrobasal complex, nociceptive neurons were found to be located in the ventral periphery of VPM and its parvocellular part (VPMpc) and in the dorsal periphery of VPMpc. The noxious stimuli used were graded pinch and radiant heat applied to the skin, and rapid cooling of the teeth, a stimulus which activates nociceptive intradental fibers exclusively.

Nociceptive neurons (n=144) either responded to these stimuli alone (nociceptive specific neurons) or in addition also to low threshold mechanical stimulation of skin and fur (multireceptive neurons). While most neurons were excited by noxious and innocuous stimulation, for four nociceptive specific and five multireceptive neurons the ongoing activity in the absence of intentional stimulation was reduced by these stimuli. For six of these nine neurons only 'inhibitory' receptive fields were found in several areas of the face, whereas three neurons in addition had excitatory receptive fields to innocuous mechanical stimulation. In one of the latter, 'inhibition' of the excitatory response to low threshold mechanical stimulation was effected by noxious pinch in a different part of the head.

The occurrence of 'inhibitory' responses to noxious as well as innocuous stimuli of nociceptive neurons shows that the organization of the nociceptive system in VPM_P is more complex than previously thought. Interaction between excitatory and inhibitory responses may play a role in the analysis of the signals which reach the thalamus with respect to their nociceptive or non-nociceptive components. This might especially hold for information processed by multireceptive neurons, which constitute 42% of the nociceptive neurons in VPM_P.

164.8 MEDULLARY SUBSTRATES MEDIATING ANTINOCICEPTION PRODUCED BY ELECTRICAL STIMULATION OF THE VAGUS IN THE RAT. A. Randich and S.A. Aicher. Department of Psychology, The University of Iowa, Iowa City, IA 52242.

Electrical stimulation of afferents of the right cervical vagus inhibited the tail-flick reflex elicited by noxious heat in barbiturate-anesthetized rats. Electrical stimulation was initiated 20 seconds prior to a tail-flick test trial and the intensity of stimulation was adjusted to produce an increase in tail-flick latency from a group mean of 3.85 seconds to a 10 second cut-off (parameters of stimulation were 20 Hz, 2 msec, and a group mean of 3.6 V). This inhibitory effect was eliminated in rats receiving local anesthetic blockade (0.5 ul microinjections of 4% lidocaine) of either the nucleus tractus solitarii (NTS), the lateral reticular nuclei (LRN), the nucleus raphe magnus and medullary reticular formation (NRM-MRF), or the nucleus raphe obscurus (NRO) regions of the medulla. Similarly, the vasodepressor and bradycardic effects of vagal stimulation were either attenuated or eliminated by local anesthetic blockade of these regions. Microinjection of the non-specific glutamate antagonist γ-D-glutamyl-glycine (DGG) into the NTS regions also eliminated vagally-evoked inhibition of the tail-flick reflex, hypotension, and bradycardia. Conversely, microinjection of glutamate into the NTS region resulted in inhibition of the tail-flick reflex, hypotension, and bradycardia. These findings with DGG and glutamate support a role for glutamate as the neurotransmitter of the primary vagal afferents mediating these antinociceptive and cardiovascular responses. These results are discussed in terms of vagal afferent influences on somatosensory, somatomotor, and cardiovascular function. This research was supported by NIH grant 18341.

164.9 DIFFERENTIAL EFFECTS OF INTRACARDIAC BRADYKININ AND CAPSAICIN ON T_2-T_4 DORSAL HORN AND ASCENDING TRACT NEURONS IN THE CAT. D.C. Bolser*, M.J. Chandler, D.W. Garrison and R.D. Foreman. (Sponsored by R. Thies) Depts. of Physiology & Biophysics and Allied Health Education, Univ. of Okla. HSC, Okla. City, OK 73190.

Intracardiac injections of an algesic substance, bradykinin (BK), stimulates cardiac receptors with sympathetic afferent fibers and excites upper thoracic dorsal horn, spinoreticular and spinothalamic tract neurons receiving viscerosomatic convergent inputs in cats and monkeys. Capsaicin (CAP) stimulates thin-fiber somatic and visceral afferents and is a potent algesic agent. The effects of intracardiac injections of CAP on thoracic spinal neurons have not been investigated. The purpose of this study was to compare the effects of intracardiac CAP and BK on dorsal horn and ascending tract neurons in the upper thoracic spinal cord.

Nine cats were anesthetized with α-chloralose, paralyzed with pancuronium, and bilaterally vagotomized. A catheter was placed in the left atrium for intracardiac injections of BK and CAP. Bipolar electrodes were placed in the C_1 spinal cord segment for antidromic activation of ascending tract neurons. The left grey matter of the T_2-T_4 spinal cord was searched with microelectrodes for extracellular potentials of neurons receiving excitatory input from both somatic and cardiopulmonary (CP) sympathetic afferent fibers.

Twelve neurons were studied (11 dorsal horn, 1 tract neuron). Left atrial injections of BK (4μg/kg) and CAP (5μg/kg) produced different effects on 9/12 neurons. Bradykinin excited these neurons while CAP produced a variety of effects (decrease in activity in 5 neurons, no change in 3, increase followed by decrease in activity in 1). Intracardiac injections of BK and CAP produced similar effects in 3 neurons (increase in activity for 1 neuron, decrease for 2 neurons). Control injections of BK or CAP into the aorta produced either no response or variable effects on neuronal firing rates that were different in direction and latency from intracardiac injections of BK or CAP for 10/12 neurons.

These results lead to the suggestion that some chemosensitive cardiac receptors can produce inhibitory effects on upper thoracic spinal neurons. These data also provide evidence that intracardiac BK and CAP may stimulate different classes of cardiac receptors, each of which can have different effects on upper thoracic spinal neurons. (Supported by NIH grants HL22732, HL07430 & NS08150).

164.10 VISCEROSOMATIC CONVERGENCE ONTO T_2-T_5 SPINAL CORD DORSAL HORN CELLS FROM DISTAL ESOPHAGUS, HEART, AND SOMATIC FIELDS IN CAT. D.W. Garrison, M.J. Chandler, and R.D. Foreman. Depts. of Allied Health Education and Physiology and Biophysics, Univ. of Okla. HSC, Okla. City, OK 73190.

Esophageal disease and spasm often produce painful sensations in the same somatic fields as angina pectoris. The referral of visceral pain to somatic fields is often the explanation for this situation. One way to account for the similarity of somatic symptoms is for esophageal, cardiac, and somatic input to excite the same dorsal horn cells. Excitatory convergence onto dorsal horn cells has been shown for cardiac afferents and for somatic afferents from the left triceps and left thoracic wall in response to noxious stimuli. It has not been shown that afferent input from the distal esophagus excites dorsal horn cells in the upper thoracic cord or that this input converges with cardiac and somatic input. The purpose of this study was to determine (1) if T_2-T_5 dorsal horn cells are excited by distal esophageal input and (2) if these same cells are excited by somatic and/or cardiac input.

Experiments were performed on 22 vagotomized cats that were anesthetized with alpha-chloralose and paralyzed with pancuronium. The left side of the T_2-T_5 spinal cord was searched for spontaneously firing dorsal horn cells that responded to distension of the distal esophagus by 3cc-15cc of water injected into a Foley catheter. Thirty eight cells that were excited by esophageal distension were analyzed for responses to (1) noxious somatic pinch of the left triceps and left thoracic wall and (2) injection of an algesic substance, bradykinin, into the left atrium.

Of the 38 cells with esophageal input, 29 (76%) had excitatory cardiac and somatic input. Four (11%) of these 38 cells had excitatory somatic input but inhibitory cardiac input. The remainder of the cells, 5 (13%) had excitatory somatic input but did not respond to bradykinin injections.

These data show for the first time that distal esophageal input excites dorsal horn cells in the T_2-T_5 region of the spinal cord and that the same dorsal horn cells are excited by noxious input from the heart and somatic fields. This relationship may help explain the similarities of somatic symptoms between esophageal disease and angina pectoris. (Supported by Univ. Okla. HSC Faculty Senate Award 43038700 and NIH Grants HL22732 & NS08150)

164.11 DIFFERENTIAL EFFECTS OF VISCERAL SPINAL AFFERENT INPUT ON LUMBOSACRAL SPINOTHALAMIC TRACT (STT) CELLS IN THE MONKEY. S.F. Hobbs, U.T. Oh*, M.J. Chandler, and R.D. Foreman. Dept. of Physiology and Biophysics, Univ. of Okla. HSC, Okla. City, OK 73190.

Stimulation of cardiopulmonary and greater splanchnic sympathetic afferents excites but does not inhibit STT cells in the upper thoracic spinal segments. These observations led to the hypothesis that sympathetic afferents excite STT cells in all segments of the spinal cord. To test this hypothesis the responses of lumbosacral STT cells to electrical stimulation of cardiopulmonary sympathetic afferents, splanchnic nerve afferents, and lumbar sympathetic afferents were tested.

Seventeen monkeys (macaca fasicularis) were anesthetized with α-chloralose and paralyzed with pancuronium. The gray matter of the spinal cord between L_1 and S_1 was searched for extracellular action potentials of cells that were antidromically activated from the ventroposterolateral nucleus of the thalamus. All cells were excited by somatic manipulation of the left hindlimb or tail and were classified as wide dynamic range, nociceptive specific, or high threshold inhibitory. Of the 34 cells studied, electrical stimulation of cardiopulmonary sympathetic afferents inhibited 28 (82%), excited 1 (3%) and did not affect 5 cells. Electrical stimulation of the splanchnic nerve inhibited 25 of 33 cells (76%), excited 5 (15%) and did not affect 3 other cells. Thus, cardiopulmonary and splanchnic sympathetic afferents, which project to the thoracic cord, primarily inhibited STT cells in the lumbosacral cord. In contrast, electrical stimulation of lumbar sympathetic afferents inhibited 12 of 34 cells (35%), excited 10 (29%), and did not affect 12 cells. These effects of lumbar sympathetic afferents were significantly related to segment level (Fisher's exact test, $P<0.001$). Of the 22 cells affected by lumbar sympathetic afferent input, all 9 STT cells located between L_1 and L_3 were excited whereas all but one cell (12/13) located between L_4 and S_1 were inhibited.

These results show that sympathetic afferent input can excite and inhibit STT cells depending on the site of afferent projection and the segments of the spinal cord being studied. Thus, sympathetic afferent input arising from an organ may excite STT cells in the region of the cord that receives the projections of these afferents and may inhibit STT cells in other segments of the spinal cord. The inhibitory effect at more distant segments may enhance the excitatory effect by improving the signal-to-noise ratio of the STT system. Presumably, the differential response facilitates the ability to localize a noxious visceral stimulus and to produce appropriate reflex and behavioral responses.

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164.12 VISCEROSOMATIC INHIBITION OF LUMBOSACRAL SPINOTHALAMIC TRACT (STT) CELLS IN MONKEYS: ROLE OF C_1-C_2 SPINAL CORD. U.T. OH*, S.F. Hobbs, M.J. Chandler and R.D. Foreman, Dept. of Physiology and Biophysics, Univ. of Okla. HSC, Okla. City, OK 73190.

Electrical stimulation of cardiopulmonary (CP) sympathetic afferent fibers and noxious pinch of forelimb triceps muscles inhibit lumbosacral STT cells. We hypothesized that either supraspinal or long propriospinal pathways are necessary for this inhibitory effect.

To test if supraspinal pathways are necessary, the inhibitory effect of electrical stimulation of CP sympathetic afferents and pinch of forelimb triceps muscles on lumbosacral STT cells was tested before and after complete spinal section at C_1. Four monkeys (Macaca fascicularis) were anesthetized with α-chloralose and paralyzed with pancuronium. STT cells in L_2 to S_1 spinal segments were antidromically activated from the ventroposterolateral nucleus of the thalamus. The same STT cells were also antidromically activated from the thoracic cord to identify cells after cord cuts. Stimulation of both visceral and somatic afferents inhibited all cells before cord cuts. After C_1 cuts inhibition of spontaneous cell activity was not markedly affected by pinching the triceps (4 of 4 cells) or electrical stimulation of CP sympathetic afferents (3 of 4 cells). These data indicate that supraspinal pathways are not necessary for this inhibitory effect and that long propriospinal pathways are involved.

Because the CP sympathetic afferents and the afferents from the forearm triceps muscles project to the upper thoracic and lower cervical cord, we hypothesized that these afferents did not reqire long propriospinal pathways originating from the upper cervical cord to produce inhibition of lumbosacral STT cells. To test this hypothesis we recorded from 11 STT cells in 11 monkeys using the same methods and protocols as above, but cord transections were made between C_2 and C_6. In 8 of 11 STT cells the inhibition resulting from pinching the triceps muscle was abolished after making these cuts. In 8 of 10 STT cells the inhibition resulting from activating CP sympathetic afferents also was abolished after these cuts were made. The effects of C_1 cuts compared to C_2-C_6 cuts were significantly different ($P<0.05$) for the inhibition of the lumbosacral STT cells resulting from triceps pinch and nearly significant ($P<0.09$) for the inhibition due to the stimulation of CP sympathetic afferents.

These results show that propriospinal cells in the C_1-C_2 cord are important for mediating the inhibition of the lumbosacral STT cells by noxious stimulation of both visceral and somatic afferents that project to the upper thoracic spinal cord. However, these data do not exclude the possibility that supraspinal pathways also play a role. Supported by NIH Grants HL22732, HL07430 and NS08150.

165.1 QUANTITATIVE ANALYSIS OF GAD mRNA LEVELS DURING CEREBELLAR DEVELOPMENT IN RAT, M.D. Willcutts*[1], W.S.T. Griffin[2], and M.R. Morrison[1], [1]Departments of Neurology and Biochemistry, UTHSCD, Dallas, Texas, 75235, and [2]Pediatrics Department, UAMS, Little Rock, Arkansas, 72205.

Glutamate Decarboxylase (GAD; EC. 4.1.1.15) catalyzes the biosynthesis of gamma-aminobutyric acid (GABA) and is the rate-limiting enzyme controlling the steady state levels of GABA in the nervous system. GABA is the principle inhibitory neurotransmitter in mammalian brain. Alterations in GABA concentrations have been associated with seizures, anxiety, and motor disorders. GABAergic neurons are widely distributed throughout the brain and are well-correlated to GAD activity.

We have analyzed the level of GAD mRNA throughout cerebellar development in the rat. We have obtained a 2.4 kb cDNA clone of feline GAD (courtesy of Dr. A.J. Tobin), subcloned into pSP65 (Kaufman et al., 1986, Science 232:1138). We generated sense and anti-sense 2.4 kb GAD RNA transcripts to serve as standards, controls, and probes for GAD mRNA. Total and poly(A)$^+$ RNA were isolated from the cerebral cortex and cerebellum of fetal rats (E20) along with 4-day (P4), 6-day, 10-day, 14-day, 21-day, 30-day, and adult rat. The RNA isolates and sense/anti-sense GAD mRNA standards were separated by MOPS/HCHO agarose gel electrophoresis and electroblotted onto Zeta Probe nylon membranes. The membranes were probed with P^{32}-labeled anti-sense GAD RNA. After hybridization and washing, GAD mRNA signals were detected by autoradiography on Kodak SB5 film and then quantitated by making densitometric comparisons to the GAD RNA standards.

GAD mRNA was present in both the cerebral cortex and cerebellum at E20. The GAD mRNA level was 0.008% of the total cerebellar poly(A)$^+$ RNA. Cerebellar levels reached one-half the adult value during the third week of postnatal life. GAD mRNA levels rose exponentially throughout cerebellar development, reaching a plateau in the adult at 0.04% of the poly(A)$^+$ RNA population.

The approximate GAD mRNA content of GABAergic neurons (purkinje, golgi, stellate and basket) was calculated assuming: (1) mRNA is confined to a spherical perikaryon in all cells; (2) that the mRNA content/cell is proportional to the perikaryon volume.

Age	Molecules GAD mRNA/Purkinje Cell
E20	70
P6	250
Adult	1500

These results indicate that E20 Purkinje cells transcribe GAD mRNA prior to their final positioning and synaptic development. The cerebellar GAD mRNA increase parallels the progression maturation of the GABAergic system, and suggests that transcription is the major site of GAD gene regulation during cerebellar development. (Supported by NIH grant HD14886 (MRM).)

165.2 IN SITU HYBRIDIZATION DEMONSTRATES VASOPRESSIN GENE TRANSCRIPTION IN HYPOTHALAMIC NEURONS OF CROSSBRED HYPERTENSIVE X DIABETES INSIPIDUS RATS. J.T. McCabe[1], K. Almasan*[,2], E. Lehmann*[,2], J. Hänze*[,2], R.E. Lang*[,2], D.W. Pfaff[1], and D. Ganten*[,2]. [1]Neurobiol. & Behav. Lab., Rockefeller Univ., NY, NY 10021-6399 and [2]German Institute for High Blood Pressure Res., Heidelberg, F.R.G.

In situ hybridization, Northern blotting, and solution hybridization assay were used to identify vasopressin (VP) gene transcription in five strains of rats: Long-Evans (LE), Wistar-Kyoto (WKY), Spontaneously Hypertensive Stroke-Prone (SHRSP), Brattleboro (diabetes insipidus: DI), and crossbred hypertensive x diabetes insipidus (SHRDI).

In situ hybridization with single-stranded VP-specific probes (exon C of VP gene, radiolabeled by SP6 transcription), identified individual magnocellular hypothalamic "VP" neurons, and parvocellular neurons of the suprachiasmatic nucleus. Confirming previous investigations (McCabe, et al., Neuroendo., 44:361, 1986), DI rats transcribe their mutant VP gene (Schmale, et al., Nature, 308:705, 1984), and the present in situ hybridization experiment extends these findings for SHRDI rats. Magnocellular hypothalamic "VP" neurons of DI and SHRDI rats appeared hypertrophic, and possessed similar levels of VP gene transcript. In a single experiment where all 5 strains of rats were processed concomitantly, the estimated relative levels of VP gene product in supraoptic nucleus neurons were LE 3.0; WKY and SHRSP 2.2; DI and SHRDI 0.8.

Quantitative solution hybridization (n=7-8/group, all 3 months of age) confirmed LE rats had the highest hypothalamic content of VP mRNA ($\bar{X}$ + SEM: 1661 + 91pg), WKY and SHRSP strains lesser amounts (1351 + 105 and 1094 + 67, respectively), and DI rats contained the lowest (571 + 40). A Newman-Keuls test (at least $p<0.05$) indicated these differences in hypothalamic "VP" mRNA content across strains were significant: i.e., LE>WKY>SHRSP>DI (relative levels 3.0; 2.4; 2.0; 1.0, respectively).

Previous studies have observed several physiological abnormalities in SHRs, including alterations in brain, pituitary, plasma, and urinary levels of VP, and VP is a potent vasoconstrictor. However, no simple causal relationship between plasma or urinary VP concentration and blood pressure is evident. Since results from solution hybridization for entire hypothalami suggest a small, but significant, 20% diminution of VP mRNA content in SHRSPs relative to WKYs, and SHRDI rats suggest VP in brain is not necessary for the development or maintenance of hypertension, further studies must determine if the level of VP mRNA in paraventricular neurons which project to the brainstem are different between hypertensive and normotensive non-diabetic rats. Descending VP cells may exhibit important alterations in mRNA content that can only be detected by careful single-cell analyses.

165.3 DIFFERENTIAL EXPRESSION OF VASOPRESSIN ALLELES IN THE BRATTLEBORO HETEROZYGOTE. T.G. Sherman and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720, U.S.A.

Several recent studies from our laboratory have shown that homozygous diabetes insipidus rats (HO) of the Brattleboro strain prove to be an interesting model system in which to study neurosecretory neurons which have a history of chronic stimuli for secretion. In many cases, neurons adapt to chronic secretion stimuli by progressing through a linear series of post-translational and translational mechanistic modifications which greatly increase the levels of available peptide for secretion. These cellular adaptations culminate in increased transcriptional activity, contributing to higher cytoplasmic levels of mRNA coding for the peptide precursors. In Sprague-Dawley rats, for example, chronic salt-loading has been shown to lead to increased cytoplasmic levels of vasopressin, dynorphin and oxytocin mRNAs in the magnocellular neurons of the hypothalamus. In the homozygous Brattleboro rat, an animal which proves to be developmentally and chronically hyperosmotic, the resting levels of oxytocin mRNA are equivalent to a salt-loaded control Long Evans (LE) rat, as expected. Similarly, dynorphin mRNA, co-localized with vasopressin mRNA in the magnocellular hypothalamus, is also increased, although to a much greater extent (8-fold) than that attainable by a six-day salt-loading regimen (3-fold). The mRNA transcripts for AVP from the mutated AVP gene in these animals, however, are found to be 40-50% of that for controls, and yet remain regulatable by further osmotic challenges, increasing their cytoplasmic pool levels in response to a part-time exposure to 2% saline over 6 days.

These results prompted us to investigate the expression of the two different alleles of the AVP gene in the Brattleboro heterozygote (HE) in response to chronic salt-loading. Using a modification of a solution-phase mRNA:cRNA hybridization/RNase A/T1 protection assay, we hoped to distinguish between the two transcripts by RNase cleavage at the point deletion site in the defective Brattleboro gene. This proved to be a very sensitive and quantitative assay, confirming all previous results obtained by Northern analysis. For example, the concentration of AVP mRNA in the LE and HE animals are 60 amoles in the PVN and 120 amoles in the SON, whereas each of these nuclei contain approximately 130 amoles of oxytocin mRNA. The HO rat, however, contains only 20-25 amoles and 70 amoles of AVP mRNA in the PVN and SON, respectively. Each of these mRNA levels increases from 60-100% with a six-day regimen of chronic intermittent salt-loading. Furthermore, we have been able to show that both AVP alleles are expressed in the Brattleboro heterozygote, although at an approximate molar ratio of 30:1, normal versus defective mRNA. This ratio changes dramatically with osmotic challenge, apparently favoring the defective gene product, resulting in an approximate 9:1 ratio. We are investigating whether this apparent differential regulation is due to differences in message half-life or differential transcription rates. Research performed with the assistance of NIMH post-doctoral grant F32-MH09239 to T.G.S.

165.4 GENE EXPRESSION OF NEUROPEPTIDES RELATED TO CRF AFTER ADRENALECTOMY. M.K.-H. Schafer*, J.P. Herman, E. Young, R. Thompson, J. Douglas, T.G. Sherman, H. Akil and S.J. Watson (SPON: E. Valenstein). Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

It has been well-established that removal of adrenal glands causes an increase of CRF biosynthesis in the parvocellular subdivision of the paraventricular nucleus (PVN). Vasopressin (AVP), normally produced in the magnocellular portion of the same nucleus, has been reported by a number of laboratories to be coexpressed with CRF after adrenalectomy. The function and the changes in expression of other neuropeptides known to coexist with subsets of CRF containing neurons such as the opioid peptides, prodynorphin and proenkephalin, and neurotensin are less well-understood.

In this study we analyzed the distribution of mRNA for CRF, AVP, oxytocin (OXY), prodynorphin (DYN) and proenkephalin (ENK) in the different subdivisions of the rat PVN and quantify the changes of their mRNA content after adrenalectomy and dexamethasone treatment using in situ hybridization.

5 male Sprague-Dawley rats were bilaterally adrenalectomized, a control group sham-operated and an additional third group dexamethasone injected (2x100 µg/day). After a period of 14 days the animals were perfused with 4% paraformaldehyde, the brains removed and frozen in liquid nitrogen. Prior to perfusion blood samples of each animal were collected and plasma levels of corticosterone and β-endorphin were established to confirm successful adrenalectomy. 10 µm serial sections were cut through the hypothalamus on a Bright cryostat. ^{35}S-UTP labeled riboprobes with high specific activity complimentary to AVP, OXY, CRF, DYN and ENK (courtesy of Dr. S. Sabol, NIH) mRNA sequences were prepared with the pSP6 and pGEM transcription systems. Hybridization to tissue sections was carried out at saturation conditions for 48 hrs at 60°C. For quantification ^{35}S-brain paste standards were prepared. Following hybridization slides were dipped in Kodak NTB2 emulsion for autoradiography. Coverslipped slides were digitized and grain densities over the subdivisions of PVN and SON, sampled at 50 µm intervals, were recorded using the Loats Inc. RAS 1000 image analysis system.

The analysis confirmed the increased expression of AVP mRNA in the parvocellular part of the PVN after adrenalectomy revealing a 7 fold increase relative to controls ($p < 0.05$), whereas signals over other areas did not change significantly. Dynorphin mRNA showed a slight increase in the medial parvocellular part, but did not change in magnocellular areas. Present studies are being carried out to quantify the number of mRNA copies for each probe detectable in the different subdivisions and to compare the results to those obtained by solution phase hybridization.

165.5 EFFECTS OF CHRONIC ELECTROCONVULSIVE SHOCK ON VASOPRESSIN mRNA IN THE RAT PARAVENTRICULAR, SUPRAOPTIC AND SUPRACHIASMATIC NUCLEI: A QUANTITATIVE IN SITU HYBRIDIZATION ANALYSIS. J.P. Herman, M.K.-H. Schafer*, E.A. Young and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Specific subpopulations of arginine vasopressin (AVP) neurons are involved in very different types of regulatory processes. In these experiments we analyzed the effect of chronic electroconvulsive shock, a treatment which markedly perturbs function of the pituitary adrenal (PA) axis, on expression of AVP mRNA in the hypothalamic paraventricular (PVN), supraoptic (SON), and suprachiasmatic (SCN) nuclei. Such analysis enables identification of which subtype(s) of AVP neurons respond to changes in PA function, and whether such a stimulus can induce AVP gene expression in parvocellular PVN neurons in a manner similar to that seen following removal of the adrenal glands.

Six male Sprague-Dawley rats received transauricular electroconvulsive shock (ECS group) once daily for a period of one week. Control rats (n=6)(CON) received no ECS. All animals were decaptiated 24h following the time of the last ECS; brains were removed and rapidly frozen in isopentane. Hypothalamic blocks were sectioned at 10 um on a Bright cryostat. ^{35}S-UTP labelled probe complimentary to AVP was prepared with an Sp6 Riboprobe transcription system and hybridized to tissue sections for 48h. Specific activity of probes and hybridization time were calculated to insure that time mRNA-cRNA binding was saturated. Following the hybridization reaction, slides were dipped in Kodak NTB2 emulsion for autoradiography. Emulsion-dipped sections (at 50 um intervals) were coverslipped, digitized and grain density over the PVN, SON and SCN subsequently quantified using Loats Associates RAS 1000 image analysis software.

Analysis revealed an 80% increase in AVP mRNA in the SON of ECS rats relative to the CON group ($p<.01$, ECS vs. CON). Quite surprisingly, no differences were evident in the magnocellular subdivisions of the PVN, whose cells are believed to subserve functions similar to those of SON neurons. In addition, there were no changes in AVP mRNA content in the medial parvocellular subdivisions of the PVN, a region where AVP gene expression is known to be induced following perturbations of the pituitary-adrenal axis. No changes were observed in the SCN. These data suggest that ECS promotes AVP gene expression in a subset of magnocellular AVP neuronal populations, and therefore that magnocellular AVP neurons may be a more physiologically heterogeneous population of neurons than had been heretofore believed. Whether the up-regulation of AVP mRNA in the SON is the result of altered adenohypophysial and/or neurohypophysial function remains to be determined.

165.6 SUBSTANCE P GENE EXPRESSION IN VENTROMEDIAL HYPOTHALAMIC NEURONS OF ESTROGEN-TREATED AND CONTROL RATS ANALYZED BY IN SITU HYBRIDIZATION. G.J. Romano, T.I. Bonner*[1] and D.W. Pfaff. The Rockefeller University, New York, N.Y. 10021 and [1]Laboratory of Cell Biology, NIMH, Bethesda MD 20892.

Neurons in the ventrolateral aspect of the ventromedial nucleus of the hypothalamus (VLVM) mediate some of estrogen's effects on reproductive behavior in the female rat. Approximately 40% of these neurons contain estrogen receptors (Morrell, et al. Exp.Br.Res.,86). Substance P (SP) is present in VLVM neurons and has been shown to facilitate lordosis (Dornan et al., NSci.Abs.,84). Since SP and estrogen receptors are colocalized in the same neurons in the VLVM (Akesson et al., NSci.Abs.,86) we hypothesized that estrogen might regulate the synthesis of SP in this nucleus. To investigate this, we employed in situ hybridization to determine whether estrogen regulates the levels of SP mRNA in the VLVM.

The probe used for in situ hybridization was a [3H] cRNA complementary to nucleotides 1-468 of exon 7 of the rat SP gene. This probe hybridizes to all three SP mRNA species. Hydrolysis of the cRNA resulted in fragments of 50-150 nucleotides. Female rats were ovariectomized, implanted s.c. with 5mm silastic capsules which were either blank (n=3) or filled with 17β-estradiol (E2)(n=3), decapitated 2 weeks later and the brains were frozen on dry ice. Cryostat sections (10 μm) were postfixed in paraformaldehyde, and in situ hybridization was carried out according to the method of Shivers et al. (Meth.Enz.,124,1986). The sections were dipped in emulsion, and exposed at 4°C for 60 days. For analysis, the mediobasal hypothalamus was scanned for labelled cells and the autoradiographic signal was cuantitated in VLVM neurons by grain counting. No labelled neurons were observed in control sections which were RNase digested prior to hybridization or hybridized with probe diluent alone.

While numerous SP mRNA-containing neurons were observed in and adjacent to the VLVM, no significant differences in grain density or in the number of labelled neurons were observed in the VLVM between the E2-treated rats and the ovariectomized controls. Surprisingly, this suggests that estrogen does not directly regulate SP mRNA levels in the VLVM neurons known to contain both SP and estrogen receptors. Although quantification was restricted to the VLVM, we have observed SP mRNA-containing neurons throughout the mediobasal hypothalamus, notably in the dorsal aspect of the ventromedial nucleus, dorsomedial nucleus, lateral hypothalamus and arcuate nucleus. Therefore, these results do not preclude an effect of estrogen on the other populations of SP mRNA-containing neurons in the MBH. The apparent lack of an estrogen effect on SP mRNA in the VLVM is especially interesting in light of our demonstration that estrogen greatly increases proenkephalin mRNA in this nucleus (Romano, et al., NSci.Abs.,86).

165.7 DIFFERENTIAL REGULATON OF PHENYLETHANOLAMINE-N-METHYL TRANSFERASE (PNMT), TYROSINE HYDROXYLASE (TH), AND PROENKEPHALIN (pEK) mRNA LEVELS IN CULTURED BOVINE CHROMAFFIN CELLS BY ACETYLCHOLINE (ACH), DEXAMETHASONE (DEX), FORSKOLINE (FSK), AND TETRABENAZINE (TBZ). M.K. Stachowiak*, R. Slepetis*, J.-S. Hong, O.H. Viveros. (SPON: G. Pollard). LBNT, NIEHS, NIH, P.O. Box 12233, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Adrenal medullary chromaffin cells contain multiple hormonal substances. In addition to epinephrine and norepinephrine, large amounts of pEK A derived peptides are costored and coreleased with catecholamines (CA). Recently we began to investigate the mechanisms which determine hormonal output from adrenomedullary cells. We have previously shown that the adaptations of the adrenomedullary cells to alterations in transmitter release involve changes in mRNA levels, synthesis, and postranslational processing of CA synthetizing enzymes and pEK. The present studies were undertaken to elucidate the role of ACH, glucocorticoids, cAMP, and CA in the regulation of these processes. Bovine adrenal chromaffin cells were exposed for 3 days to defined medium containing 2 mM Ca++ with FSK (50 uM), TBZ (100 uM), DEX (1 uM) or to ACH (50 uM) with 10 uM physostigmine. Control cultures were supplemented with ethanol (0.05%) and HCl (100 uM). The relative abundances of specific mRNAs were determined by dot blot hybridization and northern analysis using cloned TH, PNMT, and pEK cDNA probes. DEX and ACH treatments increased relative abundance of all 3 mRNAs studied. A 50% increase in PNMT mRNA occured after 24 hours of DEX treatment, whereas the effects of ACH were smaller (+30%) and became apparent at 48 hours. TH and pEK mRNA levels increased by 20-40% after DEX, while changes produced by ACH were 2-3 times larger. Striking dissociations in responses of individual mRNAs to TBZ and FSK were observed. FSK produced over 2 fold increase in both TH and pEK mRNA levels. Decrease in the cell CA content after TBZ was accompanied by a 100% increase in pEK mRNA, and relatively small (+30%) elevation of TH mRNA levels. Most interestingly relative abundance of PNMT mRNA was dramatically reduced by both treatments; over 80% decrease was observed in the presence of TBZ and a 55% decrease in the presence of FSK. Changes after TBZ occured already after 1 day treatment, 24 hours earlier than with FSK, suggesting different mechanisms may be operative for these two agents. These results are consistent with the hypothesis that the effects of ACH on PNMT mRNA levels are not mediated by cAMP or intracellular CA. In conclusion, PNMT, TH, and pEK mRNA levels are differentially controlled by extracellular and intracellular factors. Such differential regulation may provide bases for selective control of the hormonal output from adrenal chromaffin cells.

165.8 EXCESS CORTICOSTERONE INCREASES PREPROENKEPHALIN mRNA LEVELS IN THE RAT HIPPOCAMPUS. R.E. Harlan and M.M. Garcia*. Departments of Anatomy and Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112

A fundamental aspect of the response to stress is secretion of glucocorticoids from the adrenal gland. Glucocorticoids act on a variety of tissues, including the brain. A primary target site for glucocorticoid action in the brain is the hippocampus. We have begun to evaluate the effects of exposure of rats to excess glucocorticoid by determining specific mRNA levels in different brain regions. In an attempt to mimic the glucocorticoid response to chronic stress, we injected 10 mg of corticosterone (N=6), or vehicle (N=5), daily for two weeks. The rats were then decapitated, and the brains were removed rapidly and dissected into caudate-putamen, hippocampus, amygdala and hypothalamus. Samples were frozen on dry ice and stored at -80°C until use. Total RNA was extracted from each sample by the guanidinium isothiocyanate-cesium chloride method. One or 0.5 μg of each sample was dotted onto nitrocellulose by vacuum filtration, then baked in a vacuum oven. The RNA on the nitrocellulose filter was hybridized to a ^{32}P-labeled cDNA complementary to rat preproenkephalin (PPE) mRNA (gift of R. Howells). Hybrids were detected autoradiographically and quantitated by a scanning densitometer.

Results indicate that corticosterone treatment significantly increased PPE mRNA levels in the hippocampus (79% increase, $p < .01$). There was no significant effect of corticosterone on PPE mRNA levels in the amygdala, mediobasal hypothalamus or caudate-putamen. Corticosterone treatment also significantly ($p < .01$) decreased body weights. Dissected brain sample weights did not differ according to treatment group.

These results may implicate peptides derived from proenkephalin in the negative feedback regulation of corticosterone secretion mediated by the hippocampus. In addition, the results may provide insight into altered neuronal responses during stress. Supported by NS24148

165.9 TRANSCRIPTIONAL CONTROL OF ADRENAL CATECHOLAMINE AND OPIATE PEPTIDE TRANSMITTER GENES. E.F. La Gamma, & I.B. Black, SUNY at Stony Brook, 11794 and Cornell University Medical Center, NY, 10021.

In the rat, decreasing transsynaptic activity through adrenal denervation, nicotinic receptor blockade, or explantation is associated with an increase in preproenkephalin mRNA, enkephalin prohormone and peptide. In contrast, catecholamine pathways remain unchanged under similar conditions. Conversely, membrane depolarization, increased sodium or calcium ion flux, or increased intracellular levels of cAMP, all serve to inhibit enkephalin biosynthesis, lowering levels of preproenkephalin mRNA, prohormone, and peptide (see La Gamma et al., Annals NY Acad. Sci., 1987). It is not known whether changes in messenger RNA result from stabilization or increased synthesis. To begin to resolve this issue, we exploited transcription "run-off" assays to measure the rate of transmitter gene read out. Tyrosine hydroxylase message (TH-mRNA) was found to be the most abundantlty produced transcript in the unmanipulated control rat adrenal medulla. TH-mRNA was produced in excess of twice the rate of transcription of the structural gene actin. In contrast, preproenkephalin transcription occurred at a much lower rate (60% of the actin gene and only 25% of tyrosine hydroxylase gene transcription). All transcripts were inhibted by the polymerase II inhibitor, alpha-amanitin. After two days in explant culture, the rate of enkphalin transcription increased approximately 2-fold (to the same level as actin transcription); while tyrosine hydroxylase transcriptional activity fell to 30% of actin levels. Then, to analyze cellular mechanisms, explants were depolarized with potassium chloride. Enkephalin gene transcription was observed to be 2.5-fold less when grown under depolarizing conditions than in control explants. On the otherhand, tyrosine hydroxylase gene read-out was unchanged, similar to results obtained when TH catalytic activity was measured. These data indicate that membrane depolarization can selectively regulate expression of a transmitter gene product and are consistent with a proposed transsynaptic regulatory mechanism controlling biosynthesis of adrenal opiate peptides. Additionally, these observations represent a molecular mechanism through which physiologically relevant stimuli can modify transmitter expression to alter neurohumoral function or transmitter pheontypic plasticity.

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165.10 REVERSE TRANSCRIPTION OF MESSENGER RNA IN FIXED TISSUE SECTIONS. L.H. Tecott, J.D. Barchas and J.H. Eberwine. Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305.

A method is under development in which the synthesis of DNA complementary to a specific mRNA is achieved in fixed tissue sections. The in situ transcription (IST) procedure is an extension of in situ hybridization (ISH) technology. An oligonucleotide complementary to an mRNA of interest is hybridized in situ and serves as a primer for the polymerase activity of the enzyme reverse transcriptase. Using the mRNA as a template, a complementary radiolabeled DNA strand may be synthesized. This strand will remain hybridized to the mRNA and may thus enhance the ISH signals obtainable with oligonucleotide probes. In addition, the IST procedure may provide information regarding the translational state of mRNA.

A 36-mer was synthesized complementary to the sequence coding for amino acids 100-111 of rat proopiomelanocortin (POMC). The unlabeled oligonucleotide was hybridized in situ to 10 micron paraformaldehyde-postfixed cryostat sections of rat pituitary. Following hybridization and washes, transcription was performed on the tissue sections in reverse transcriptase buffer in the presence of radiolabeled deoxynucleotides. Following reverse transcription, sections were washed, dehydrated through an ethanol series and exposed to X-ray film.

The IST procedure resulted in intense hybridization signals in the intermediate lobe, requiring very brief film exposures (10 min). Heat inactivation of the reverse transcriptase enzyme prior to the reaction eliminated the signal. The signal was also eliminated by omission of the oligonucleotide primer and by substitution of a heterologous oligonucleotide of similar length and base composition. Prior treatment of rats with 4 daily i.p. doses of 3 mg/kg haloperidol resulted in increased intermediate lobe signals relative to controls, consistent with known effects of the drug on POMC mRNA levels.

The above results indicate that the IST signal is enzyme-dependent and primer-specific. The regional pattern of signal and its haloperidol-induced increase provide support for the specificity of the method. The signal intensity produced by the method suggests its potential usefulness for the detection of low-abundance messages. We are currently investigating the utility of the method as a means for the nonisotopic detection of in situ hybrids. (NIDA 01207 and NIMH 23861.)

165.11 REGULATION OF G-PROTEIN mRNA LEVELS IN ACTH/ENDORPHIN-PRODUCING PITUITARY CELLS. E.A. Thiele*, R.R. Reed* & B.A. Eipper. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Guanine nucleotide-binding proteins (G proteins) are essential components of various signal transduction systems, including the adenylate cyclase/cAMP system. We find significant alterations in G protein mRNA levels in AtT-20 cells and rat neurointermediate pituitary lobes in response to agents affecting secretion, suggesting that regulation of G protein levels may be an important mechanism in determining the responsiveness of a cell to stimuli.

In AtT-20/D16v mouse corticotrope tumor cells, corticotropin releasing factor (CRF), vasoactive intestinal peptide (VIP) and β-adrenergic agonists stimulate the synthesis and secretion of pro-ACTH/endorphin (PAE) by increasing adenylate cyclase activity. Pretreatment of AtT-20 cells with dexamethasone (DEX), a synthetic glucocorticoid, suppresses this stimulation. In intermediate pituitary melanotropes the synthesis and secretion of PAE are inhibited by dopamine via inhibition of adenylate cyclase.

The cDNAs corresponding to $G\alpha_s$, $G\alpha_i$, and $G\beta$ were obtained from a rat olfactory bulb cDNA library (D. Jones and R.R. Reed, manuscript submitted) and used to probe total cellular RNA on Northern blots. AtT-20 cells contained RNA species hybridizing with cDNA for $G\alpha_s$, $G\alpha_i$, $G\beta$. Rat neurointermediate pituitary contained RNA species hybridizing with cDNAs for $G\alpha_s$ and $G\alpha_i$; $G\beta$ mRNA was not detected in the rat neurointermediate pituitary.

AtT-20 cells were treated with 100 nM CRF for 4 hours, or with 1 μM DEX for 2 days. CRF treated cells exhibited a 1.5-fold increase in secretory rate of PAE-derived peptides and DEX treated cells exhibited a 3-fold decrease. Northern analysis indicated parallel changes in PAE mRNA. $G\alpha_s$ and $G\alpha_i$ mRNA levels increased several-fold in the CRF treated cells. The increase in $G\beta$ mRNA levels was smaller but still significant. In DEX treated cells, there were similar, although less dramatic, increases in the levels of all three G protein mRNA levels. Cholera toxin and pertussis toxin treatment of AtT-20 cells both decreased G protein mRNA levels.

To investigate possible regulation of G protein mRNA levels in the neurointermediate pituitary lobe, adult male rats were treated for 3 weeks with bromocriptine (1 mg/kg/day) or haloperidol (2 mg/kg/day). Neurointermediate lobes were removed, and extracted for peptides or total cellular RNA. Radioimmunoassay indicated a 5.3-fold decrease in PAE content following bromocriptine treatment and a 2.3-fold increase following haloperidol treatment. PAE mRNA levels showed parallel changes. Following bromocriptine treatment there was a decrease in levels of $G\alpha_s$ mRNA, and no apparent change in levels of $G\alpha_i$ mRNA. Following haloperidol treatment there was a significant increase in $G\alpha_s$ mRNA levels and a decrease in $G\alpha_i$ mRNA levels.

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165.12 HORMONAL MODULATIONS OF VIP GENE TRANSCRIPTS: I. Gozes and F. Baldino, Jr. Department of Hormone Research, The Weizmann Institute of Science Rehovot 76100, Israel and Neurobiology Group, Medical Products Department E.I. du Pont de Nemours and Co., Wilmington, Delaware 19898, U.S.A

VIP-vasoactive intestinal peptide is a widely distributed multifunctional neuromodulator. As a neurohormone VIP has been proposed as an inducer of prolactin release. A sensitive RNA detection assay which utilizes in vitro transcribed RNA probes corresponding to specific exons of the VIP gene identified a 2,000-base long RNA encoding VIP. This RNA also contains the coding sequences for the VIP-related peptide PHM/I (peptide histidine methionine amide or isoleucine amide). During lactation a two fold increase in the hypothalamic VIP mRNA was demonstrated (Gozes, I. and Shani, Y. Endocrinology 119, 2497, 1986. To precisely localize the hypothalamic areas involved and more particularly the cells associated with the changes in VIP-PHM/I gene activity an in situ hybridization histochemical procedure was adapted which employs either radioiodinated or ^{35}S-labeled synthetic oligodeoxynucleotide probes (Card, J.P., Fitzpatrick-McElligott, S., Gozes, I. and Baldino, F. Jr., submitted for publication). Our in situ hybridization results show a most pronounced increase in VIP and PHM/I transcripts during lactation in the suprachiasmatic nucleus as well as in the thalamus areas not directly involved in the regulation of pituitary hormone secretion. Thus, an indirect role for VIP gene products additional to the prolactin releasing activity is suggested. The modulation of VIP gene transcripts during lactation may be associated with alteration of steroid levels at this period which in turn may interact directly with steroid binding sites on the VIP gene (Gozes, I., Giladi, E., Shani, Y., Chao, H.M., McEwen, B.S. and Rostène, W.H., submitted for publication, Gozes, I., Werner, H., Fawzi, M., Shani, Y. and Koch, Y., in preparation, and Gozes, I. (1987) In: Krieger - Brain Peptides Update, Martin & Brownstein, eds., Chapter 10).

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166.1 FASTING INCREASES AND REFEEDING REINSTATES NEUROPEPTIDE Y (NPY) CONCENTRATIONS IN THE PARAVENTRICULAR NUCLEUS (PVN). A. Sahu*, S.P. Kalra and P.S. Kalra, Dept. OB-Gyn, Univ. Fla., Col. Med., Gainesville, FL 32610

We have shown that a bolus intraventricular injection of NPY elicits a robust feeding response lasting 120 min and continuous NPY infusion provokes an episodic feeding pattern in satiated rats in a manner seen during the night when rats normally eat. To demonstrate further that NPY may be a physiological neurochemical signal that normally activates feeding, we have studied the effects on NPY concentrations in various brain sites either of fasting (F) for 2, 3 or 4 days to increase appetite or in rats fasted for 4 days and allowed ad libitum (FA) access to rat chow for one day to reinstate satiety. Male rats were decapitated and various hypothalamic sites were microdissected by the technique of Palkovits and processed for measurement of NPY immunoreactivity by RIA. The hypothalamic sites selected were those shown previously to be innervated by NPY neurons and implicated in the control of feeding behavior. Water was available ad libitum throughout the experiment. Body weight decreased as a function of time of fasting by 26% and regained 12% after 1 day of free access to rat chow. The arcuate nucleus, rich in NPY perikarya, showed a progressive increase in NPY concentrations during food deprivation (Control (C): 103 ± 9; F2d: 114 ± 4; F3d: 184 ± 26; F4d: 221 ± 18; pg/µg protein, F = 13.542, ANOVA) and the levels remained elevated even in the FA group (243 ± 15). However, two types of responses in NPY concentrations were seen in those regions that are either sparsely or richly innervated by projections of NPY perikarya located in the arcuate nucleus. NPY concentrations in the median eminence and dorso- and ventromedial nucleus remained unchanged in response to either food deprivation or followed by free access to rat chow. Likewise, fasting failed to change NPY levels in the medial preoptic area, but surprisingly, availability of rat chow for one day after 4 days of fasting significantly increased NPY levels (C: 45 ± 2.7; FA: 82 ± 6.4, $p < 0.05$). In contrast, in the PVN, a site implicated in the control of feeding behavior and richly innervated by NPY neurons, NPY levels increased significantly in response to food deprivation. The NPY increments were apparent within two days and continued to rise in a time-related fashion for 4 days of fasting (C: 63 ± 12; F2d: 97 ± 21; F3d: 137 ± 18; F4d: 169 ± 17; pg/µg protein, F = 4.736, ANOVA) and fell precipitously in FA rats (86 ± 16, $p < 0.05$) and returned to the range found in satiated control rats. Thus, these results show for the first time that hunger and satiety evoke regionally specific reciprocal changes in the secretory function of NPY innervations in a site implicated in control of feeding behavior. Consequently, these findings strongly imply that NPY may be a physiological neurochemical signal that normally evokes the eating response. (Supported by NIH DK 37273).

166.2 CONTINUOUS INTRAVENTRICULAR INFUSION OF NEUROPEPTIDE Y (NPY) EVOKES EPISODIC FOOD INTAKE IN SATIATED FEMALE RATS: EFFECTS OF ADRENALECTOMY AND CHOLECYSTOKININ (CCK). S.P. Kalra, M.G. Dube* and P.S. Kalra, Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610

We have reported that a bolus intraventricular injection of NPY stimulated components of feeding behavior in male and female rats. We have now studied the effects on feeding behavior of continuous intraventricular infusion of 117, 470 or 1175 pmol/h for 4 h and for 2 h postinfusion in satiated female rats. The components of feeding behavior analyzed were: cumulative food intake, number of feeding episodes, mean episode length, total time eating, eating rate (g/min) and interepisode interval. Rats were implanted with permanent cannulae in the third ventricle of the brain and 6-14 days later they were connected to an infusion assembly on the morning of the experiment. Saline (18 µl/h) or various concentrations of NPY in saline were infused for 4 h. Only 1 of the 6 saline-infused control rats displayed some feeding (0.7 g) which consisted of 4 episodes of an average 33 sec duration, and 2.2 min total feeding time. In contrast, a dose-dependent stimulation of all components of feeding behavior monitored was seen with 117 and 470 pmol/h NPY infusion rates; at 1175 pmol/h rate, NPY produced a plateau effect. In addition, despite continuous NPY receptor activation, these rats displayed discrete episodes of feeding, the duration of which varied from a mean of 50 sec to 170 sec/rat. The episodes were interrupted mostly by grooming, drinking and exploratory behavior and to a lesser extent by restful postures and sleeping. Moreover, the elicitation of feeding episodes was dose-dependent as from a mere 4 episodes seen in saline-infused or 117 pmol/h NPY-infused rats, they increased characteristically to 40 after 1175 pmol/h NPY infusion. Remarkably, the incremental pattern of food intake was sustained during the 2 h post-NPY infusion period. CCK (1175 pmol/h) infused concurrently with NPY (470 or 1175 pmol/h) failed to block the NPY stimulation of any parameters of feeding monitored. In contrast, adrenalectomy dramatically attenuated ($p < 0.05$) the cumulative food intake by 50% in rats receiving NPY at the hourly rate of 470 pmol, this attenuation was apparent only during the 3rd and 4th h of NPY infusion. Further analysis showed that the reduction in food consumption was due to a dramatic increase in the interepisode interval ($p < 0.05$) resulting in significant decrease (> 50%) in the number of feeding episodes and in time spent feeding. Therefore, these studies show for the first time that continuous central NPY receptor activation can evoke episodic pattern of feeding behavior which is not blocked by the putative satiety influence of CCK. Adrenalectomy attenuated, but did not eliminate food consumption and affected the inter episode intervals and related feeding parameters. In summary, our findings of episodic feeding behavior in response to continuous NPY infusion taken together with the observations (reported elsewhere) of changes in NPY levels occurring specifically in the paraventricular nucleus in response to food deprivation, and augmentation of ongoing feeding response by NPY administration during the night, support the hypothesis that NPY may be a physiological neurochemical signal that normally activates the feeding response. (Supported by NIH DK 37273).

166.3 INTRAVENTRICULAR (IVT) ADMINISTRATION OF NEUROPEPTIDE Y BOTH INCREASES FOOD INTAKE AND CAUSES CONDITIONED TASTE AVERSIONS. A. J. Sipols*, D. J. Brief, K. Ginter*, S. Saghafi*, and S. C. Woods. Department of Psychology, University of Washington, Seattle, WA 98195.

Neuropeptide Y (NPY), a 41-amino acid peptide found in the brain and the gut, elicits rapid and large increases of food intake when administered in microgram quantities into either the cerebrospinal fluid or the paraventricular nuclei in rats. Preliminary work in our lab revealed that as the dose of IVT NPY is increased, food intake may actually decrease. The purpose of this experiment, therefore, was to determine if NPY might cause the formation of food aversions in addition to enhancing meal size. Male Long-Evans rats (N=8) were surgically implanted with cannulas aimed at the third cerebral ventricle. Patency and location within the ventricle were assured when each subject consumed at least 10 ml of water within 2 hours following IVT administration of carbachol. Rats were food-deprived 6 hours daily. Food was then presented and intake recorded over the subsequent hour. Following stabilization of intake after IVT saline, all animals were administered 9.5 µg NPY. Food intake increased 44.9 +/- 8.6% ($p<.01$, paired t-test) over the previous saline day. The animals were then adapted to a schedule of ad lib food. Water was available only 20 min/day. Following water intake stabilization (11 days), a novel aqueous solution of non-caloric lime-flavored Koolaid was offered instead of water. After 20 minutes, half the rats were injected with saline IVT and half with 9.5 µg NPY. On the subsequent day, all subjects were presented with a two-bottle choice of lime Koolaid vs. water. Animals receiving NPY drank significantly ($p<.01$, t-test) less Koolaid (12.4 +/- 3.1% of total intake as Koolaid) than animals receiving saline on the previous day (50.0 +/- 4.7%), with no reliable difference of total intake between the two groups. These results indicate that NPY causes at least two consequences associated with ingestion. Through one IVT cannula, it both stimulates food intake and causes the formation of a flavor aversion depending upon the paradigm.

166.4 INTRAVENTRICULAR NEUROPEPTIDE Y INJECTIONS STIMULATE FOOD INTAKE IN LEAN, BUT NOT OBESE ZUCKER RATS. D.J. Brief ,A. Sipols*, K. Ginter*,D. Amend*,& S.C. Woods. U. of Washington, Dept. of Psychology, NI-25, Seattle WA 98195.

Intraventricular and intrahypothalamic injections of neuropeptide Y (NPY) increase food and water intake in satiated rats. Because NPY receptors exist in many areas of the brain containing insulin receptors, and because obese Zucker rats have relatively few insulin receptors in the brain and do not alter food intake in response to intraventricular insulin administration, we injected either saline or NPY (.95, 3.0, 9.5, & 30.0 µg/rat) into the third ventricle of satiated homozygous fat (fa/fa), heterozygous lean (Fa/fa), and homozygous lean (Fa/Fa) Zucker rats and measured food and water intake at 1, 4, and 22 hours postinjection.

Neither .95 nor 30.0 µg NPY had any effect on food or water intake in any group. However, a significant ($p \leq .05$) increase of food intake was observed in Fa/Fa rats at 1 and 4 hours after 3.0 µg NPY and at 1, 4, and 22 hours after 9.5 µg NPY. Both 3.0 and 9.5 µg injections of NPY also increased 1 and 4 hour food intake in Fa/fa rats above intake following saline, but this increase was only significant ($p \leq .05$) 4 hours after 3.0 µg NPY. In constrast, there was no effect on food intake of fa/fa rats at any dose of NPY.

The only significant increases in water intake occurred in Fa/fa rats 1 hour after a 3.0 µg injection ($p \leq .05$) and in Fa/Fa rats 4 hours after a 3.0 µg injection ($p \leq .05$).

Lean Zucker rats, like rats of other strains, have elevated food intake following intraventricular injections of NPY. Obese Zucker rats, however, show a similar lack of response to the effects of centrally administered insulin and NPY on food intake. These results are consistent with the hypothesis that NPY and insulin receptors may interact to influence food intake.

166.5 CCK RECEPTOR BINDING IN THE PYLORUS OF GENETICALLY OBESE MICE (ob/ob). A. J. Strohmayer, D. Greenberg, T. Moran, and P. McHugh. Depts. of Psychiatry, Cornell Univ. Med. Coll., Manhasset, NY 11030 and Johns Hopkins School of Med., Baltimore, MD 21205

The hyperphagia of the genetically obese mouse (ob/ob) has been characterized as increased meal size and abnormal satiety behavior (Strohmayer & Smith, Physiol. & Behav. 22:1157-62, 1979). Cholecystokinin (CCK) has been shown to be a satiety peptide which reduces meal size and stimulates behavioral satiety (Smith & Gibbs, Ann. NY Acad. Sci. 448:417-423, 1985). An attractive hypothesis is that ob/ob mice either do not make or release active CCK in response to food stimuli or that receptor or effector mechanisms are dysfunctional. Strohmayer & Smith, (Brain Res. Bull. 17:571-73, 1986) have demonstrated that exogenous CCK administration was effective in reducing meal size and restoring some satiety behavior with appropriate equal sensitivity between obese and lean mice (2 ug. kg). However, this dose may be supra-physiological. Moran and McHugh (AJP, 242: R 491-97, 1982) have suggested a possible mechanism for CCK satiety is stimulation of pyloric constriction via CCK receptors increasing gastric distension. In order to determine if these receptors were intact in ob/ob mice, we compared the specific binding of CCK-33 in the pylorus of obese and lean mice. Specimens of the pyloric junction were obtained from 3 obese and 4 lean male mice frozen in methyl butane and 25 micron tissue sections were prepared with I^{125} labelled CCK-33 with or without 1u molar cold CCK-8, (Brain Research, 362:175-179, 1986). Autoradiographic images were quantified using a computer microdensitometry system. Specific binding (the difference between images with or without cold CCK-8) is reported as units of relative optical density.

The median specific binding for obese mice was .478 (range .406-.648) vs .229 (range .147-.343) for lean mice. Thus there was greater binding for the obese mice ($p = .028$ by Mann-Whitney U Test). We can conclude CCK receptors are present and bind CCK in obese mice. The increase in specific binding is consistent with an up regulation suggesting lower endogenous levels of active CCK in the obese mouse. Further experiments will attempt to measure endogenous CCK release.

166.6 TOTAL ABDOMINAL VAGOTOMY, BUT NOT GASTRIC OR HEPATIC VAGOTOMY, BLOCKS THE SYNERGISTIC SATIETY EFFECT OF SIMULTANEOUS INJECTION OF PANCREATIC GLUCAGON AND CHOLECYSTOKININ ON SHAM FEEDING. J. LeSauter* Z. Pap* M.K. Albertson* & N. Geary. Dep't. Psychology, Columbia Univ., New York, NY 10027.

Simultaneous intraperitoneal injection of pancreatic glucagon (PG) and cholecystokinin (CCK) inhibits rats' sham intake of milk more than the sum of the inhibitions elicited by the peptides individually (LeSauter et al., Soc. Neurosci. Abstr. 11: 38, 1985). To investigate the peripheral neural mechanism of this synergism, we tested the effects of PG and CCK alone and in combination after selective hepatic or gastric vagotomy or total abdominal vagotomy. Rats with chronic gastric cannulas were maintained on Purina chow ad lib and sham fed condensed milk. Simultaneous injection of CCK and PG still elicited large inhibitions of sham feeding after either selective vagotomy:

Mean ± SEM % Inhibition of 30 min Sham Intake

	PG 400 µg/kg	CCK 0.3 µg/kg	PG + CCK
Surg. Control (n=8)	7 ± 10	34 ± 13*	74 ± 13*
Hepatic Vagox (n=8)	-2 ± 6	44 ± 10*	73 ± 6*
Surg. Control (n=6)	12 ± 3	25 ± 15	53 ± 11*
Gastric Vagox (n=7)	7 ± 5	25 ± 17	75 ± 15*

*Inhibition of sham feeding, $p < .01$, Tukey's test after ANOVA.

In contrast, total abdominal vagotomy greatly attenuated, but did not entirely block, the inhibitory effect of simultaneous PG and CCK:

Mean ± SEM % Inhibition of 30 min Sham Intake

	PG 400	CCK 0.15	CCK 0.30	PG + .15 CCK	PG + .3 CCK
Surg. Contr (n=7)	7 ± 5	32 ± 8	58 ± 10*	65 ± 13*#	73 ±12*
Total Vagox (n=5)	15 ± 18	-5 ± 6	-5 ± 7¢	19 ± 10¢	29 ±13*¢

*Inhibition of sham feeding, $p < .05$, Tukey's test after ANOVA.
#$p < .08$ vs. sum of PG alone and CCK alone.
¢Smaller inhibition than surgical controls, $p < .01$.

These results suggest that subdiaphragmatic vagal neural traffic is necessary for the synergistic inhibition of sham feeding elicited by PG and CCK, but that neither the hepatic branch nor the gastric branch alone is necessary. Two possibilities are that coeliac vagal fibers mediate the synergism or that the hepatic and gastric branches provide redundant contributions that are each sufficient to mediate the synergism. This contrasts with the reported dependence of the satiety effect on real feeding of CCK on the gastric branch (Smith et al., Science 213: 1036, 1981) and of PG on the hepatic branch (Geary and Smith, Physiol. Behav. 31: 391, 1983).

Research supported by NIH grant AM 32448.

166.7 DIFFERENTIAL POTENCY OF FOUR ANTAGONISTS OF CHOLECYSTOKININ (CCK) FOR THE PYLORIC, PANCREATIC, AND SATIATING EFFECTS OF CCK-8. R.B. Murphy, G. Haraminian#, L.H. Schneider, and G.P. Smith. Department of Psychiatry, New York Hospital-Cornell Medical Center, White Plains, NY 10605, and Department of Chemistry, New York University, New York, NY 10003

Receptors for CCK in the pyloric sphincter, abdominal vagus, and hindbrain are candidates for mediating the satiating potency of peripherally administered CCK. In order to identify which of these candidate populations are involved, we have examined the potency of 4 CCK antagonists to inhibit contraction induced by CCK-8 at a half-maximal dose (8×10^{-10} M) in the isolated rat pyloric sphincter (Murphy et al Peptides 8: 127, 1987), and compared the results to the reported potency of these compounds to reverse the inhibition of CCK-8-induced satiety in the rat (Schneider et al, in press) and to antagonize the effect of CCK-8 on pancreatic acinar cells (Niederau et al, Am. J. Physiol. 251:G856, 1986; Jensen et al, Am. J. Physiol. 249:G214, 1985; Chang and Lotti, P.N.A.S. 83:4923, 1986).

Results

Drug	Pyloric IC_{50} (M)	Relative Potencies: Pylorus	Acinar Cell	Satiety
Proglumide	1×10^{-3}	1	1	1
PGDPA	$4 \pm 0.5 \times 10^{-6}$	250	133	1000
CR-1409	$5 \pm 1 \times 10^{-7}$	2000	4000	1000
L-364,718	$4 \pm 2 \times 10^{-9}$	250,000	800,000	100,000

Discussion. Pyloric and pancreatic acinar cell receptor populations have very similar absolute and rank-ordered potencies for the 4 antagonists. These potencies are also similar to those found for the inhibition of CCK-8-induced satiety, except for that of PGDPA which is considerably more potent for antagoninizing CCK-8-induced satiety than it is in the pyloric sphincter or on the pancreatic acinar cell. The differential potency of PGDPA suggests that although CCK receptors in the rat pyloric sphincter and/or pancreatic acinar cells could contribute to CCK-8-induced satiety, additional receptor populations or control mechanisms are also involved. Supported in part by RSA MH00149, MH40010.

166.8 L-364,718 ABOLISHES THE INHIBITION OF FOOD INTAKE INDUCED BY CCK-8 IN RATS. L.H. Schneider, R.B. Murphy, P.M. Perez# and G.P. Smith. Dept. of Psychiatry, Eating Disorders Inst. and Bourne Lab, NY Hospital-Cornell Med. Ctr., White Plains, NY 10605 and Dept. of Chemistry, New York University, NY, NY 10003.

L-364,718 is presently the most potent antagonist of cholecystokinin octapeptide (CCK-8) in vitro (Chang & Lotti, 1986). We investigated L-364,718 for its potency to antagonize the inhibition of food intake produced by exogenous CCK-8.

Methods. 44 male Sprague-Dawley rats were adapted to 30-min access to sweet milk after an 18h food deprivation and ip injections at -15 and -5 min. They were tested with vehicle + saline on Mondays, Tuesdays, and Thursdays, and with vehicle + CCK-8 (8 mcg/kg) on Wednesday. The purpose of the Wednesday test was to assure that the inhibitory effect of CCK-8 on food intake was balanced across the Friday test groups. On Fridays, 4 groups of 11 rats each received CCK-8 (8 mcg/kg)and one of the following doses of L-364,718: 0 (the control group); 2, 20, 200 pg/kg; 2, 20, 200 ng/kg; 2, 20, 200 mcg/kg. We also tested the effect of 200 mcg/kg of L-364,718 on the inhibition of food intake produced by bombesin (12 mcg/kg; n=11).

Results. L-364,718 antagonized the inhibition of food intake produced by CCK-8 in a dose-related manner [$F(9,90)=10.56$; $p<.01$]. Doses of L-364,718 of 2, 20, and 200 mcg/kg completely reversed the effect of CCK-8; mean (±SE)% inhibition of baseline intake (vehicle + saline, ip) was 3.4 (6.2), 4.9 (6.0) and -13.7 (5.7), respectively, as compared with 55.9 (3.3) in the control group. The lowest dose that produced a reliable partial reversal of the effect of CCK-8 was 200 ng/kg (28.0±7.3% inhibition; $p<.05$ Dunnett's t-test, 2-tailed). The highest dose of L-364,718 did not antagonize (42.8±5.8%) the inhibitory effect of bombesin (12 mcg/kg) on food intake (49.7±9.8%).

Conclusions. L-364,718 is the most potent antagonist of the inhibition of food intake produced by exogenous CCK-8 we have tested, being about 100,000 times more potent than proglumide and about 100 times more potent than the two proglumide analogues PGDPA and CR-1409 (Schneider et al, 1986, 1987) under our experimental conditions. We confirmed that L-364,718 is selective for reversing CCK-8, but not bombesin, induced satiety. Our results indicate a much higher potency than that reported in a recent abstract (Hanson and Strouse, 1987) that L-364,718 administered p.o. (ED50=334 mcg/kg) antagonized CCK-8 induced satiety (12 mcg/kg), in rats, even when taking account of several differences in experimental conditions.

Given its behavioral potency and selectivity, we conclude that L-364,718 will be a valuable probe into further investigations of a role of endogenous CCK in normal satiety mechanisms.

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166.9 SOMATOSTATIN AND GLUCAGON BLOCK THE INHIBITORY EFFECT OF CCK8 ON FOOD INTAKE IN DOGS. T.J. Kalogeris*, R.D. Reidelberger*, T.E. Solomon* and V.E. Mendel (SPON: V.V. St. Omer). Depts. of Medicine and Physiology, University of Missouri Medical School and Truman V.A. Hospital, Columbia, MO 65201.

We studied the dose-response effects of somatostatin and glucagon alone and in combination with CCK8 on food intake in dogs. Seven pure-bred beagles were adapted to ad libitum access to solid food for 18 h followed by a 4 h food deprivation prior to a one hour test session. In one feeding study dogs received intravenous infusions of somatostatin (0, 40, 400, 4000 pmol/kg-h, one dose per day) for 15 min prior to presentation of the test meal and during the subsequent 45 min feeding period. No effect on feeding was observed except at 4000 pmol/kg-h which increased 45 min meal size by 21%. In a second study, a similar dose range of somatostatin was tested in combination with each of three doses of CCK8 (0, 50, 400 pmol/kg-h). In the absence of somatostatin, CCK8 at 50 pmol/kg-h did not affect food intake, whereas CCK8 at 400 pmol/kg-h depressed food intake by 55%. This inhibition was blocked at all doses of somatostatin tested. Combination of a physiologic dose of CCK8 (50 pmol/kg-h, which alone is subthreshold for inhibition of feeding) with any dose of somatostatin did not affect food intake. Somatostatin alone at 4000 pmol/kg-h increased food intake by 60%. In a third study the effects of glucagon (0, 50, 500, 5000 pmol/kg-h) in combination with each of three doses of CCK8 (0, 50, 400 pmol/kg-h) on food intake were tested. Infusions were given for 25 min prior to and during the subsequent 35 min feeding period. When glucagon was given in combination with 0 and 50 pmol/kg-h of CCK8, food intake was not affected. At 400 pmol/kg-h, CCK8 alone depressed food intake by 53%; glucagon produced a dose-dependent attenuation of this effect. Insofar as a potential endocrine role for these peptides in the control of food intake in dogs is concerned, these results demonstrate: 1. physiologic intravenous doses of CCK8, somatostatin and glucagon alone, and combinations of CCK8 and somatostatin or CCK8 and glucagon do not inhibit food intake; 2. somatostatin and glucagon each block the inhibition of food intake caused by a supraphysiologic dose of CCK8; and 3. somatostatin alone increases food intake at a supraphysiologic dose.

166.10 **INHIBITORY EFFECTS OF CHOLECYSTOKININ DEVELOP THROUGH INTERACTION WITH ABDOMINAL SIGNALS.** **J.E. Cox & M.J. Smith*, Dept. Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.**

We sought to further specify the nature of the interaction between cholecystokinin (CCK) and feeding-contingent signals in decreasing feeding motivation. Our previous research revealed large decrements in runway performance if tests were preceded by the combination of 0.25 - 1.0 ug/kg CCK-8 and prefeeding of 30% sucrose (Cox, Behav. Brain Res., 21, 29, 1986). We have subsequently assessed the effectiveness of sham feeding (SF) and intragastric feeding (GF) for reproducing the capacity of normal prefeeding to interact with CCK-8.

Male Sprague-Dawley rats underwent surgery for gastric implantation of stainless steel cannulas for SF (N=7) and silastic catheters for GF (N=5). All test protocols included 21 h food deprivation, a prefeeding interval, an i.p. injection of 1 ug/kg CCK-8 (Sincalide, Squibb) or 0.9% saline 2 min later, and 6 runway trials for 0.3 ml of 30% sucrose beginning 10 min after the injection. For the GF group there were 4 prefeeding conditions: 9 ml of 30% sucrose consumed normally (F), gastric infusion of 9 ml of 30% sucrose (GF), infusion of 9 ml of 0.9% saline (GS), and no prefeeding (NF). Performance after CCK-8 was similar on NF (7% decrease compared to saline injections) and GS tests (5% decrease). Intragastric sucrose was, by contrast, as effective as normal prefeeding in interacting with CCK-8. The impairment produced by the drug was 39% on F tests and 46% on GF tests. For the SF group, there were 3 prefeeding conditions: normal prefeeding, of 30% sucrose (F), sham feeding (SF), and no prefeeding (NF). On NF tests CCK-8 produced a small decrease in running speed (9%), whereas in the F condition, rats ran 43% slower after CCK-8 than after 0.9% saline. SF failed to mimic normal prefeeding. CCK-8 produced very little reduction in running speed (2%) after sham feeding. These results suggest abdominal origin of the critical feeding-contingent signals; signals generated by intragastric sucrose were as effective as normal prefeeding, and signals from the oropharynx were without apparent effect. Subsequent experiments will more specifically examine the contribution of the stomach.

We should add the proviso that the above conclusions apply most clearly to tests using relatively small doses of CCK-8. In subsequent tests, we found evidence of synergism between SF and CCK-8 with larger doses. After SF, 2 and 4 ug/kg CCK-8 decreased running speed by 29% and 41%, respectively, though these doses produced little inhibition (5% and 12%) without prefeeding. Thus, increasing doses of CCK-8 appear to engage additional inhibitory mechanisms involving signals from the oropharynx.

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166.11 COMPARISON OF POSTPRANDIAL PLASMA LEVELS OF CCK WITH PLASMA LEVELS OF CCK ANALOGS THAT INHIBIT FOOD INTAKE IN DOGS. R.D. Reidelberger*, T.J. Kalogeris*, C.M. Turkelson*, and T.E. Solomon* (SPON: R.C. McClure). Depts. of Medicine and Physiology, University of Missouri Medical School and Truman V.A. Hospital, Columbia, MO 65201.

Cholecystokinin (CCK) is postulated to be a satiety hormone. While peripheral administration of large doses of CCK inhibits feeding, it is not known whether physiological doses produce satiety. We used specific, sensitive radioimmunoassays of CCK to compare postprandial blood levels of CCK with plasma levels of CCK analogs that inhibit food intake. Eight pure-bred beagles were adapted to ad libitum access to solid food for 18 h followed by 4 h food deprivation and a 1 h test session. Dogs received intravenous infusions of CCK8, cerulein, or Thr(28),Nle(31)-CCK9 (0, 25, 50, 100, 200, 400, and 800 pmol/kg-h, one dose per day) for 15 min prior to presentation of the test meal and during the subsequent 45 min feeding period. Each peptide produced a similar dose-dependent inhibition of food intake. The minimal effective dose for each analog was the same (200 pmol/kg-h). At this dose food intake was suppressed 36, 41, and 55 % by CCK8, Thr(28),Nle(31)-CCK9, and cerulein, respectively. In a second study the same dogs received scalar doses of CCK8 or Thr(28),Nle(31)-CCK9 (0, 25, 50, 100, 200, and 400 pmol/kg-h), or cerulein (0, 16.7, 50, and 150 pmol/kg-h), each dose for 30 min, and each peptide given on different days. Blood samples were collected at the end of each dose for determination of plasma peptide concentrations. Peptide levels were highly correlated with dose: correlation coefficients were 0.94, 0.95, and 0.87 for CCK8, Thr(28),Nle(31)-CCK9, and cerulein, respectively. Peptide levels produced by the minimal effective dose for inhibition of food intake were 67, 72, and 55 pM, respectively. In a third study, blood samples were collected before food presentation and at 15, 45, and 90 min after access to food, and plasma CCK concentrations were determined. CCK levels increased significantly from 2.7 $\pm$ 0.2 pM before food intake to a peak of 5.0 $\pm$ 0.7 pM at 45 min. These results do not support a role for CCK as a satiety hormone, since plasma levels of CCK analogs required to inhibit feeding in dogs were 10 to 14 times larger than physiological levels of plasma CCK produced by food intake.

166.12 SUPPRESSION OF SHAM FEEDING BY INTRAINTESTINAL OLEATE IS ATTENUATED BY A SPECIFIC CCK RECEPTOR ANTAGONIST. D Yox, H Stokesberry*, RC Ritter. Department of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843

Suppression of food intake following systemic administration of exogenous cholecystokinin (CCK) is well-documented. Recently, specific and potent CCK receptor antagonists have become available. Using these antagonists, several laboratories have attempted to assign a role in the termination of feeding to endogenous CCK. Nevertheless, attempts to increase the size of meals utilizing CCK antagonists have met with very limited success. It is likely, however, that CCK is involved in mediating only a subset of multiple postingestive signals involved in control of food intake. Consequently, the role of endogenous CCK in ingestive control may be more easily appreciated if its participation in the suppression of intake by individual gastrointestinal stimuli can be established. Therefore, we have examined the effect of the potent CCK antagonist, CR1409 (Rotal), on the suppression of feeding by intraintestinal sodium oleate infusions. The effect of CR1409 on suppression of sham feeding by intraintestinal oleate infusion (0.65 kcal/min) is shown in the table below.

INJECTION	INFUSATE	% SUPPRESSION[a]
saline	saline	---
saline	oleate	36.4 $\pm$ 4.9
CR1409 (300 ug/kg)	oleate	22.7 $\pm$ 3.8[b]
CR1409 (600 ug/kg)	oleate	5.5 $\pm$ 4.9[c]
CR1409 (300 ug/kg)	saline	-3.5 $\pm$ 2.1
CR1409 (600 ug/kg)	saline	0.5 $\pm$ 4.8

a $\frac{[(\text{saline \& saline}) - (\text{experimental treatment})]\ [100]}{[\text{saline \& saline}]}$

b, c Differs from saline/oleate, b=$p<0.05$, c=$p<0.001$

CR1409 (300 ug/kg) attenuated oleate-induced suppression of sham feeding while a dose of 600 ug/kg abolished the suppression entirely. Neither dose of the antagonist altered sham feeding in the absence of oleate. As a test of CR1409's specificity for attenuating suppression of intake by CCK, we examined the effect of CR1409 on feeding following systemic treatment with exogenous CCK-8 or bombesin. CR1409 failed to attenuate the suppression of intake induced by bombesin but completely abolished suppression of intake induced by 2 ug/kg of CCK-8. These results suggest that endogenous CCK may participate in the mediation of suppression of ingestion by intestinal chemical signals such as long chain fatty acids. Supported by NIH grant R01 NS20561.

167.1 MECHANISMS OF FORMATION OF THE RETINAL GANGLION CELL AND OPTIC FIBER LAYERS. M. Watanabe*, U. Rutishauser, and J. Silver (SPON: A.L. Acheson). Neuroscience Program, Department of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

The neural retina is an example of nervous tissue which begins as a simple neuroepithelium and differentiates into a multi-stratified structure with alternating cell body and neurite domains. In order to begin to understand the mechanism by which such layers form, the early development of the retinal ganglion cells was examined using EM immunohistochemical techniques. The row of ganglion cells with its associated fiber layer are the first two layers to form within the retinal anlage.

A newly characterized monoclonal antibody directed to a neuron-specific cytoskeletal component was used to specifically mark retinal ganglion cells that have left the mitotic cycle and have elaborated an axon. Wedges of E4-6 chicken neural retina were immunostained after permeabilization of the tissue in PLP fixative (McClean and Nakane, 1974, J. of Histochem and Cytochem. 29, 775) and prepared for observation by light and electron microscopy. In retina of 4-6 day chicken embryos, most of the retinal ganglion cells which were stained with the neuron-specific antibody had already migrated to the vitreal surface. However approximately 3% of the stained cells at E4 and E6 had cell bodies and processes in the middle or ventricular zone of the retinal neuroepithelium and of these several had radial processes connected to vitreal endfeet. Ultrastructural observations of the vitreal margin revealed that several endfeet were stained with the ganglion cell specific antibody. Furthermore, stained axons were observed protruding directly from stained and as well as unstained vesicle-filled vitreal attachments. Such primitive appearing ganglion cells that possess simultaneously axons and vitreal endfeet were described at the light microscope level in Golgi stained material by Morest (1970, Z. Anat. Entwickl.-Gesch. 131, 45-67). We have identified and examined at the ultrastructural level cells with the morphology which fit into his proposed sequence of ganglion cell differentiation. According to Morest, the axonogenic retinal ganglion cell is anatomically bipolar with both a ventricular and vitreal attachment (vitreal endfoot). It first releases only the ventricular attachment, generates its axon directly from the vitreal endfoot, translocates its cell body to the vitreal position while maintaining the vitreal attachment and finally, without ever freely migrating, eliminates the vitreal endfoot once translocation is complete. Our results provide evidence that this is a viable mechanism for the formation of the first 2 layers in the retina of the chick embryo.

167.2 REMODELLING OF CAT RETINAL GANGLION CELLS DURING FETAL AND POSTNATAL DEVELOPMENT. A.S. Ramoa, G. Campbell* and C.J. Shatz. Dept. Neurobiology, Stanford U. Sch. of Med., Stanford, CA 94305.

The retina of the adult cat contains a variety of morphologically distinct ganglion cell types. Among these, alpha cells have the largest somata and long, sparsely branched dendrites while beta cells have medium sized somata and very small extensively branched dendritic trees. Here we have examined when the adult cell classes first can be recognized in development and whether the acquisition of adult form is simply the result of progressive growth or whether morphological remodelling is also involved.

Retinae were dissected from cats aged between embryonic day 35 (E35; gestation is 65 days) and adult and maintained alive in a tissue-slice chamber. To distinguish retinal ganglion cells from other cell types early in development, they were identified by the retrograde transport of rhodamine labeled microspheres (Katz et al, Nature 310,498,1984) injected 48 hours earlier into central retinal targets (superior colliculus and lateral geniculate nucleus). The fine morphology of 283 ganglion cells was then revealed by intracellular filling with Lucifer Yellow (LY). Between E35 and E45 (gestation=65 days), LY filled ganglion cells are morphogically immature, with a few simple dendrites often aligned parallel to the trajectory taken by axons en route to the optic disc. From E52 onwards a much more elaborate pattern of dendritic branching is present. At this age some of the LY-filled cells resemble the alpha and beta classes of the adult based on comparisons of the soma size and dendritic spread of cells located at a similar eccentricity. By E57 cells resembling the gamma class could also be recognized. In other respects, however, the fetal and neonatal ganglion cells display several morphological features not present in the adult including 1) an enormous number of dendritic and somatic spines, 2) more highly branched dendrites, 3) short sidebranches along the intraretinal course of axons, and 4) axons that (for about 10% of the cells) bifurcate, giving rise to one or more collaterals. Quantification of these transient morphological features indicates that they are all especially evident between E57 and P7, mostly gone by P31 and not found in the adult cat.

These results show that some retinal ganglion cells can be assigned to classes analogous to those present in the adult as early as two weeks before birth. However, the transient expression of excessive dendritic, axonal and somatic protrusions implies that a great deal of remodelling occurs in the retina before the final adult morphology is established. Thus basic ganglion cell form may be laid down even before photoreceptor outer segments are present, but final maturation may require visual experience. (Supported by NSF grant BNS 8616798 and the March of Dimes.)

167.3 DO AMACRINE CELLS EXTEND, THEN RETRACT A CENTRALLY-PROJECTING AXON? G.Campbell*, A.S.Ramoa and C.J.Shatz (SPON: M.W. Siegel). Dept. of Neurobiology, Stanford Univ. Sch. of Medicine, CA 94305.

We have investigated the possibility that amacrine cells of the mammalian retina derive from ganglion cell precursors that lose a centrally-projecting axon (Hinds and Hinds, J.Comp.Neurol. 213, 1, 1983). We examined whether, during fetal development in the cat, amacrine cells project an axon beyond the optic chiasm.

To test whether amacrine cells transiently project to central targets, large injections of rhodamine labeled microspheres were made in vivo into the optic tract, diencephalon and midbrain in order to retrogradely label any retinal cells with centrally-projecting axons at the time of injection. In one set of experiments, microspheres were injected at embryonic day 56 (E56; 9 days before birth) or older (up to postnatal day 32) and 24-48 hrs later the retinae were removed and placed as living wholemounts in vitro. Somata containing retrogradely transported microspheres were intracellularly injected with Lucifer Yellow (LY) to reveal their morphology. Almost every cell double-labeled with LY and rhodamine microspheres could be morphologically identified as a ganglion cell and had an axon in the optic fiber layer. Moreover, no double-labeled cells, even those with the smallest somata, resembled adult amacrine cell types. Amacrine cells could only be injected when the microelectrode was advanced into cells devoid of microspheres, in which case by E62 many narrow-, small-, medium- and wide-field types of cat amacrine cell (Kolb, Vis. Res. 21: 1081, 1981) could be identified and distinguished from ganglion cells. Moreover at this age and thereafter, none of these cell types had axons in the optic fiber layer. Thus amacrine cell types resembling those in the adult may be present as early as E62, but at this age, they do not send an axon into the optic tract or beyond.

In a second set of experiments, the microspheres were injected at E35-E38 but the retinae from injected animals were not removed for the LY injections in vitro until E62 or older in order to examine whether amacrine cells have axons in the optic tract early that are retracted by E56. E35-38 was selected because many retinofugal axons have just arrived at their central targets and very few growth cones are left in the optic nerve. Again no double-labeled cells resembling amacrine cells were found at E62 or older, while 227/237 double-labeled cells were identified as ganglion cells.

These results firstly show that many amacrine cells acquire their basic form prenatally, at least by E62. Secondly, while it is possible that during development amacrine cells extend an axon for short distances, it is unlikely that amacrine cells derive from ganglion cell precursors that shed a centrally-projecting axon. Supported by NSF grant BNS 8616798, and the March of Dimes.

167.4 CLUSTER ANALYSIS OF CAT RETINAL GANGLION CELLS DURING POSTNATAL DEVELOPMENT. J.S. Tootle and M.J. Friedlander Neurobiology Research Center and Dept. of Physiology & Biophysics, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Despite the enormous volume of data supporting the division of adult cat retinal ganglion cells (RGCs) into the three (X-, Y- and W-) functional classes, the recognition of functional cell classes in the neonate remains controversial. In order to identify the appropriate number and nature of the functional groups present during development, we have applied cluster analysis to electrophysiological data acquired from retinal ganglion cells. Action potentials in response to visual and electrical stimulation were recorded from single retinal ganglion cell axons within the optic tract just beneath the lateral geniculate nucleus. Recordings were made from RGC axons at 3 postnatal ages (4½ weeks n=40; 6½ weeks n=33; adult n=36). The spatial resolution, temporal resolution, latency of response to electrical stimulation of the optic chiasm (OX) and degree of linearity of spatial summation of each cell were quantified. The scores on each variable were standardized to a mean of zero and unit variance and were used with Ward's hiearchical clustering method (SAS, Inc.) to form from 1 to 5 clusters at each age. R-Squared and pseudo-F statistics were plotted as a function of the number of clusters formed and indicated that two groups (clusters) were present at all ages tested. The cells in one cluster had on average low spatial and high temporal resolution, short response latencies to OX stimulation and nonlinear spatial summation. Cells in the other cluster had on average high spatial and low temporal resolution, long response latencies to OX stimulation and linear spatial summation. In the adult the two clusters corresponded closely to X- and Y- cells (cluster 1: 13/14 X-cells; cluster 2: 15/18 Y-cells). The direction of differences on the clustering variables were the same for the kitten and adult, but many kitten cells did not reach adult criteria for X- or Y- classification on some variables. The results demonstrate that 1) two groups of cells, which correspond closely to X- and Y- cells, are identified by the application of cluster analysis to data acquired from retinal ganglion cell axons in adult cats and 2) two functional classes of retinal ganglion cells which show the same relative differences on the clustering variables as adult X- and Y- cells can be recognized in the neonatal kittens as young as 4 to 5 postnatal weeks.

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167.5 CHANGES IN THE BASIS OF THE RETINAL SPECIALIZATION AS THE GOLDFISH RETINA MATURES. A.D. Springer and A.S. Mednick. Dept. of Anatomy, New York Medical College, Valhalla, NY 10595.

The goldfish has a retinal specialization (RS) that is situated along the temporal boundary between the dorsal and ventral retina. This RS was observed in retinae that varied in size from 10-50 mm^2. Retinal ganglion cell density was at least 500% higher in the RS as compared to the surrounding retina. Similarly, the inner nuclear and cone mosaic layers of the RS also had a high density of cells as compared to the surrounding retina.

The ganglion cell layer contains cells that do not backfill when cobaltous-lysine is applied to the cut optic nerve. Many of these unfilled, hematoxylin stained, cells appear to be morphologically similar to the amacrine cells of the inner nuclear layer. Ganglion cells range in size from 10-310 um^2, and most are 40-50 um^2. Presumptive displaced amacrine cells are 10-60 um^2 in size, and most are 30 um^2. Since there is appreciable overlap in the soma area distributions for ganglion and displaced amacrine cells, many cells would be misclassified unless the various cell types were distinguished by backfilling the ganglion cells with cobaltous-lysine.

In small retinae, the percentage of neuronal cells in the ganglion cell layer that are ganglion cells (95%) or displaced amacrine cells is constant across the retina. However, neuronal cell density is 25% higher in the RS. In large retinae, the RS, where ganglion cells are numerous, has a low density of displaced amacrine cells. The density of displaced amacrine cells is greater in the region that surrounds the RS. Adding the displaced amacrine cells to the ganglion cells results in a homogeneous distribution of cells across the ganglion cell layer of large retinae. Therefore, unlike the small retinae, the RS in large retinae contains a disproportionately higher percentage of ganglion cells relative to the surrounding retina. Furthermore, although ganglion cell density diminishes as retinal area increases, displaced amacrine cell density increases as retinal area increases. The data suggest that several mechanisms (e.g., differential cell death, differential cell transformation) may serve to maintain the RS as the retina grows. Supported by NEI 03552.

167.6 A POSITIONAL MARKER FOR THE EMBRYONIC MOUSE RETINA. S. A. Rabacchi* and U. C. Drager. Harvard Medical School, Dept. Neurobiology, Boston MA 02115.

We are trying to identify molecular mechanisms involved in the establishment of topographical maps in the visual system of the mammalian embryo. As work on cold-blooded vertebrates indicates that hypothetical specification events for the topographical coordinates of the retina take place just before the first retinal ganglion cells start to differentiate, we generated monoclonal antibodies to the corresponding stage of the mouse embryo at embryonic day 11 (E11). One of our monoclonal antibodies, "Jula", labels the early embryonic retina in a position- but not maturation-dependent fashion. At late E10 or early E11, when antibodies to differentiation antigens--neurofilaments and HNK-1 epitope--do not yet label the eye, the Jula antigen appears in the dorsal retina opposite to the optic fissure; it first covers about one third of the extent of the retina, where it appears to distribute diffusely in the cytoplasm of all cells. As maturational processes, starting at E11.5, advance from the center to the periphery, the Jula antigen becomes gradually pushed toward the dorsal edge; as soon as cells turn positive for neurofilaments or HNK-1, they lose the Jula antigen. By E16 the Jula antigen has practically disappeared from the neural retina, but occupies only a small sector on the dorsal rim of the eye cup which sprouts to form the iris.

The Jula antigen is not restricted to the retina, but it appears transiently also in central locations: at E13 in the optic nerve, at E14 in the diencephalon in the region of the lateral geniculate body and in the internal capsule of the forebrain.

Two other antigens with initially similar distribution, but which persist in differentiated regions of the embryonic eye, have been identified with monoclonal antibodies: "TOP" (Trisler et al.) detects a cell-surface glycoprotein, and "JONES" (Constantine-Paton et al.) a ganglioside in the dorsal retina. To test whether the Jula antigen corresponds to one of these, we are trying to determine its biochemical nature. So far we have found that Jula immunoreactivity is destroyed by SDS, high temperature, low pH, alcohols, pronase and chymotrypsin; it is partially affected by periodate; and it is resistant to lipid solvents (Triton, ether, chloroform, xylene, acetone) and a large number of enzymes including neuraminidase.

These preliminary biochemical observations and the apparent cytoplasmic labeling pattern suggest that the Jula antigen may be a novel cytoplasmic protein, and its topographical distribution suggests that it may be relevant for the setting-up of the dorso-ventral axis in the embryonic eye. Supported by EY 01938.

167.7 CLASS SPECIFIC CELL DEATH DURING DEVELOPMENT SHAPES THE DISTRIBUTION AND PATTERN OF CENTRAL PROJECTION OF CAT RETINAL GANGLION CELLS. A.G. Leventhal, J.D. Schall, S.J. Ault, J.M. Provis, D.J. Vitek. Anatomy Dept., University of Utah School of Medicine, Salt Lake City, Ut. 84132; Dept. of Brain and Cognitive Science, E25-634, MIT, Cambridge, MA 02139; and Dept. of Clinical Ophthalmology, Sydney Eye Hospital, Sydney, Australia 2011.

The development of the nasotemporal division in cat retina was studied. We find that in the normally pigmented neonatal cat many of the ganglion cells in temporal retina project to the contralateral dorsal lateral geniculate nucleus (LGNd). Since very few cells in temporal retina project contralaterally to the LGNd in the adult, most of these cells must be eliminated during development.

Experimental interruption of one optic tract in the neonate results in the retrograde degeneration of the ipsilaterally projecting ganglion cells in the temporal retina ipsilateral to the lesion. Consequent to the loss of the ipsilaterally projecting cells in this hemiretina, many of the ganglion cells projecting to the intact contralateral LGNd survive and grow to be abnormally large (Ault et al, Soc. Neurosci. Abstr., 11:15, 1985). All classes of retinal ganglion cells comprise this "stabilized exuberant" projection. Contralaterally projecting alpha cells extend up to four mm into temporal retina; contralaterally projecting beta cells extend up to two mm into temporal retina and contralaterally projecting epsilon and other ganglion cell types extend to the temporal margin. Also, unlike in the normal cat in which very few of the small ganglion cells in temporal retina project to the LGNd, in optic tract lesioned cats many of the smallest ganglion cells in the temporal retina ipsilateral to the lesion project contralaterally to the intact LGNd.

A quantitative analysis of ganglion cell density in the temporal retina contralateral to the lesion (ipsilateral to the intact hemisphere) indicates that there is a reduction in the population of ipsilaterally projecting ganglion cells which is complementary to the abnormally large number of contralaterally projecting cells which survive in the temporal retina ipsilateral to the lesion. A loss of alpha cells is evident as far as 4 millimeters from the nasotemporal division; a loss of beta cells is evident within 2 millimeters of the nasotemporal division. As a result of this complementary loss, the intact LGNd receives inputs from normal numbers of alpha and beta cells. These results suggest that class specific cell death during development helps to shape to the retinal distribution and pattern of central projections of the different classes of ganglion cells in the mature cat retina. (Supported by EY04951 to AGL, EY05863 to SJA and EY05767 to DJV).

167.8 EVIDENCE FOR EARLY SPECIFICITY OF THE RETINOGENICULATE X CELL PATHWAY. M.Sur, A.W.Roe* and P.E.Garraghty. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

The development of the retinogeniculate pathway in cats and ferrets involves specific connections between retinal X and Y afferents and their target geniculate neurons within eye-specific laminae. How early in development is the specificity of retinal X and Y axons for their target cells laid down?

In cats, retinogeniculate axons have segregated into eye-specific laminae by birth (E64); ablating area 17 (the target of A-laminae X cells) as well as area 18 at birth causes degeneration and loss of LGN X cells (McCall et al., '86) and transneuronal loss of retinal X cells (Tong et al., '82). In ferrets, the temporal order of retinogeniculate development matches almost exactly that in cats (Linden et al., '81; Shatz '83), yet ferrets are born much earlier (E42) and axons from the two eyes overlap extensively in the LGN at birth. In the present experiments, we ablated areas 17, 18 and parts of 19 in newborn ferrets, and asked whether this would still cause a reduction in the X cell retinogeniculate pathway.

In ablated animals reared to adulthood, the LGN was only about half its normal size but eye-specific laminae were well-formed. Physiologically, while only 20% of cells in the normal LGN can be classified as Y cells (Esguerra et al., '86), we recorded almost exclusively Y cells in the LGN of ablated animals. Receptive field properties of these cells were similar to normal Y cells. Though there was some clustering of On and Off cells, On/Off sublaminae seen in the A-laminae of normal animals did not form clearly. This is consistent with a reduction in the retinogeniculate X cell pathway; in normal ferrets, retinogeniculate X arbors closely follow the On/Off sublaminar segregation while Y axons do not (Roe et al., '86). Finally, retrograde labeling of retinal ganglion cells following small injections into the surviving LGN and comparison with normal animals indicated a substantial reduction in backfilled medium-sized (200-350 um2) ganglion cells.

While ablation of cortex can have other effects, these data suggest that early removal of target neurons, even before retinogeniculate axons from either eye have segregated or most axons have elaborated a recognizable terminal arbor (eg., Sretavan and Shatz '86), still causes a loss of the retinogeniculate X cell pathway. Therefore, at least the X retinal axons must have a high degree of specificity for their LGN targets by E42.

Finally, contrary to the cat LGN (Murphy and Kalil '79), surviving cells in the A-laminae were smaller than normal. These cells are contacted by Y axons; the smaller size of these cells is consistent with a role for geniculocortical factors in controlling cell structure independent of the retinogeniculate pathway.

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167.9 LAMINAR SEGREGATION OF BINOCULAR AFFERENTS TO THE RAT TECTUM INDUCED BY NEONATAL LESIONS OF THE OPTIC TRACT. C.A. Serfaty* & R. Linden, Instituto de Biofísica da UFRJ, Rio de Janeiro, Brazil.

The normal retinotectal projections of rodents show no segregation of crossed and uncrossed components, although this can occur after induction of recrossed projections in hamsters (Mooney et al., Dev. Brain Res., 19: 297, 1985). We report a form of aberrant laminar segregation in the superior colliculus (SC) of rats following neonatal lesions of the opposite optic tract. Tectopretectal lesions (Linden & Perry, Neurosci., 7: 2813, 1982) were made on the left side of the brain of newborn rats, and the retinal projections to the right SC were traced with HRP or ^{3}H-proline injected in the eye. In operated rats the uncrossed retinotectal projection formed an aberrant subpial lamina of terminal labeling, in addition to the puffs of termination normally placed at the lower edge of the superficial gray layer. The subpial lamina varied in size but was consistently found at the rostral 2/3 of the SC, especially in its lateral half. The lamina tended to form at the expense of the deep puffs, but the total volume of uncrossed projection was greatly increased. In 6 out of 9 rats tested the crossed retinotectal projection retracted from the surface of the SC, forming holes similarly located as but smaller than the subpial uncrossed lamina. Double labeling showed that the crossed and uncrossed projections segregated only where the density of the ipsilateral lamina was the highest. The uncrossed retinotectal projection normally develops along a rostrolateral to caudomedial gradient, as shown by the progressive condensation of the deep terminal zones. In operated rats the condensation of the subpial uncrossed lamina was apparent at 3 days after birth, and developed along a gradient similar to normal rats, cotemporal with natural neuronal death among retinal ganglion cells. The results indicate that segregation of binocular afferents to the rodent tectum is critically dependent on the relative densities of projection from each eye. The data are also consistent with the interpretation that the anomalous retinotectal projection induced by neonatal optic tract damage in rats is due to a change in the course of developmental neuronal death in the retina.

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167.10 Prenatal Development of Retinogeniculate Axon Arbors in the Presence of Tetrodotoxin - D.W. Sretavan[1], C.J. Shatz[1] & M.P. Stryker[2]. [1]Dept. of Neurobiology, Stanford Univ. Sch. Med., CA. 94305 & [2]Dept. of Physiology, Univ. Calif. San Francisco, CA. 94143

During the cat's prenatal development, retinal ganglion cell (RGC) axons from both eyes are initially intermixed within the lateral geniculate nucleus (LGN) and are morphologically simple, consisting of one main axon with a few side branches. Between embryonic days 43 and 58 (E43-E58) (birth=E65), axons from the two eyes sort out from each other by losing side-branches in parts of the nucleus destined to belong to axons from the other eye while simultaneously forming terminal arbors in parts of the LGN appropriate to eye of origin. By E58, axons are like those in the adult consisting of a smooth main trunk and a narrow cylindrical arbors strictly localized to an eye-specific layer (Sretavan & Shatz, J. Neurosci. 6:234, 1986).

To examine whether action potential activity is involved in shaping RGC axon terminal arbors during this period of fetal development, we have used tetrodotoxin (TTX), a Na^{+} channel blocker that eliminates action potentials and is known to prevent the formation of the eye-specific layers (Shatz & Stryker, Soc. Neurosci. Abstr. 12:589,1986). TTX (0.3mM) in citrate buffer (0.32 mM) was infused intracranially via osmotic minipumps starting at E42. (Infusion of citrate vehicle alone does not affect formation of the layers.) At E57 or E58, fetuses were delivered and RGC arbors were filled with horseradish peroxidase using an in vitro method. Preliminary saxitoxin competition binding assays of CSF samples at E57/58 showed 160-720 nM of TTX, within the range of the level needed to block action potentials in neonatal rat optic nerves (Foster et.al. Dev. Br. Res.3:371, 1982).

RGC axon arbors (n=27) in fetuses treated with TTX from E42-58 were highly abnormal, branching widely along their entire traverse across the LGN. TTX axons at E58 formed broader terminal arbors (TTX:253um ± 88um, n=14; Normal:90um ± 33um, n=28) and in addition elaborated more total axon length within the LGN (TTX:3910um ± 1116um, n=14; Normal:2520um ± 810um, n=28). Eye injections of ^{3}H-leucine in these same fetuses showed that the pattern of eye-specific layers, normally present at this age, was missing.

Our results show that in the presence of TTX, retinal axons fail to elaborate the restricted axon arbors that form the basis of the eye-specific LGN layers. In addition TTX-treated axons are not slowed in their axonal growth nor do they resemble normal axons arrested at earlier stages of arbor development. These observations imply that during fetal development, activity dependent mechanisms are likely to be important in directing the formation of axon terminal arbors that are both of the appropriate morphology and in the correct location within CNS targets.
Support: NSF BNS 8616798 & March of Dimes(CJS); NIH EY 02874 (MPS).

167.11 **LAMINATION OF GLIAL CELL MARKERS PRECEDES THE FORMATION OF NEURONAL LAMINATION IN THE LATERAL GENICULATE NUCLEUS (LGN).** **James B. Hutchins and Vivien A. Casagrande**, Dept. of Cell Biology, Vanderbilt Univ. School of Medicine, Nashville, TN 37232.

The development of cell layers in the lateral geniculate nucleus (LGN) occurs after LGN cells are born and have migrated from the third ventricle to the lateral edge of the thalamus. At present, it is unclear what mechanisms are involved in producing LGN layers from an apparently homogeneous mass of cells. We have been examining this issue using the tree shrew (*Tupaia belangeri*) as a model system. In this species, retinal afferents segregate into layers prior to birth (at postnatal day zero, or P0) within the cytoarchitecturally-homogeneous nucleus. Six cell layers gradually emerge during the first postnatal week. Several investigators have proposed that glial cells play a role in trophic and/or mechanical guidance of neurons to their final locations. We have used antisera against glial fibrillary acidic protein (GFAP) and vimentin (Vim), two intermediate filament proteins known to be expressed in glia during different times in development. Both antigens were localized using either peroxidase/antiperoxidase or avidin/biotin-complex immunocytochemical methods. At P0, prior to the formation of cell layers, there is a laminar pattern of GFAP+ astrocyte-like cells and a shadow-like staining of very fine processes. The GFAP+ LGN laminar pattern at P0 resembles the retinal afferent lamination pattern more closely than it does the cell layer pattern that will form later. At P0, Vim+ radial glia are seen in the midbrain, thalamus and cortex. Radial glia still traverse the dorsal-most segment of the LGN; they are absent from the main body of the LGN where astrocyte-like cells prevail. GFAP+ and Vim+ fibers are also seen within the optic tract and radiations at P0. During the period of LGN cell layer formation, a GFAP+ laminar pattern is still apparent. However, by P9, it has come to more closely resemble the cellular pattern of lamination. Faint bands of Vim+ LGN lamina are also apparent during the first postnatal week. At the end of this period, Vim+ radial glia are no longer apparent in any part of the LGN and have begun to disappear elsewhere. In the adult, GFAP+ and Vim+ LGN lamination is no longer evident. Fine, perivascular GFAP+ processes, and evenly-distributed Vim+ cells and fibers, are seen in the LGN. Since glial-like elements are laminated within the LGN early in development, they are potentially in a position to guide the process of cell layer formation. However, it remains to be demonstrated whether the neuronal cellular lamination is dependent upon glial lamination, and whether glial lamination follows or precedes the lamination of retinal terminals.

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167.12 A GOLGI STUDY OF DENDRITIC DEVELOPMENT IN THE DORSAL LATERAL GENICULATE NUCLEUS (dLGN) OF FERRETS. J. Keith Sutton, Darrell A. Agee*, and Judy K. Brunso-Bechtold. Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

The dLGN of ferrets, Mustela putorious, is anatomically similar to that in cats, having 2 distinct layers (A and A1) and 4 narrow, unseparated layers (collectively called C layers). Prior studies have documented several morphological classes of neurons within the adult dLGN of various species. We have employed the Riley modification of the Golgi-Kopsch technique (Riley, Brain Res. Bull., 4: 127, 1979) to demonstrate the dendritic arbors of neurons within the dLGN of ferrets aged P0 (first postnatal day) to adults. Our main focus has been to study the postnatal maturation of dendritic arbors in an effort to begin an examination of possible correlations between changes in these dendrites and other developmental events. In adult ferrets, cells of the dLGN were similar to those described previously in cats (Guillery, J. Comp. Neurol., 128: 21, 1966). Large multipolar neurons (35-45 µm somata) with highly-branched dendrites arranged in a spherical array were seen as well as medium multipolar neurons (20-30 µm somata) with tufts of dendritic appendages clustered at regular intervals. Both of these cell classes were found throughout the dLGN except for the outer C layers. Some medium neurons (20-30 µm somata) with dendrites oriented perpendicular to the axes of the cell layers were found in layers A and A1. Within the C layers, other neurons (20-25 µm somata) were found oriented parallel to the axes of the cell layers. In immature animals, all neurons examined were from layers A or A1 due to increased overlap of filled cells and increased artifact in the C layers. At P0, neurons were small (10-15 µm somata) and had few dendrites with occasional branches. At subsequent ages, dendrites increased in number and in extent of branching. At P0, some neurons appeared more immature with thick dendrites and many appendages while other neurons appeared more mature with thin dendrites and short, less frequent appendages. Long (4-5 µm), thin-stalked appendages were also seen arising from neuronal somata between P0 and P21. Somatal appendages were not seen at P56 although, at that age, dendritic appendages were still more apparent than in adults. Cell classes could first be distinguished at P21, when dendritic arbors first began to resemble those of adults and when increases in size (15-25 µm somata) were apparent. (Supported by NIH grant EY05028).

167.13 POSTNATAL DEVELOPMENT OF GABAERGIC SYNAPSES IN THE LATERAL GENICULATE NUCLEUS OF NORMAL AND DEPRIVED CATS: AN ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. R.E. Kalil and A.N. Lies*. Dept. of Ophthalmology and Neurosciences Training Program. University of Wisconsin, Madison, WI 53706

There are several reasons to believe that cells in the lateral geniculate nucleus (LGN) of the cat that contain gamma-aminobutyric acid (GABA) are probable interneurons. Specifically, GABA containing cells are uniformly small in size, they cannot be labeled retrogradely from visual cortex with horseradish peroxidase, and they have synaptic endings that frequently contain pleomorphic vesicles. Light microscopic evidence suggests that the development of GABA-positive cells in the LGN is completed during the third postnatal month, but no information is available regarding the maturation of terminals in the LGN that may release GABA as a neurotransmitter. We have therefore combined electron microscopy with immunocytochemistry to study the development of GABAergic synapses directly.

Antibodies made in rabbits against GABA conjugated to bovine serum albumin were affinity purified, and their specificity confirmed by immunoblotting. Following incubation in the primary antibodies, immunoreactive terminals in the LGN were stained with the biotin-avidin method. Normally reared cats that ranged in age from newborn to adult were examined, as were animals that had been raised to adulthood with monocular or binocular eyelid suture.

In thin sections from newborn animals, GABA-positive cell bodies and dendrites can be recognized easily, but very few immunoreactive profiles can be found that also contain synaptic vesicles. Immunostained terminals increase in number slightly during the first three postnatal weeks, but they are not prominent in the LGN until the fourth week. In the second postnatal month GABAergic terminals enlarge in size and occur frequently in complex synaptic zones, in association with retinogeniculate boutons and LGN cell dendrites. This configuration is characteristic of the mature pattern. GABA containing terminals in adult cats raised with monocular or binocular visual deprivation do not appear qualitatively different from those in normal animals of the same age.

These results indicate that GABAergic synaptic profiles in the LGN, which probably derive from interneurons and presumably are inhibitory, develop chiefly between the third and eighth postnatal weeks. During the first month, these synaptic terminals are not common in the LGN. This suggests that many of the GABA-positive "puncta" seen with the light microscope at early ages represent sections through immunostained dendrites and axons, and therefore do not reflect synaptic endings reliably.

167.14 DIFFERENTIATION BETWEEN DISUSE AND BINOCULAR COMPETITION EFFECTS IN VISUAL CORTEX CELLS OF MONOCULARLY DEPRIVED SPLIT BRAIN KITTENS. U.Yinon* and M.Chen (SPON: Z.Elazar). Physiol. Lab., Goldschleger Eye Inst., Tel-Aviv Univ. Faculty of Medicine, Sheba Med. Ctr., Tel-Hashomer, 52621, Israel.

To differentiate between the effect of disuse developmentally induced in visual cortex cells by deprivation and the effect of the resulting interocular competition , visual split brain was surgically produced in kittens concurrently with monocular deprivation. Unit recording was made in cortical areas 17,18 and their boundary, >4 months after the surgical procedures. In all cats in which split brain was induced, a full separation was found between the two hemispheres as reflected in the absolute ipsilateral eye dominance. In the monocularly deprived split brain cats the relation between the visually responsive cells in the visually experienced hemisphere (ipsilaterally to the nondeprived eye) and the responsive cells in the inexperienced hemisphere (ipsilaterally to the deprived eye) was 2:1. In comparison, a relation of nearly 3:1 was found between cells driven by the nondeprived and the deprived eye in the monocularly deprived control cats. It has been concluded that visual disuse resulting from monocular deprivation has an effect on visual cortex cells independently of binocular competition. The binocular competition in our experimental condition enhances the effect of disuse on cortical cells by a factor of 1.5; this competition-factor however, is age dependent.

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DEVELOPMENT OF INVERTEBRATES I

168.1 CHANGES IN TRANSMITTER PHENOTYPE DURING DEVELOPMENT IN IDENTIFIABLE NEURONS IN THE AMERICAN LOBSTER. *Barbara S. Beltz.* Biology Dept., Wellesley College, Wellesley, MA 02181, and *Edward A. Kravitz* (SPON: R. Tuttle). Neurobiology Dept., Harvard Med. Sch., Boston, MA 02115.

Within a 2-4 week period, newly hatched larval lobsters undergo a dramatic anatomical and behavioral metamorphosis. We want to identify neuronal changes during this period, in order to understand the cellular bases for the development of behavior. Specifically, we have been examining the timing of appearance and disappearance of amines and peptides in the nervous system using immunocytochemical methods.

We see distinct patterns in the timing of transmitter acquisition for a variety of amines and peptides. The earliest embryos studied (at 50% development) already show immunoreactivity for serotonin. The stained neurons and processes are similar in most ways to those seen in adults. We believe that the function of one unpaired medial neuron in the first abdominal ganglion may change during development: it reaches full adult size and stains densely for serotonin during embryonic life, while staining only faintly in adults. In contrast to early staining for serotonin, immunocytochemical studies of proctolin show only a few hundred cell bodies staining in embryos, while the full complement of adult neurons (1500) does not stain until the fifth larval stage. The acquisition of proctolin immunoreactivity generally follows an anterior - posterior gradient, with the largest increase in numbers of cells staining at the third and fourth larval stages. Of particular interest is a large unpaired anterior, medial cell in the second abdominal ganglion that stains strongly for proctolin during embryonic and early larval life, but loses the peptide phenotype by the fifth larval stage. Immunostaining for FMRFamide-like peptides during development shows a pattern distinct from the other substances examined. FMRFamide immunoreactivity first appears in mid-embryonic life and the development of staining does *not* follow an anterior-posterior gradient. The first cells to stain are paired medial abdominal neurons and anterior subesophageal cells, while thoracic cells acquire the FMRFamide phenotype only during late larval stages. Many cells that stain prominently in embryonic and larval life stain weakly, or show no FMRFamide immunoreactivity in juvenile animals. Future studies will focus on (1) anatomical and physiological characterization of the neurons showing transmitter plasticity during development, to see what properties of these cells are changing, and (2) developmental immunocytochemical studies of additional peptides and amines, to further explore the possible guiding principles in the expression of transmitter phenotype in neurons. (Supported by NIMH and NINCDS.)

168.2 **DISTRIBUTION OF PEPTIDE NEUROMUSCULAR JUNCTIONS IN WILD-TYPE AND HOMEOTIC MUTANT *DROSOPHILA*** . M.E. Halpern*, M.-D. S. Anderson, J. Johansen and H. Keshishian. Dept. of Biology, Yale University, New Haven, CT 06511.

On the basis of transmitter phenotypes specific neuronal populations in the *Drosophila* CNS can be identified. Similarly, patterns of peripheral synaptic connections made by motoneurons expressing a given transmitter can be described. We have recovered the peptide neurotransmitter proctolin from the CNS, hindgut and segmental bodywall musculature of *Drosophila melanogaster* third instar larvae by reverse phase HPLC. The peptide is expressed in segmentally iterated clusters of dorsal and ventral neurons in the larval ganglion as shown by immunocytochemistry (M.E. Halpern *et al. Soc. Neurosci. Abstr.*, 1986). We propose that some of these cells are motoneurons that project to the bodywall musculature, which accounts for the high level of proctolin associated with it. The larval segmental musculature consists of internal, external and superficial fibers arranged in a highly stereotypic pattern of from 7 to 30 fibers per hemisegment. Since each muscle fiber is uniquely identifiable, this is an ideal preparation for studying the development and organization of specific neuromuscular junctions.

Immunocytochemistry of whole-mount larval bodywalls with a proctolin antiserum reveals that the peptide is present in nerve terminals on a subset of the oblique and longitudinal muscle fibers (muscles 4, 12, 13 and 19). Proctolin innervation is found on these fibers in the third abdominal segment and all segments posterior to it. The stained endings are less than 0.5 um in diameter and contain regularly spaced varicosities ranging between 1 and 3 um in size. Proctolinergic endings rarely extend the full length of a muscle fiber, in contrast to the glutamate and aspartate immunoreactive nerve terminals described by J. Johansen *et al.*, this volume. Experiments are in progress to discover whether other transmitters are co-localized with the proctolin endings.

Since the peptide innervation is arranged in a muscle fiber and segment-specific pattern, it can be examined in *Drosophila* homeotic mutants where segmental identities are altered. We began the analysis using third instar larvae bearing viable mutations at the Bithorax Complex (BX-C). The BX-C is responsible for specifying thoracic and abdominal segmental identity in ectodermal and mesodermal derivatives (E.B. Lewis, *Nature*, 1978). Haplo-insufficient BX-C gene function (*i.e.* a wild-type/deficiency larvae) results in the regular appearance of proctolinergic endings on muscles of A2, one segment anterior to the wild-type innervation. The novel endings in the second abdominal segment are found on the expected muscle homologs, indicating that overall fiber specific patterns are retained. However, there is also evidence for the innervation of muscle fibers that do not usually receive proctolinergic input in wild-type larvae. Using appropriate genotypes, we can rearrange patterns of neuromuscular endings as defined by transmitter phenotype. We plan to determine whether these changes are due to novel axonal projections or switches in transmitter expression.

We thank C. Bishop and M. O'Shea for the proctolin antisera.

168.3 FATE OF MOTOR AXONS LACKING TARGET MUSCLES IN THE DROSOPHILA TS MUTANT SHIBIRE. M.R. Hummon and W.J. Costello, Dept. Zool. & Biomed. Sci./Col. Osteo. Med., Ohio Univ., Athens, OH 45701.

Insect flight muscle requires innervation for survival and function; however, insect motor axons may survive in the absence of targets. In an adult flightless grasshopper, certain muscle fibers are reduced or absent, but motor axons from earlier instars persist and maintain presynaptic terminals (Arbas & Tolbert, 1986). In the Drosophila mutant sr/df(3), certain target muscles are eliminated, and motor axons form neuromas with numerous presynaptic terminals (hemisynapses) (Costello & Wyman, 1986). We are investigating the development of the giant fiber pathway in the Drosophila mutant shi, in which we can generate adults with some but not all flight muscle fibers absent. shi flies have largely normal development, morphology and behavior at 22C, but continuous exposure at 30C is lethal. A 6h heat pulse (HP) during pupal development results in adults with predictable, time-dependent anomalies in adult morphology and function.

In wild-type and non-HP shi flies, DLM (wing depressor) has 6 fibers, each singly innervated. The 5 motor axons exit the thoracic ganglion in a peripheral nerve (PDMN). In shi exposed to 6h HP starting at 30h of pupal development at 22C (at 25C ≅ 20h; eclosion at 96h), adult DLM fiber number is only 1 or 2, not 6 (3 flies, 6 sides). The extant fibers retain their normal anterior - posterior orientation. The fibers can be activated by the giant fiber pathway, contain synaptic profiles, and have the normal 3:1 thin:thick filament ratio.

The 5 DLM motor axons are within the PDMN (confirmed by TEM, 4 sides), which extends dorsally as normal. However, in all cases, the PDMN divides before the expected level of the ventral-most DLM fiber. Typically, 2 motor axons (confirmed by TEM, 2 sides) continue dorsally toward the targets. The remaining motor axons extend for about 50 um and terminate in a tangled neural mass, or neuroma, always located posterior to the PDMN. The neuroma contains numerous smaller axon profiles and many presynaptic terminals (hemisynapses) facing other axons, glia, or space.

The regularity in the altered pattern of muscle and motor axons is striking, and suggests that the greater apparent autonomy of invertebrate motor axon survival can be mediated by target muscle survival. This regularity should allow us to follow the development of the final pattern, to learn more of the interaction of nerve and muscle during formation of the motor system which mediates escape in Drosophila. Supported by NIH-NRSA (MRH), Muscular Dystrophy Association (WJC) and research funds of the Col. Osteo Med., Ohio Univ. (WJC).

168.4 TRANSFORMATION OF IDENTIFIED MUSCLE AND ITS MOTORNEURON IN BITHORAX DROSOPHILA. A.M. Schneiderman, M.L. Tao* and R.J. Wyman, Dept. Biology, Yale University, New Haven CT 06511.

Although the cuticular transformations caused by mutant bithorax-complex (BX-C) genes of Drosophila have been well characterized, the effects of these genes on neurons and muscles are less well understood. Not all tissues are transformed: most striking is that the mutant metathorax (T3) is virtually devoid of flight muscle [Ferrus & Kankel, Dev. Biol. 85:485, 1981; Costello & Wyman, Roux's Arch. 194:373, 1985]. Not all classes of sensory cells appear to be transformed [Palka & Ghysen, Trends Neurosci.5:382, 1982], and the extent of transformation of adult cuticle and of underlying central nervous system do not correspond [Teugels & Ghysen, Nature 304:440, 1983]. We are using identified cells in the pathway for the jump response to recognize transformation of neurons and muscles in mutant abx bx^3 pbx/Df(3R)P2 flies, in which the T3 (metathoracic) cuticle is transformed to T2 (mesothoracic).

In wildtype flies, the T2 tergotrochanteral muscles (TTMs) provide the thrust for the escape jump, which is elicited by extracellular stimulation of the giant fiber (GF), the command neuron for the response [Tanouye & Wyman, J. Neurophys. 44:405, 1980]. Within the T2 neuromere, the GF axon makes a characteristic tuft and lateral bend where it synapses with the TTMn, the motorneuron innervating the T2 TTM [Koto et al., Brain Res. 221: 213, 1981]. Developmental studies show that the GF never enters T3. Thomas and Wyman [Devel. Biol. 102: 531, 1984] observed that in mutant flies the GF, which as a head segment (brain) cell should not be affected by the BX-C genes, enters T3 where it makes a supernumerary tuft and lateral bend. They suggested that transformed neurons in T3 induced the extra GF branches, but did not directly study cells intrinsic to T2 and T3.

We have found that the mutant T2 TTM is anatomically similar to its wildtype form. In T3, although the flight muscles are greatly reduced or absent, there is a TTM-like muscle, approximately half the volume of a T2 TTM, but larger than any muscle found in wildtype T3. It has points of insertion in the transformed cuticle similar to those of the T2 TTM. The motorneurons to both the T2 and T3 muscles have dendritic branching patterns that closely resemble that of the wildtype T2 TTMn, and are similarly driven at fast latency and frequency by GF stimulation. We thus suspect that the cells we identify as the T3 TTM and TTMn are transformed, either from homologous cells normally in T3 or as cells totally new to T3. Supported by NSF BNS-85-10678 and Helen Hay Whitney Foundation.

168.5 *FACET-GLOSSY*, A *NOTCH* MUTANT, AFFECTS *DROSOPHILA* PUPAL EYE DEVELOPMENT. Ross L. Cagan* and Donald F. Ready. Dept. of Biology, Princeton University, Princeton, New Jersey 08540

We have taken advantage of the simplicity and accessibility of the retina of *Drosophila melanogaster* to study an allele of *Notch*, *facet-glossy*. In flies without normal *Notch* product, neuroblasts proliferate at the apparent expense of epidermis; loss of *Notch* product in adult tissue is lethal, with a variety of defects observed (Deitrich and Campos-Ortega, 1984). Models of *Notch*'s role in development would benefit from studying mutant alleles in a system in which each cell's developmental history was known. We have undertaken an electron and light microscope analyses of the development of the pigment and bristle cells. We compared normal development with that of *facet-glossy*, and find that *facet-glossy* fails to develop normal primary pigment cells. This in turn appears to lead to a cascade of later defects.

The fly's compound eye contains 700-800 unit eyes, or ommatidia. Towards the end of larval development, eight photoreceptor cells and four cone cells group into prospective ommatidia. Development of these twelve cells uses local cell contacts to cue each cell to its fate (Tomlinson and Ready, 1987). At about 10% of pupal development, eight to ten undifferentiated cells surround each maturing ommatidium. Two cells, one contacting the anterior cone cell, the other contacting the posterior cone cell, stretch around the ommatidium in both directions, displacing cells in their path. These cells will ultimately touch each other at both ends, encircling the ommatidium. They will become the primary pigment cells. The displaced cells are pushed out between ommatidia, and appear to compete to become the remaining secondary and tertiary pigment cells; excess cells die to leave nine pigment cells shared between clusters. Later, the primary pigment cells will accumulate brown pigment; the secondary and tertiary pigment cells accumulate red pigment.

In the *Notch* allele *facet-glossy*, eye development in the larva is essentially normal. During early pupal stages, two cells touching the anterior and posterior cone cells begin to stretch around the ommatidium, displacing cells much as in wild type. However, these cells fail to fully encircle the ommatidium, leaving not two but generally four cells surrounding the ommatidium. Most of the displaced cells remain between the clusters and excess cells fail to die. Furthermore, cells that should become primary pigment do not synthesize brown pigment, but accumulate the red pigment characteristic of secondary pigment cells.

168.6 OCTOPOD, A HOMEOTIC MUTATION OF THE MOTH MANDUCA SEXTA, INFLUENCES THE FATE OF PATTERN ELEMENTS WITHIN THE CNS. R. Booker* and J.W. Truman (Spon. J. Palka). Dept. of Zoology, Univ. of Washington, Seattle, WA 98195

In insects much progress has been made in understanding the process of segmental determination, primarily due to the existence of homeotic mutations, which result in the transformation of one body part to another. While it has been firmly established that the homeotic mutations transform epidermal structures, there is less certainty about their role in establishing the segmental specificity observed within the CNS. Recently we isolated the first homeotic mutation of the moth Manduca sexta. It is an autosomal dominant mutation resulting in the appearance of ectopic thorax-like legs on the first and less often the second abdominal segments. We have named this mutant Octopod. This mutation also influences the fate of pattern elements within the CNS.

During adult development new neurons are added to all of Manduca's segmental ganglia. These new neurons are generated during larval life by an array of individually identifiable stem cells (neuroblasts (NBs)). The number of NBs found within a ganglion varies in a segment specific manner. The third thoracic ganglion contains 45 NBs while in the first abdominal ganglion a homologous but reduced array of 24 NBs are found. There are also regional differences in the numbers of progeny generated by homologous NBs, with those in the thorax generating substantially more progeny than their abdominal homologs.

An examination of the first abdominal ganglia of Octopod moths revealed that they were partially transformed with the appearance of from 3 to 9 ectopic NBs. These Nbs were homologous to Nbs found in the thoracic ganglia based upon morphological criteria and the number of progeny they generated. The distribution of the ectopic neuroblasts was not uniform, as they were found only in the anterior half of the ganglion. In addition, the fate of some of the NBs normally present in the first abdominal ganglion was transformed. By the end of the larval stage some of the NBs in the first abdominal ganglion had generated the number of progeny typical of their thoracic homologs. Our analysis demonstrates that the fate of pattern elements within the segmental ganglia of insects can be influenced by homeotic mutations. (supported by NIH grant # NS-13079)

168.7 EMBRYONIC ASSEMBLY OF A MUSCLE LAYER IS ORGANIZED BY A SINGLE COMPLEX CELL IN THE LEECH. J. Jellies and W.B. Kristan, Jr. Department of Biology, B-022, UCSD, La Jolla, CA 92093.

We have found a single cell in the leech (Hirudo medicinalis) that serves as a necessary organizer for one of the three muscle layers in the body wall. These layers consist of an inner layer of longitudinal muscle, an outer layer of circumferential muscle, and an orthogonal grid of obliquely oriented muscle fascicles between them. The oblique muscle grid first appears at about embryonic day 12, after the rudiments of longitudinal and circular muscle layers have assembled.

Intracellular dye-filling revealed a bilaterally represented cell in each segment of the embryonic leech that had a complex morphology reminiscent of the oblique muscle layer. Each such cell ("centipede", or C-cell) has a longitudinally oriented spindle containing a single nucleus. Each spindle projects 35 thin, parallel processes toward the ventral midline at a posteriorly directed angle of 45-60° and an oppositely directed set of 35 processes toward the edge of the germinal plate, the future dorsal midline. The processes of bilaterally homologous C-cells cross perpendicular to one another, forming an orthogonal grid that is coincident with the grid of oblique muscle. By filling C-cells and individual muscle fibers with different fluorescent probes we found that individual muscle cells align themselves with C-cell processes during development, forming the oblique muscles.

The necessity of the C-cell for oblique muscle formation was tested by photoablating it in living embryos before oblique muscle assembly began. Each of 36 such animals exhibited a complete loss of the oblique muscle fascicles corresponding to the predicted location of the missing C-cell and its processes when examined 6-10 days after the ablation. The fact that a single cell organizes such an extensive array of muscle bands makes the C-cell quite different from the "muscle pioneers" found in insects.

The striking morphology of the C-cell makes it interesting to us not only because of its role in organizing muscle assembly, but also as a "model" cell for examining how its many processes navigate long distances in a highly ordered fashion in vivo. It appears that the 70 or so individual "growth cones" of a single C-cell interact with each other and with other cells in the embryonic environment to accomplish this task.

This work was supported by an NIH fellowship, #NS 07684 (JJ) and an NIH grant, #NS 20746 (WBK).

168.8 **PERIPHERAL TARGET CHOICE BY IDENTIFIED NEURONS DURING EMBRYOGENESIS OF THE LEECH.** C.M. Loer and W.B. Kristan, Jr. Dept. of Biol., Univ. of Calif., San Diego, La Jolla, CA 92093.

The acquisition of a unique phenotype by a neuron during embryogenesis is under the direction of both internal instructions and external influences. We have been investigating the importance of target interactions in the development of differences between segmentally homologous neurons in the leech, *Hirudo medicinalis*. A pair of large serotonergic Retzius (Rz) cells is found in each segment of the CNS. In most segments, Rz cells diffusely innervate the body wall of their own and each adjacent segment. Rz cells in segments 5 and 6 [Rz(5,6)] instead heavily innervate the reproductive tissue. During embryogenesis, Rz(5,6) initially have the same pattern of outgrowth as their segmental homologues, but their processes stop growing into the body wall and interganglionic connectives following apparent contact with the reproductive primordia. Rz(5,6) subsequently become different from their segmental homologues in a variety of morphological and physiological characteristics. These observations suggest that some interaction between Rz(5,6) and the reproductive primordia causes these neurons to assume their segment-specific phenotype. We have shown previously that when their normal target is ablated, Rz(5,6) develop more like standard Rz cells. For example, they frequently extend axons into the connectives and body wall. Here we ask whether standard Rz cells are capable of developing like Rz(5,6) when given the opportunity to innervate reproductive tissue.

Reproductive primordia were transplanted into standard midbody segments at a time before Rz cell processes have exited the CNS. Body wall not containing reproductive primordia was transplanted into control embryos. Almost all embryos survived the operation and ectopic reproductive tissue was incorporated into standard segments in more than half of the experimental embryos. 6 to 7 days later, embryos were dissected and assayed either with a serotonin antiserum which marks all Rz cell processes or by injection of HRP into individual Rz cells.

Transplanted male reproductive tissue in experimental embryos was almost as heavily innervated by standard Rz cell processes as it would normally be by Rz(5,6). Furthermore, the tissue was sometimes innervated through additional nerve branches similar to the sex nerves normally found only in segments 5 and 6. In control embryos, transplanted body wall was innervated similarly to normal body wall. Injecting HRP into individual standard Rz cells revealed that the neurons innervate ectopic reproductive tissue more densely than body wall; however, they also appeared to retain other morphological characteristics of standard Rz cells. These results could mean that standard Rz cells differ intrinsically from Rz(5,6) in their ability to be modified by interaction with the reproductive tissue. Another possibility is that we have not yet provided the conditions necessary for standard Rz cells to interact appropriately with reproductive tissue and thereby express the Rz(5,6) phenotype. Further experiments are being performed to distinguish between these possibilities.

This work was supported by an NIH Traineeship, GM07313 (CML) and an NIH grant, NS20746 (WBK).

168.9 **PERIPHERAL CELLULAR CONTACTS THAT MAY REGULATE THE CENTRAL MORPHOLOGY OF DEVELOPING IDENTIFIED LEECH (*Hirudo medicinalis*) NEURONS.** K.A. French, S.M. Furgal* and W.B. Kristan, Jr. Department of Biology, B-022, UCSD, La Jolla, CA 92093.

A set of identified neurons, the Retzius (Rz) neurons, in the leech central nervous system assume during embryonic development essentially the same morphology in every segment of the ventral nerve cord, except in the two segments that contain the reproductive structures. Rz cells in segment 5 (the male segment) and segment 6 (the female segment) possess unique central and peripheral morphology. The results of previous experiments have shown that their distinctive morphology arises only after peripheral processes of these Rz cells - Rz(5,6) - have reached the vicinity of the primordial reproductive tissue in the two segments, an event that occurs on the 10th day of development. Furthermore, when the primordial reproductive tissue is ablated before this time, the morphology of Rz(5,6) develops much more similarly to that of Rz neurons in standard, non-reproductive segments (Loer, Jellies, and Kristan, *J. Neurosci.*, in press).

When Rz(5) were filled with horseradish peroxidase (HRP) on the 10th day of development and their peripheral processes were examined with EM, the filled growth cones and processes were observed to be in contact with cells that surrounded the definitive reproductive primordium, but were not integrated into it. The cells contacted could be identified by two distinctive features in sections; they had irregular and often tortuous boundaries, suggesting active extension and ruffling of the plasma membrane, and many of the cells contained one or more large (approximately 1 μm across), electron-lucent vesicles. We have named these cells V cells, for their distinctive intracellular inclusions. Filled profiles contacted V cells in two regions in which Rz processes possess large growth cones, structures that suggest that the processes are actively choosing a pathway: the near-vicinity of the primordial male reproductive duct and the region between the duct and the as-yet-undifferentiated genital pore, which eventually becomes heavily innervated by Rz(5).

As a step toward identifying the function of V cells, we wished to learn whether the vesicles were pinocyotic. A solution of 6% HRP and 0.1% fast green in leech saline was injected into whole embryos on the 9th day of development. After 24 hours, the embryos were dissected, fixed, reacted for HRP, and observed with the EM. In all 4 embryos examined in this way, most cells with the characteristics of V cells contained one or more typical large vesicles filled with HRP reaction product. Few such vesicles were observed that were not filled with HRP. From their irregular external shape and the HRP-filled vesicles, we conclude that V cells share many characteristics with cells in other systems that have been activated by exposure to growth factors or by transformation by oncogenes. Future work will focus on identifying the developmental fate of V cells and characterizing the nature of the interactions between these cells and Rz(5).

Supported by an NIH Research Grant (NS20746) to WBK and a University of California, San Diego, OGSR grant to KAF.

168.10 MALE SPECIFIC NEURONS ARE STAINED IN THE NEMATODE CAENORHABDITIS ELEGANS WITH ANTIBODIES AGAINST MOUSE NEURAL CELL ADHESION MOLECULE (N-CAM). S. Siddiqui. Laboratory of Molecular Biology, Toyohashi University of Technology, Toyohashi 440, Japan and Department of Biochemistry, Molec. Biology and Cell Biology, Northwestern University, Evanston, IL 60201.

The nematode Caenorhabditis elegans exists in two sexual forms, hermaphrodites, the self fertilizing with two X chromosomes, and males with one sex chromosome. Using polyclonal antibodies raised against mouse neural cell adhesion molecule(N-CAM), we have shown that N-CAM like antigen is limited to the male specific neurons in C. elegans. Anti-N-CAM antibodies(kindly provided by Dr. U. Rutishauser, Case Western Reserve U.) stain four cephalic neurons in the male head, when wholemount animal squashes are stained. In addition, a small number of neurons are stained in the male tail. We have identified the neurons stained in the head as male specific CEMDL, CEMDR, CEMVL, and CEMVR neurons. This identification was confirmed by staining mutants of ced-3 and ced-4(in these hermaphrodite animals CEM cells survive due to a block in programmed cell death, H. R. Horvitz and others, MIT Cambridge, Mass.). The male tail neurons, we have tentatively identified as ray neurons B. Among the non-neural tissues stained in both sexes are pharyngeal gland cells, excretory H-cell and sperm. The immunoreactivity is observed from the early embryonic development and it continues during the entire growth through larval phases to adulthood. This is consistent with immunoblot analysis of embryonic, larval and adult tissues which show a large number of smeary bands from 200 k daltons to 16 k daltons in both sexes. We have also stained tra-1, tra-2, and tra-3(transformer mutants in which XX animals are transformed into males, J. Hodgkin, MRC, Cambridge, England) animals, and found that the transformed animals stain like wild type males.

We have recently screened a genomic library of the C. elegans DNA with a 600 b.p. probe of NCAM cDNA (coding region), given by Dr. C. Goridis(CNRS-CIML,Marseille, France) and found many positive clones on hybridization. Currently we are trying to characterize these putative clones of the C. elegans NCAM like gene. Work in Evanston was done in the labs of Prof. J. Culotti (cytochemistry) and Prof. R. Holmgren (Molecular genetics), their support is appreciated. Ms T. Orenic helped in teaching the cloning work, for which I am grateful.

168.11 NEURONAL CHANGES DURING THE METAMORPHOSIS OF THE GENITAL SEGMENTS IN A HOLOMETABOLOUS INSECT. R.S. Thorn* and J.W. Truman. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195

In the tobacco hornworm moth, Manduca sexta, metamorphosis triggers a sex-specific differentiation of the genital segments of the abdomen into the male or female genitalia of the adult. Giebultowicz and Truman (1984, J.Comp.Neurol. 226: 87-95) demonstrated that this transformation is accompanied by an extensive bout of sex-specific motorneuron death that sculpts the different adult neuronal complements from an identical set of larval neurons. We have carried this analysis further by asking, first, whether this sex-specific neuron death correlates with a sex-specific loss of target muscles, and, secondly, how does the adult nervous system then accomodate the production of new targets in these segments, namely the muscles associated with the reproductive tract(s).

By dissection and cobalt backfilling, we have followed the fates of specific nerve muscle pairs in the genital segments through metamorphosis. We find that all of the muscles degenerate during the early stages of metamorphosis in both sexes. After this decline, there is a sex-specific regrowth of specific muscle remnants to generate the two adult muscle patterns. Reuse of a larval muscle remnant is invariably accompanied by retention of its larval motorneuron in the adult, while loss of a remnant is always paralleled by loss of the motorneuron. This pattern is inviolate for skeletal muscle motorneurons.

Visceral muscle motorneurons show greater plasticity. Larval visceral motorneurons of the genital segments can survive or perish along with their targets, but some appear to be able to switch targets during metamorphosis. This usually involves switching from a purely larval structure, such as the rectal cryptonephridium, to the adult reproductive tract. The adult reproductive tract also receives innervation from several classes of post-embryonic cells specific to the adult which first differentiate during metamorphosis.

168.12 INDUCTION OF A NEURONAL ARBORIZATION BY NON-TARGET TISSUE IN C. ELEGANS: C. Li and M. Chalfie, Department of Biological Sciences, Columbia University, New York, New York 10027

We are assessing the role of target cells and surrounding tissues on the innervation of the vulva in Caenorhabditis elegans. Two cell types, the VC and PVT neurons, arborize extensively at the vulva where they synapse onto the vulval muscles and a pair of neurons, the HSN cells. The extent of this arborization can be seen immunocytochemically with an antibody raised against the neuropeptide FMRFamide (kindly donated by R.L. Calabrese). In mutants in which the vulva and target vulval muscles are displaced anteriorly, the arborization is similarly displaced, suggesting that external factors are influencing the VC and PVT projections.

The region around the vulva contains the muscle and neuronal targets, vulval and non-vulval hypodermal cells, and gonadal cells. By examining mutants affecting the development of these cell types, we have found that the vulval hypodermal cells and not the muscle or neuronal targets cells are necessary for the induction of the vulval arborization. Specifically, perturbations of the target cells, such as displacement of the vulval muscles in egl-15 mutants or removal of the HSN neurons in egl-1 mutants, had no effect on the pattern or position of the arborization. By contrast, mutants in which the vulval hypodermal cells are absent but the target cells are present, for example in lin-2, lin-3, lin-7, and lin-10 mutants, have no detectable arborization.

Examination of cell lineage mutants that have ectopic clusters of hypodermal cells revealed that vulval hypodermal cells, irrespective of their positions, can induce neuronal branching. Specifically, when the clusters are composed of vulval hypodermal cells, immunoreactive processes are observed projecting into the clusters. When the clusters are composed of non-vulval hypodermal cells, however, no processes are observed.

These experiments suggest that vulval hypodermal cells are necessary for the induction of vulval branches by the VC and PVT neurons. The target cells have no inductive capacity, but may serve a role in the final shaping of the vulval arborization.

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PRESYNAPTIC MECHANISMS II

169.1 POTASSIUM-EVOKED ATP SECRETION FROM A HIGHLY PURIFIED PREPARATION OF MOUSE BRAIN SYNAPTOSOMES. J. A. Bitran*, H. B. Pollard and E. Rojas*. Laboratory of Cell Biology and Genetics, NIDDK/NIH, Bethesda, MD. 20892

Secretion of neurotransmitters from presynaptic terminals, in synaptosomal preparations, occurs by a very fast process. For this reason it has been difficult to study the kinetics of synaptosomal secretion in a quantitative manner and the mechanisms involved remain to be elucidated. To resolve the time course of transmitter secretion from mouse brain presynaptic terminals, we have measured the stimulated secretion of ATP from synaptosomes using a luciferin-luciferase mixture and monitoring the light emitted by the reaction. ATP is not only a transmitter itself, but is also co-stored and co-secreted with transmitters from synaptic vesicles. From the crude synaptosomal preparation (P2), we obtained a highly purified fraction using an isotonic continuous metrizamide/sucrose gradient. The use of biochemical markers and electronmicroscopy indicates that this fraction is highly enriched in synaptic endings and the luciferin-luciferase method shows that this fraction has the highest ATP content. Depolarizing the synaptosomes with elevated K^+ causes the synaptosomes to secrete ATP, and the time course could be fit to a single exponential, thus indicating a first order reaction. Two different processes could be detected, however, depending on whether depolarization occurred in the presence or absence of calcium in the medium. In the presence of Ca^{2+}, secretion was rapid, having a time constant in the range of c. 1 sec. However, in the absence of Ca^{2+} secretion was much slower, having a time constant of c. 7 sec. In the presence of $[Ca^{2+}]_o$, increasing $[K^+]_o$ induced a moderate decrease in the time constant. In the absence of Ca^{2+} similar changes in $[K^+]_o$ did not significantly affect the time constant of release. At each $[K^+]_o$ the extent of the secretory response was evaluated. Taken together, the data showed that an e-fold change in the extent of secretion could be obtained over a 2-fold smaller range of voltage for Ca^{2+}-dependent than Ca^{2+}-independent secretion. At each $[K^+]_o$, decreasing $[Ca^{2+}]_o$ from 1 to 0 mM induced c. a 4-fold increase in the time constant required to fit the time course of release. The results suggest that, in addition to the Ca^{2+}-dependent mechanism for the release of neurotransmitters from presynaptic terminals, synaptosomes are also equipped with another mechanism that does not require extracellular Ca^{2+}. It is possible that this latter mechanism might involve the activation of voltage-dependent Na^+-channels and/or the activation of intracellular messengers to induce Ca^{2+} release from intracellular stores.

169.2 DIFFERENTIAL ION DEPENDENCY OF GLUTAMATE INDUCED ^{3}H-GABA RELEASE FROM OLFACTORY BULB in vitro. E.H. Jaffé*, M. L. Vaello* and N.H. Hernández* (SPON: J.A. González V.). Laboratorio de Neuroquímica, IVIC, Caracas, Venezuela.

It is well established that GABA is the neurotransmitter of a population of granule cells of the olfactory bulb of the rat. These cells establish dendro-dendritic reciprocal synapses with the mitral cells which is apparently of glutaminergic nature. These dendrites project densily to the external plexiform layer (EPL) of the olfactory bulb making it possible in this way to microdissect these neuronal elements. Previously we could show that the glutamate (Glu) agonist, kainic acid (KA) is able to release ^{3}H-GABA from EPL but not other Glu agonists as NMDA or aspartic acid. Glu itself was only effective when the tissue was preconditioned with 25 mM K^+, 12 minutes before adding 5 mM Glu. This effect on the ^{3}H-GABA release was apparently receptor mediated since specific Glu antagonists could block it (Jaffé, E.H., Vaello, M.L., submitted to Neuroscience). We were interested to characterize the ion dependency of the ^{3}H-GABA release from rat EPL using 25 mM K^+, 0.5 mM KA and 25 mM K^+ - 5 mM Glu stimulation. The microdissected tissue was incubated in 0.1 µM ^{3}H-GABA and superfused with Krebs bicarbonate under continuous oxygenation. Fractions were collected every 2 minutes and radioactivity measured using a liquid scintillation counter. The K^+ stimulated ^{3}H-GABA release was completely inhibited when the tissue was superfused with $0Ca^{++}$/EGTA, 2 mM Co^{++} and partially inhibited by 25 mM Mg^{++}. In a similar way, KA induced ^{3}H-GABA release was completely inhibited by 2 mM Co^{++}. However, no Ca^{++} dependency of the K^+ - Glu stimulated ^{3}H-GABA release could be observed in the presence of $0Ca^{++}$/EGTA, 2 mM Co^{++} or 25 mM Mg^{++}. This was not an unspecific effect of the K^+ prestimulation since the same treatment of the KA induced ^{3}H-GABA release was completely blocked by 2 mM Co^{++}. The Ca^{++} dependency of the KA induced release was not at the receptor level since 5 mM Mg^{++} which completely blocked the K^+ - Glu release did not inhibit the KA one. To study the Na^+ dependency of this ^{3}H-GABA release mechanism from the EPL, the tissue was superfused with 300 nM TTX or with $0Na^+$ solution. A complete inhibition of the K^+ - Glu as well as the KA induced ^{3}H-GABA release was observed with no effect on the K^+ induced release. Our results suggest the presence of two different release mechanisms of ^{3}H-GABA with a different Ca^{++} dependency when stimulated with two different glutamate agonists. Interestingly, no Na^{++} dependency of the K^+ stimulate ^{3}H-GABA release was observed, however, the complete Na^+ dependency of glutamate and KA stimulation brings further evidence for a Na^+ dependent depolarization mechanism for this two glutamate receptor agonists.

169.3 ELECTRICALLY EVOKED RELEASE OF TRANSMITTER FROM CULTURED SYMPATHETIC NEURONS IS FACILITATED BY FORSKOLIN AND VASOACTIVE INTESTINAL POLYPEPTIDE - ROLE OF cAMP AND Ca. T.D. Wakade,* S.V. Bhave,* R.K. Malhotra* and Arun R. Wakade. (SPON: J. Jakway) Dept. of Pharmacol., SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

A number of compounds facilitate stimulation-evoked release oftransmitter from sympathetic neuroeffector organs. However, the mechanism of such a facilitation has remained unresolved, the major reason being that measurements of key substances involved in release process can not be made in sympathetic neuroeffector organs. For this reason, we have used a homogenous population of cultured sympathetic neurons to determine the effects of forskolin and vasoactive intestinal polypeptide (VIP) on electrically evoked release of ^{3}H-norepinephrine (^{3}H-NE). The major aim was to know if these agents influence the release by altering intracellular messengers such as cAMP and Ca. Sympathetic neurons derived from peripheral ganglia of 10- to 12-day-old chick embryos were cultured in serum-free medium. After 2 to 3 days, cultures were loaded with ^{3}H-NE to study the release of tritium in the absence and presence of electrical stimulation (1 Hz for 15 sec). We show here that VIP or forskolin in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), enhanced neuronal cAMP by about 7- to 10-fold without affecting Ca^{45} uptake and the release of ^{3}H-NE. Electrical stimulation of sympathetic neurons (1 Hz for 15 sec) evoked a marked increase in ^{3}H-NE release over the spontaneous release, without altering cAMP levels but enhancing the net uptake of Ca^{45}. Stimulation in the presence of forskolin or VIP caused a pronounced facilitation of ^{3}H-NE release without an additional increase in Ca^{45} uptake. If 10 nM phorbol 12, 13-dibutyrate, an activator of protein kinase C, was added along with forskolin and IBMX, the evoked release was even further potentiated, and it was associated with an almost 10-fold increase in the net uptake of Ca^{45} but cAMP content was not further enhanced. It is concluded that facilitation of sympathetic transmitter release is a result of either an increase in intracellular concentrations of cAMP, Ca, or both; the major role of cAMP appears to be in the utilization rather than mobilization of Ca ions.

169.4 A ROLE FOR BOTH cAMP AND PHOSPHATIDYL INOSITOL IN SEROTONIN FACILITATION IN CRAYFISH. D. Dixon* and H.L. Atwood. Dept. of Physiology, University of Toronto, Toronto, Ontario M5S 1A8.

Facilitation of synaptic transmission by serotonin at the crayfish neuromuscular junction has been shown to have two phases of decay after induction (Dixon and Atwood, J. Neurobiol. 16:6, 1985), as in lobster (Glusman and Kravitz, J. Physiol. 325, 1982). The second long lasting phase was shown to involve cAMP as a second messenger in the lobster. We report here that the second phase of facilitation in the crayfish is cAMP mediated, while the first phase involves phosphatidyl inositol metabolism.

Brief applications of activators of adenylate cyclase (e.g. Forskolin) or inhibitors of phosphodiesterase (eg. IBMX) can facilitate transmitter release (as recorded intracellularly) at a relatively low level (100%) for long periods of time (1+ hr.), as in the second phase of serotonin facilitation. Presynaptic injection (while recording intracellularly) of Protein Kinase Inhibitor (PKI) or SQ 22,536 (a cyclase inhibitor) blocks the development of the second phase of facilitation. Only a brief, high level of facilitation remained after serotonin application. The spread of PKI in the pre-synaptic region was monitored using a Rhodamine tag on the protein. Post-synaptic sites were selected, one within the region of PKI influence, one distant from the injection site. Normal facilitation developed at the distant site, whereas the second phase of facilitation was absent at the site near the point of injection.

The injection of RA 233 (a phospholipase C inhibitor) blocked the development of both phases of serotonin facilitation. Release of intracellular calcium induced by inositol tri-phosphate may be responsible for the early, high level facilitation. In calcium-free medium, some release is restored during serotonin application; this is indicative of increased intracellular free calcium. C kinase activation may lead to stimulation of the adenylate cyclase since both phases of facilitation are blocked by RA 233.

This work was supported by MRC Canada and a University of Toronto Open Scholarship.

169.5 **PAIRING PRE- AND POSTSYNAPTIC NEURONS LOCALIZES FACTORS AFFECTING EFFICACY AND VARIABILITY OF SYNAPTIC CURRENTS.** Daniel Gardner. Department of Physiology, Cornell University Medical College, New York NY 10021.

At these meetings last year, I reported (*Soc. Neurosci. Abstr.* 12: 740, 1986) that both duration and amplitude of synaptic currents vary, that each may be altered over the short term by manipulating presynaptic activity, and that these variations affect synaptic efficacy. In order to localize the sites of variability as either pre- or postsynaptic, I now report results from analyzing variations recorded in identified three-cell networks of *Aplysia* buccal ganglia. Two protocols were used: either a single identified postsynaptic neuron innervated by paired presynaptic neurons B4 and B5, or else a single presynaptic cell innervating two similar postsynaptic neurons. In either case, peak amplitude g_{peak} and time constant of decay τ of inhibitory postsynaptic currents (PSCs) could be recorded with low noise. All PSC were evoked by intracellular stimulation of specific presynaptic neurons. Time constants of digitized PSCs were measured using least-squares fits to semilog plots. For this population, as before, coefficients of variation CV (σ/mean) were uncorrelated with g_{peak} or τ ($r < 0.15$).

PAIRED PRESYNAPTIC INPUTS TO SINGLE POSTSYNAPTIC NEURONS: The similarity of paired inputs on common followers itself differed from cell to cell. To determine if the postsynaptic neuron constrained variability, paired presynaptic inputs on single postsynaptic cells were compared to those for the unpaired population. Fractional differences between paired g_{peak} (0.30 ± 0.08) were as large as those for the population (0.43 ± 0.04). Similarly, ANOVA for g_{peak} showed paired variance equal to unpaired. In contrast, fractional differences between τ produced by paired inputs (0.11 ± 0.03) were significantly ($p < 0.05$) smaller than those of the population (0.27 ± 0.02).

Tetanization or DC polarization of a single presynaptic interneuron modulated input from that neuron alone, producing a significant ($p < 0.01$) change in both g_{peak} and its CV for PSCs evoked from the modulated neuron, without any effect on any parameter of input from from the unmodulated neuron. This result is consistent with a presynaptic locus for short-term variability.

SINGLE PRESYNAPTIC CELLS INNERVATING PAIRED POSTSYNAPTIC NEURONS: In complementary protocols, two postsynaptic neurons innervated by a common presynaptic cell were simultaneously voltage-clamped and PSCs recorded synchronously in both neurons. As before, values for parameters determining synaptic efficacy and variability resembled those of the unpaired population. Following tetanic stimuli of the presynaptic neuron, PSCs in both postsynaptic cells showed post-tetanic potentiation, usually of similar amplitude and time course.

I conclude, on the basis of differences in variability seen in three-cell networks, that both presynaptic and postsynaptically determined factors contribute to short-term plastic changes affecting PSC amplitude and duration, and therefore efficacy. Additionally, synapse-to-synapse variability of some parameters is greater than similarity introduced by a common pre- or postsynaptic element.

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169.6 IS LONG-TERM POTENTIATION IN HIPPOCAMPUS ASSOCIATED WITH AN INCREASE IN TRANSMITTER RELEASE? S. S. Chirwa* and B. R. Sastry. Neuroscience Research Laboratory, Department of Pharmacology & Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Tetanic stimulation of stratum radiatum in the hippocampus results in a subsequent long-term potentiation (LTP) of the excitatory postsynaptic potential (EPSP) in the CA_1 neurons. Whether this LTP is pre- or postsynaptic is unclear. At the skeletal neuromuscular junction it is known that in the presence of extracellular Ba^{2+}, stimulation of the motor nerve terminals result in not only the synchronous end-plate potential (EPP) but also a tail of miniature EPPs (Silinsky, E. M., J. Physiol. (Lond.), 274: 157, 1978). In the present investigation, we examined whether Ba^{2+} has similar effects at the synapses between stratum radiatum and CA_1 neurons in the guinea pig hippocampal slices; and if it did, determined if the number of miniature EPSPs in the tail was increased during LTP.

Intracellular recordings were made from CA_1 neurons with CH_3COOK filled electrodes while the slices (the CA_2, CA_3 and CA_4 somata were dissected out) were in the normal medium. After obtaining controls (stable membrane potential above -60 mV, input resistance greater than 25 M ohms), the slices were exposed for 2 min to a medium containing 3.5 mM Ba^{2+}, 0.5 mM Ca^{2+} and 0.1 mM picrotoxin. Stimulation of stratum radiatum (1-5 pulses at 50 Hz, stimulation strength was enough to evoke an action potential in the CA_1 neuron) at 0.02-0.2 Hz resulted in a tail of miniature EPSPs that followed the evoked action potential for at least 3 s (24 of 31 neurons). After obtaining adequate control records of the tails of miniature EPSPs, slices were reexposed to normal medium for 15 min. LTP could then be induced (in 18 of the above 24 cells) by tetanizing stratum radiatum (400 Hz, 200 pulses). When slices were reexposed to Ba^{2+}-containing medium 15 min after the tetanic stimulation, the number of miniature EPSPs in the tail was at least doubled (in 14 of the above 18 cells). In control experiments, if Ba^{2+}-containing medium was applied twice with a 30 min interval in between, the tails were not significantly different. During Ba^{2+} application the input resistance was increased, spikes were broadened, membrane potential depolarized and the after-spike hyperpolarization decreased. The passive electrical properties of the neurons during the control tails and post-tetanic tails were similar.

The size of the miniature EPSPs was small (less than 2 mV). It was, therefore, not possible to do further quantal analysis to determine that the increase in the number of miniature EPSPs in the tail during LTP does in fact reflect an increase in transmitter release. We, therefore, tentatively conclude that LTP is associated with what appears to be an increase in transmitter release. Experiments are currently underway at mossy fiber - CA_3 synapses, where an analysis of size distribution of the miniature EPSPs is feasible.

(Supported by the Canadian MRC. SSC is a WHO Fellow.)

169.7 PRESYNAPTIC, VOLTAGE-DEPENDENT CONTROL OF LONG-TERM FACILITATION (LTF) AT THE CRAYFISH NEUROMUSCULAR SYNAPSE. J.M. Wojtowicz, H. L. Atwood, Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8.

Crustacean neuromuscular synapses can be facilitated for periods of several hours when subjected to bouts of intense activity lasting a few minutes. LTF was studied in a crayfish opener muscle by means of pre- and post-synaptic electrophysiological recordings. A presynaptic microelectrode was used to measure membrane potential and to pass depolarizing and hyperpolarizing current pulses via a standard bridge circuit. A postsynaptic electrode recorded quantal release of neurotransmitter. Preparations were perfused with crayfish saline at 13°C with the addition of 0.1 mM 4-AP to reduce potassium conductance in the nerve terminal and 0.1 μM TTX to abolish voltage sensitive sodium conductance. In such a medium a bout of stimulation delivered to the terminal (3-5 msec depolarizing pulses, 30-60 mV in amplitude, repeated at 20Hz for a period of 10 minutes) resulted in 81% enhancement (range 30-117%, s.d. = 33, n=5) of post-synaptic response 40 minutes after the tetanus. It was determined that the induction of this long-lasting effect was not critically dependent on the influx of sodium ions into the terminals since TTX together with replacement of 90% of sodium ions with either choline or N-methyl-D-glucamine had only a small effect on the magnitude of LTF (mean = 62%, range 20-91%, s.d. = 37, n=3,). Similarly omission of calcium ions and their replacement by either 6 mM manganese or 30 mM magnesium did not reduce LTF significantly (mean = 67%, range 0-171%, s.d. = 55, n=7). Thus it appeared that repeated depolarizations and not ionic fluxes were primarily responsible for the establishment of LTF. We can be less certain as to the exact alteration produced by the depolarization. Two mechanisms proposed for other types of long-lasting plastic changes, viz, reduction in input conductance of the terminals and accumulation of calcium ions, have been ruled out: no change in the slope of the synaptic transfer curves or synaptic delays was observed and there was no increase in the rate of occurrence of spontaneous release. We propose that LTF is produced by a direct structural (or conformational) change in the presynaptic membrane which in turn alters release of transmitter substance. Alternatively a second messenger system, triggered by membrane depolarization, may be involved. Experiments are in progress to identify the exact mechanism.

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169.8 MATHEMATICAL MODEL OF TWO CELLULAR MECHANISMS CONTRIBUTING TO DISHABITUATION AND SENSITIZATION IN APLYSIA. K.J. Gingrich and J.H. Byrne, Albany Medical College, Albany, NY and University of Texas Medical School, Houston, TX. 77225.

Presynaptic facilitation contributing to dishabituation and sensitization in Aplysia appears to be mediated by two mechanisms, broadening of the presynaptic spike and mobilization of transmitter (Gingrich & Byrne, 1985; Hochner et al, 1986; Rankin & Carew, 1986). While spike broadening can be observed directly, mobilization has been inferred from mathematical models and from changes in kinetics (shape) of the EPSP. Using a modification of a model of transmitter release developed previously (Gingrich & Byrne, 1985), we were interested in determining if mobilization in conjunction with spike broadening could account for the electrophysiological results obtained by Hochner et al (1986).

The model includes a description of membrane Ca^{2+} influx, diffusion of Ca^{2+} away from the plasma membrane, transmitter release proportional to the product of a depletable Ca^{2+} pool of transmitter and the square of the concentration of Ca^{2+} in the submembrane compartment (5 nm deep), and a postsynaptic membrane modeled by an RC circuit.

In normal (undepressed) cells, the PSP amplitude is a steep function of the duration of presynaptic voltage clamp pulses. In contrast, this function is relatively flat in depressed cells (Hochner et al, 1986). The model simulates changes in PSP amplitude produced by spike broadening. Broadening the pulse duration without modulating the releasable pool enhanced release in normal cells. In depressed cells, the releasable pool is depleted, and increasing pulse duration alone does little to enhance the PSP. In order to restore the effectiveness of pulse widening, the size of the releasable pool must be increased (i.e. mobilization).

The model also supports the hypothesis that the shape of the PSP can be used to gain insight into the relative contribution of spike broadening and mobilization to facilitation. We found that mobilization alone is associated with enhanced PSPs with constant time-to-peak and increased rate-of-rise, while pulse broadening alone is associated with enhanced PSPs with a reduced rate-of-rise and increased time-to-peak. To simulate dishabituation, we approximated spikes from Hochner et al (1986) with pulses and adjusted the size of the releasable pool. The model simulated the experimentally observed complex changes in the amplitude and shape of the PSPs (Hochner et al, 1986).

Our model demonstrates that mobilization of neurotransmitter in conjunction with spike broadening can account for the physiological changes in PSPs produced by presynaptic facilitation and can be used to gain insights into the relative contribution of these mechanisms in experimental studies.

169.9 EVIDENCE THAT THE ARACHIDONIC ACID CASCADE GENERATES SECOND MESSENGERS THAT MEDIATE PRESYNAPTIC INHIBITION IN AN IDENTIFIED CELL CIRCUIT OF APLYSIA. E. Shapiro, D. Piomelli* and J.H. Schwartz. Howard Hughes Med. Inst., Columbia Univ. Col. of Phys. & Surg., New York, NY 10032.

In the abdominal ganglion of Aplysia, the transmitter output of cell L10 can be reduced by stimulating L32 neurons. Histamine, the putative transmitter of L32, mimics the synaptic actions of L32 and reduces the transmitter output of L10 (Kretz et al., J. Neurophysiol. 55:113 and 131, 1986). It also stimulates the formation of metabolites of arachidonic acid in Aplysia neurons (Piomelli, Shapiro, Feinmark & Schwartz, Soc. Neurosci. Abst. 12:1340, 1986), and this prompted us to examine the role of the arachidonic acid cascade in the L32 circuit.

In ganglia prelabeled with [^{3}H]arachidonic acid, physiological intracellular stimulation of L32 neurons generated labeled metabolites of arachidonic acid including 12-hydroxyeicosatetraenoic acid (12-HETE) and prostaglandin E_2. Application of arachidonic acid onto L10 (0.1 mM puffed from a broken micropipette) reversibly hyperpolarized L10 through an apparent increased conductance mechanism, and reduced its transmitter output by 26%, mimicking both the stimulation of L32 and the application of histamine. Application of seawater or eicosamonoenoic acid had no effect.

The responses of L10 to histamine are blocked with the phospholipase inhibitor 4-bromophenacyl bromide (BPB), providing further indication for the involvement of arachidonic acid. Histamine (10^{-4} M) was puffed onto L10 to elicit hyperpolarizing responses; BPB (20 uM in 0.1% DMSO) within 30 min inhibited this response by more than 90%. Using 50 uM BPB we were also able to reduce the L32-L10 IPSP. Histamine and L32 responses were stable for over 1 h in 0.1% DMSO alone.

We suggest that presynaptic inhibition in L10 is produced by a cascade initiated by a histamine receptor which activates a phospholipase to liberate arachidonic acid from membrane phospholipids. Cell-specific metabolism of arachidonic acid might generate a variety of second messengers that affect characteristic membrane conductances and thereby reduce transmitter release. FMRFamide has also been found to produce inhibition by a similar mechanism in sensory neurons (Piomelli et al., Nature, submitted). We are now attempting to identify the active metabolites and are examining the conductance pathways that they modulate in L10.

169.10 PERTUSSIS TOXIN-SENSITIVE G PROTEIN TRANSDUCES THE RESPONSE TO HISTAMINE THAT PRODUCES PRESYNAPTIC INHIBITION IN L10, AN IDENTIFIED APLYSIA INTERNEURON. S.S. Vogel*, E. Shapiro, G.J. Chin and J.H. Schwartz (SPON: M.E. Swanson). Howard Hughes Med. Inst., Columbia Univ. Col. of Phys. & Surg., New York, NY 10032.

A M_r 40,000 protein, labeled by pertussis toxin (IAP)-catalyzed [^{32}P]ADP-ribosylation (Critz et al., Neur. Lett. 64:145, 1986), is also recognized by antibodies raised against bovine G protein synthetic peptide sequences (G.J. Chin et al., These Abstracts) in 100,000 x g Aplysia neural membrane fractions; it is not present in the supernatant. In both neural and muscle membranes a single component on SDS-PAGE appears to be labeled with IAP. An identical component was labeled in pooled isolated cell bodies. Western blots on the cell bodies with an antibody that recognizes the IAP substrate revealed that the G protein is present in L10 (Chin et al., ibid.). Partial proteolysis of these substrates from neural and muscle membranes with V8 protease and chymotrypsin reveal similar peptide patterns, suggesting that the proteins from both tissues are alike. In contrast, rat brain and squid optic lobe contain two components that are labeled with IAP; these are likely to be G_o and G_i.

Transmitter output from the Aplysia cholinergic interneuron L10 is reduced by application of histamine, which causes slow hyperpolarization mediated by a decreased Ca^{++} current and an increased K^+ current (Kretz et al., J. Neurophysiol. 55:131, 1986). Intracellular injection of IAP (0.2 mg/ml) within 50 min reduces the peak response to histamine more than 90% without affecting L10's resting potential or input resistance. Inhibition was dose-dependent: injection of 0.1 mg/ml decreased the histamine response by 75%. Injection of heat-inactivated IAP (80°C, 30 min) had no effect. Application of arachidonic acid mimics the histamine response in L10; this suggests that histamine acts as a neurotransmitter and liberates arachidonic acid from L10's membrane through a receptor-linked phospholipase (E. Shapiro et al., These Abstracts). We saw no significant decrease in the arachidonic acid-induced hyperpolarization after the histamine response had first been blocked by injecting IAP; this suggests that the histamine receptor is linked to a phospholipase through an IAP-sensitive G protein.

169.11 12-HYDROPEROXYEICOSATETRAENOIC ACID (12-HPETE), A LIPOXYGENASE METABOLITE OF ARACHIDONIC ACID THAT PRODUCES PRESYNAPTIC INHIBITION IN APLYSIA, IS METABOLIZED TO THE HEPOXILINS A AND B IN NEURAL TISSUE . D. Piomelli*, S.J. Feinmark* and J.H. Schwartz. Depts. of Pharmacology, Medicine, and Howard Hughes Med. Inst., Columbia Univ. Col. of Phys. & Surg., New York, NY 10032.

Several inhibitory responses in Aplysia are mediated through release and metabolism of arachidonic acid. Histamine and FMRFamide, two transmitters that cause presynaptic inhibition, stimulate the formation of the lipoxygenase metabolite, 12-hydroxy eicosatetraenoic acid (12-HETE). Application of 12-HPETE, the short-lived precursor of 12-HETE, mimics these responses in the appropriate identified neurons (Piomelli et al., J. Neurosci., in press, 1987; Piomelli et al., Nature, submitted, 1987; E. Shapiro et al., These Abstracts). 12-HETE, a major metabolic end product of 12-HPETE, was found to be inactive. Does 12-HPETE act as the actual second messenger, or, rather, must it be transformed to another product to be active? To answer this question, we are examining the metabolism of 12-HPETE in Aplysia neurons.

In mammalian tissues, 12-HPETE is converted to other products beside 12-HETE: these include two epoxy alcohols, the hepoxilins A and B (Pace-Asciak et al., J. Biol. Chem. 258:6835, 1983). In Aplysia, we find that [^{3}H] arachidonic acid can be transformed in substantial yields to products with the chromatographic properties of the hepoxilins, as assessed by normal phase HPLC and by thin layer chromatography (after HPLC purification). The identity of these compounds was confirmed using gas-chromatography/mass spectrometry. These products were isolated after incubation of neural components dissected from Aplysia ganglia with either 12-HPETE (50 uM for 10 min) or arachidonic acid (50 uM for 30 min). After they were purified by HPLC, the metabolites were converted to the corresponding methyl esters trimethylsilyl ethers. Hepoxilin A eluted from the GC with an equivalent chain length (ECL) of 23.3 as expected (Pace-Asciak et al., ibid.); characteristic ion fragments for hepoxilin A were generated from this material (m/z 407, 391, 311, 281 and 255). Hepoxilin B had the expected ECL of 22.1, and yielded characteristic ions (m/z 311, 282 and 269). Pace-Asciak et al. (ibid.) has provided evidence that the hepoxilins participate in modulating glucose-stimulated insulin secretion in pancreatic islets. We are testing whether these products are active in Aplysia neurons that are inhibited by histamine and 12-HPETE.

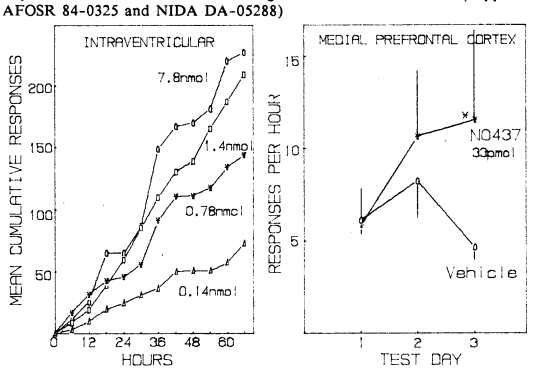
169.12 STIMULATION OF STRIATAL PRESYNAPTIC DOPAMINE D-2 AUTORECEPTORS DIFFERENTIALLY AFFECTS THE ACTIVATION OF TYROSINE HYDROXYLASE BY 2-CHLOROADENOSINE AND PHORBOL DIESTERS. P.Onali, B.Bunse* and M.C.Olianas. Department of Neurosciences, University of Cagliari, via Porcell 4, 09100 Cagliari, Italy.

In striatal dopaminergic terminals tyrosine hydroxylase (TH) activity is under inhibitory control by dopamine (DA) acting on presynaptic D-2 autoreceptors. Previous studies have shown that stimulation of D-2 sites is associated with inhibition of either adenylate cyclase activity or phospholipase C-induced hydrolysis of inositol phospholipids. We have investigated the possible involvement of these second messenger systems in the presynaptic control of TH by examining the effect of the D-2 agonist quinpirole on the stimulation of TH by 2-chloroadenosine (2-CADO), which increases cyclic AMP levels via adenosine A-2 receptors, and by phorbol diesters, which are potent activators of protein kinase C. Striatal synaptosomes were incubated in the presence of the various agents, pelleted by centrifugation and lysed by sonication. TH activity was assayed in the supernatant fraction of lysed synaptosomes using 0.1mM L-(1^{14}C) tyrosine in the presence of pterin cofactor 6-methyl-5,6,7,8-tetrahydropterin (6-MePH4). Exposure of synaptosomes to quinpirole produced an inhibition of TH activity which was maximal (40% decrease of control activity) at 1μM quinpirole, whereas preincubation of tissue with 2-CADO (from 10 to 3000μM) maximally increased TH activity by fourfold. The inhibitory effect of quinpirole was associated with an increase in the Km of TH for 6-MePH4, whereas the enzyme activation by 2-CADO was accompanied by a decrease in the Km for 6-MePH4. Quinpirole reduced by approx. 50% the enzyme stimulation produced by 2-CADO. This effect was antagonized by l-sulpiride but not by d-sulpiride. On the other hand, quinpirole failed to inhibit the activation of TH (50% increase) produced by 1μM phorbol 12-mystrate 13-acetate (PMA). Indeed, in the presence of the phorbol diester the inhibitory effect of quinpirole was significantly smaller than that observed under basal conditions. These results strengthen the idea that inhibition of a presynaptic adenylate cyclase activity is involved in the negative feedback on TH activity mediated by D-2 autoreceptors. Moreover the antagonistic action exerted by PMA on D-2 inhibition of TH activity suggests that the diacylglycerol-protein kinase C pathway may also be operative in the autoreceptor mediated control of striatal DA synthesis.

BEHAVIORAL PHARMACOLOGY: DOPAMINE AND NEUROLEPTICS

170.1 **DOPAMINE D2 "REWARD" RECEPTORS IN MEDIAL PREFRONTAL CORTEX.** K.E. Stevens, N. Nourbakhsh*, J.D. Belluzzi and L. Stein. Department of Pharmacology, College of Medicine, University of California, Irvine CA 92717

Brain self-stimulation studies are contradictory in assessing the relative importance of dopamine D1 and D2 receptors in behavioral reinforcement; some studies implicate the D2 receptor (Gallistel & Davis, 1983), while others implicate the D1 receptor (Nakajima, 1986). In the conditioned place preference test, we found that the specific dopamine D2 receptor agonist N0437 exerted a rewarding effect, whereas the specific D1 receptor agonist SKF38393 did not (Gilbert et al. 1986). Here we used our intraventricular self-administration method (Belluzzi & Stein, 1977) to determine whether rats would learn to work for N0437 solutions delivered directly into the lateral ventricle. A bar-press response delivered one of the indicated doses of N0437 (1μl in 0.9 sec). Rates of self-administration in the 66-hr test were monotonically related to dose (left graph). We also used the cortical self-administration method of Goeders and Smith (1983) to determine whether rats would self-administer N0437 directly in the medial prefrontal cortex. Each bar press delivered 100nl of N0437 (33pmol) or vehicle in 5 sec. On the third test day, hourly self-administration rates of N0437 significantly ($p<0.05$) exceeded that of the vehicle control (right graph). This effect of N0437 was blocked when the D2 antagonist sulpiride (33pmol) was added to the reinforcing solution (not shown). The results support the suggestion of Goeders and Smith (1986) that the medial prefrontal cortex contains reinforcement-relevant dopamine D2 receptors that may be important sites for the reinforcing actions of cocaine. (Supported by AFOSR 84-0325 and NIDA DA-05288)



170.2 BEHAVIORAL SUPPRESSANT EFFECTS OF NEUROLEPTICS IN MONKEYS:RELATION TO DOPAMINE D_2 RECEPTOR BINDING. J. Bergman*, B.K. Madras, D. Canfield*, R.D. Spealman*. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

The potencies of a series of neuroleptics for altering schedule controlled behavior were compared with their potencies for binding to dopamine D_1 and D_2 receptors in monkeys. Behavioral experiments were conducted in squirrel monkeys trained to respond under a fixed-interval (FI) schedule of reinforcement. Binding studies were conducted in striatal membranes derived from both fresh and stored (-85°C) brains of cynomolgus monkeys. Dopamine D_1 receptors were labelled with tritiated ^{3}H-SCH 23390 using cis-flupentixol to determine non-specific binding; dopamine D_2 receptors were labelled with ^{3}H-spiperone using S-butaclamol as the baseline drug.

All drugs produced dose-related decreases in response rate under the FI schedule; dose-response curves were approximately parallel for all drugs. The rank order of potency for suppression of schedule-controlled behavior was: cis-YM-09151-2 (YM) > raclopride (RAC) ≈ haloperidol (HAL) > clozapine (CLZ) ≈ remoxipride (REM). All drugs also produced concentration-dependent inhibition of ^{3}H-spiperone or ^{3}H-SCH 23390 binding with pseudo-Hill slopes close to unity. The rank order of potency for displacing ^{3}H-spiperone from D_2 binding sites was similar to the rank order for suppressing schedule-controlled behavior (YM > RAC ≈ HAL > CLZ ≈ REM). However, the rank order of potency for displacing ^{3}H-SCH 23390 from D_1 binding sites differed considerably from the rank order for suppression of behavior (HAL > CLZ > YM > RAC > REM).

These results are consistent with the view that the behavioral suppressant effects of neuroleptics in monkeys are more closely linked to their antagonistic actions at D_2 than D_1 receptors. (Supported by USPHS Grants RR 00168, DA 03773, DA 00499.)

170.3 **EFFECT OF MEDIAL PREFRONTAL CORTEX LESIONS ON DOPAMINE TURNOVER AND DOPAMINE AGONIST INDUCED BEHAVIORS.** G.E. Jaskiw*, F. Karoum, W.J. Freed, J.E. Kleinman, and D.R. Weinberger. Clinical Brain Disorders Branch, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032

Indices of augmented dopaminergic transmission at subcortical sites in the rat have been demonstrated after various lesions of the medial prefrontal cortex (MPFC) and frontal cortex (FC). While it has been suggested that FC and particularly MPFC tonically inhibit subcortical dopamine (DA) systems, it is still not clear how destruction of MPFC neurons alone affects these systems.

Bilateral MPFC lesions were made in male rats (Sprague-Dawley 200-250 gr) using stereotactic injections of ibotenic acid (IA) (5 μg in 0.5 μl), an excitotoxin which preferentiallly affects neuronal bodies while sparing axons of passage. The lesions were confirmed histologically and neurochemically. Behavioral sensitivity to apomorphine was tested using a cumulative dose response schedule (.05, .25, .5 and 1.0 mg/kg) on post-lesion days 7, 14 and 21. Responses to d-amphetamine (2.5 mg/kg) on day 28 were also tested. After a 30 min. habituation phase, locomotion (photocell apparatus) as well as stereotypy (observational rating) scores were recorded for 90 min. following injections of saline and drug. Rats were killed on day 42. Quick frozen brains were sectioned on a cryostat (150 μm thickness) and punch biopsies taken of MPFC, nucleus accumbens, striatum, olfactory tubercle, substantia nigra and ventral tegmental area, for analysis of DA and its metabolites using HPLC. There were no significant differences between sham and IA lesioned animals in stereotypy or locomotion scores at any point during the testing. Behavioral results were also compared to results from biochemical analysis. Our findings are different from those reported following MPFC electrolytic lesions (Carter, C.J. et al. Br. J. Pcl., 62:402, 1978), and FC aspiration lesions (Scatton, B. et al. Brain Res., 232:331, 1982). Selective destruction of MPFC cell bodies may produce different effects than lesions which also affect axons of passage. These data also suggest that the influence of MPFC on subcortical DA systems is not one of simple tonic inhibition.

170.4 ATYPICAL ANTIPSYCHOTICS, LIKE D-1 ANTAGONISTS, INCREASE DOPAMINE METABOLISM BUT NOT RELEASE AT BEHAVIORALLY EFFECTIVE DOSES. C. A. Altar, W. C. Boyar, A. M. Wasley*, S. Gerhardt, J. M. Liebman and P. L. Wood. Neuroscience/Cardiovascular Res. , Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Behaviorally effective doses of clozapine (Wood et al, Prog. Neuropscyhopharm. Biol. Psych. 7:765, 1983) and CGS 10746B (Altar et al, Life Sci. 39:699, 1986) decrease or do not change 3-methoxytyramine (3-MT) levels and increase dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations. Because D-1 antagonists also increase dopamine metabolism but not release (Boyar and Altar, J. Neurochem. 48:824, 1987), the neurochemical activity of some antipsychotics may depend upon a D-1 mechanism, as suggested for clozapine and fluperlapine (Anderson and Braestrup, J. Neurochem. 47:1822, 1986). Using GC/MS analysis, 3-MT, DOPAC, HVA, and dopamine levels were measured in the caudate-putamen 60 min after the oral administration of 14 experimental or clinically effective antipsychotic drugs.

Male Swiss-Webster mice (18-26 g; Mbf:(SW)) received either the cornstarch vehicle (10 ml/kg) or a dose equal to or 6 times the ED_{50} dose for the inhibition of cage climbing induced by a subcutaneous injection of 2.0 mg/kg apomorphine.

DOPAC and HVA were increased by at least one dose of every drug tested. The drugs could, however, be readily categorized by their effects on 3-MT. Among the "atypical" antipsychotics (those relatively free of motor system side effects) or atypical antipsychotic candidates, clozapine and CGS 10746B decreased 3-MT at doses that increased or did not alter DOPAC and HVA. Fluperlapine, flumezapine, CL 77,328, BW 234U and RMI 81582 did not change 3-MT levels at doses that increased DOPAC and HVA levels. Thioridazine and mesoridazine increased 3-MT at the higher dose and increased DOPAC and HVA at each dose. Melperone and perlapine consistently increased 3-MT, DOPAC, and HVA, as did the "typical" (side-effect prone) antipsychotics haloperidol, chlorpromazine, and metoclopramide.

In summary, drugs associated with a low incidence of extrapyramidal side effects in humans or animal models increase dopamine metabolism but not release at doses effective in the apomorphine climbing test. Thus, antagonism of a cyclase-linked population of D-1 receptors may be a mechanism that contributes to the atypical profile on dopamine release identified here.

170.5 BLOOD LEVELS OF NEUROLEPTICS AND METABOLITES AND CLINICAL RESPONSE IN SCHIZOPHRENICS. Robert D. Edkins*, Thomas Mattio*, Samuel D. Shillcutt* and Douglas W. Hoffman (SPON: B. Manyam). Neurochemistry Lab., SIU Sch. of Med., Springfield, IL 62708.

There are both clinical and basic scientific difficulties in the current methods of neuroleptic quantification. Radioreceptor assays (RRA), relative to high performance liquid chromatography (HPLC), have the advantages of greater sensitivity combined with a measure of biological activity, but they cannot distinguish active metabolites or nonspecific interference from parent drug, as can HPLC. To resolve these problems, we are performing HPLC of neuroleptic drugs and metabolites in patient plasma followed directly by RRA of all the HPLC fractions, which provides the sensitivity of detection of the RRA coupled with the selectivity and resolution of HPLC.

Receptor binding assays for D_2, $alpha_1$ and sigma receptors are performed, as neuroleptic drugs and their metabolites have been shown to be pharmacologically active at several classes of neurotransmitter receptors. The antagonism of D_2 receptors by neuroleptic drugs is central to the dopamine theory of schizophrenia, but the activities of neuroleptics and their metabolites at other receptor sites may play a significant role in both their therapeutic and toxic actions, and so raise questions regarding an exclusive role for D_2 receptors in schizophrenia and neuroleptic activity.

Up to 50% of patients respond poorly to these medications or experience serious toxicity or severely disabling or discomforting side effects. We are using our method to compare the plasma levels of phenothiazine and metabolites in patients who are clinically defined as responders or non-responders to drug therapy, and in patients in whom side-effects develop. We have identified several different patterns of metabolism in patients with positive response to neuroleptic medication.

Acknowledgements: This work has been supported in part by the SIU School of Medicine Central Research Committee (DWH) and E.R. Squibb and Sons (DWH and SDS).

170.6 MOTOR AND POSTURAL EFFECTS OF HALOPERIDOL AND OTHER SIGMA LIGANDS AT A NON-DOPAMINERGIC RECEPTOR SITE. R. R. Matsumoto, W. D. Bowen, D. L. Gans*, K. D. Jones*, F. O. Walker*, J. M. Walker. Brown University, Providence, RI 02912 and Wake Forest University, Winston-Salem, NC 27103.

It has been suggested that the motor side effects of haloperidol and other antipsychotic drugs are mediated by dopamine systems. However, many antipsychotic drugs, including haloperidol, have been shown to bind to a non-dopaminergic subclass of sigma receptors. The possible involvement of this haloperidol-sensitive sigma receptor in posture and movement was tested in rats. Di-ortho-tolylguanidine (DTG), a selective ligand for the haloperidol-sensitive sigma receptor, and two other sigma ligands (haloperidol and +SKF10,047) were unilaterally microinjected into the red nucleus and substantia nigra pars reticulata, two areas rich in sigma receptors and known to be involved in posture and movement.

Unilateral microinjections of haloperidol hydrochloride and DTG acetate (9.3, 3.72, and 1.49 nmol) into the red nucleus produced dose-dependent torsional head angles that resembled the abnormal postures of clinical torticollis, a motor side effect that can be precipitated by antipsychotic drug treatment. +SKF10,047, a sigma ligand that is less potent than haloperidol and DTG in binding assays for the sigma receptor, also produced torticollis, but to a lesser degree than haloperidol and DTG. In addition to the abnormal postures, a noticeable reduction in spontaneous movement was observed in the animals. Although the actions of haloperidol, DTG, and +SKF10,047 in the red nucleus resemble some side effects of antipsychotic drug treatment, the effects are thought to be mediated by sigma receptors and not dopamine receptors since: (1) DTG binds sigma, but not dopamine receptors, and (2) the red nucleus is virtually devoid of any other type of receptor that may interact with either haloperidol or +SKF10,047 (i.e. the red nucleus contains almost no dopamine, norepinephrine, PCP, opiate, or 5-HT2 receptors).

A second group of animals received unilateral microinjections of one of five doses (5.0, 1.25, 0.31, 0.078, or 0.019 ug) of DTG acetate in the substantia nigra pars reticulata. Significant dose-dependent contralateral rotations were produced at doses as low as 0.078 ug. These activating effects of DTG in the pars reticulata are among the most potent effects ever reported in the nigra and suggests that an endogenous ligand for sigma receptors may be involved in movement. Haloperidol and +SKF10,047 were not tested in the pars reticulata since they cross-react with other non-sigma receptors that are also present in the nigra.

The findings suggest that sigma receptors may be involved in movement and posture. It also appears that at least some of the motor effects of antipsychotic drug therapy that are normally attributed to dopaminergic systems may in fact be mediated by haloperidol-sensitive sigma receptors.

170.7 ROLE OF THE A10 DOPAMINE NEURONS IN SENSITIZATION TO COCAINE AND AMPHETAMINE, P.W. Kalivas*, and B. Weber, Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA.

It is known that daily treatment with cocaine or amphetamine produces a progressive increase in the motor stimulant effects of these drugs. Since both of these drugs produce an acute psychostimulant effect by augmenting dopamine (DA) neurotransmission, it is generally presumed that an enhancement in the capacity of cocaine or amphetamine to augment DA neurotransmission may underlie the behavioral sensitization following daily administration. However, in one study it was found that daily microinjection of amphetamine into the nucleus accumbens (a DA terminal field) did not augment the behavioral response to subsequent peripheral amphetamine administration (Life Sci., 1981). Since it is now known that behavioral sensitization to peripheral opioid administration results primarily from an action in the A10 DA region (J. Pharmacol. Exp. Ther., 1987), we designed experiments to determine if amphetamine and cocaine may be acting in the A10 DA region to produce behavioral sensitization.

Male S.D. rats were implanted with chronic injection cannulae into the A10 or A9 DA region, or into the nucleus accumbens, striatum or lateral ventricles. Amphetamine (0.5-15.0 µg/brain) was bilaterally injected into the implanted brain region once a day for two days. One to two weeks later the rats were adapted to a photocell cage for 60 min, followed by injection of saline, ip. The next day following a 60 min adaptation the rats were injected with cocaine (15 mg/kg, ip) or amphetamine (1.0 mg/kg, ip) and motor behavior monitored for 120 min. It was found that rats pretreated with amphetamine in the A10 or A9 regions demonstrated a greater motor stimulant response to amphetamine and cocaine. However, the threshold dose for the A10 region was 0.5 to 1.5 µg of amphetamine, while it was 1.5 to 5.0 µg for the A9 region. In contrast, pretreatment with 15 µg amphetamine into the nucleus accumbens, striatum or lateral ventricles did not alter the motor stimulant effect of subsequent peripheral administration of cocaine or amphetamine. These data argue that the capacity of daily amphetamine or cocaine administration to produce behavioral sensitization may result from an action on the A10 and perhaps A9 DA perikarya. Since behavioral sensitization to these psychostimulants in humans can produce paranoid psychotic symptoms, these data argue that a drug-induced change in the function of DA perikarya, not terminal fields, may mediate this action.

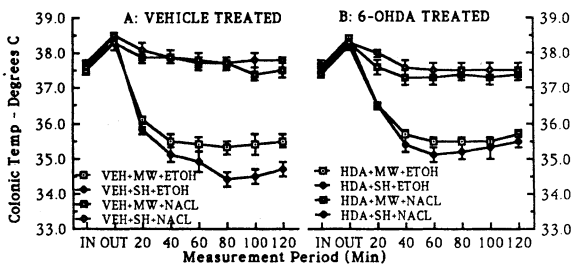
170.8 STRESS-INDUCED INSOMNIA: OPIOID-DOPAMINE INTERACTIONS W.Fratta, M.Collu*, M.C.Martellotta*, M.Pichiri*, G.L.Gessa*. Department of Neurosciences-University of Cagliari - Via Porcell,4 09124 Cagliari; Italy.

Platform technique of REM deprivation (REMD) in rats involves several factors such as isolation, immobilization, falling into the water, soaking and others which cause a heavy stress. A peculiar feature of this model is that the period of REMD is immediately followed by a constant period of wakefulness characterized by a high degree of motor activity, exploratory behaviour, increased alertness and reactivity toward enviromental stimuli. At the end of this period the rats fall asleep and show the first episode of REM sleep whithin few minutes. For the above reasons we have regarded this particular feature as a model of stress-induced insomnia. In order to understand the possible biochemical mechanisms involved in this phenomenon, we have investigated whether different treatments, known to affect opiatergic, dopaminergic and gabaergic neurotransmission, could specifically interfere whith this behaviour. The results obtained indicate that this peculiar phenomenon of excitement and insomnia is related to an hyperactivity of opiatergic and dopaminergic systems. In fact, excitement and sleep latency were significantly reduced by Naloxone while Morphine, β-Endorphin and DADLE have an opposite effect. Neither Diazepam nor the benzodiazepine antagonist Ro 15-1788 had any effect, suggesting that GABA systems do not play a specific role in this situation. A dopaminergic control, specifically mediated through D1 type receptors seems to be evident. Thus, while Sulpiride was ineffective, the selective D1 antagonist SCH 23390 was extremely potent in reducing the sleep latency and excitement following REMD. On the contrary, the administration of the specific D1 agonist SKF 38393 induced a marked opposite effect. These data do not exclude the possibility that other neurotransmitter systems might actively interact in this situation. However, these results indicate that opioid and dopaminergic systems, the latter specifically through D1 receptors, play a crucial role in this experimental model of stress-induced insomnia.

170.9 6-HYDROXYDOPAMINE BLOCKS 2.45 GHz MICROWAVE ATTENUATION OF ETHANOL-INDUCED HYPOTHERMIA IN RATS. D.L. Hjeresen and J.M. O'Donnell. Biophysics/Neurobiology Group, Los Alamos National Laboratory, Los Alamos, NM 87545

Recent work in our laboratory has focused on the possible interaction between low-level microwave irradiation and central neurotransmitter systems. Specifically, we have confirmed the report by Lai et al. (Bioelectromagnetics 5:213, 1984) that low-level microwave energy (Specific Absorption Rate = 0.3 to 0.6 W/kg, 2.45 GHz, CW) attenuates the hypothermic effects of ethanol (EtOH). Additional studies suggest that this effect is mediated in part by an interaction between microwave energy and beta-adrenergic neurotransmitter systems. That is, the microwave attenuation can be blocked by pretreatment with propranalol or sufficient doses of CGP-12177, both beta-adrenergic antagonists. The present study was conducted to determine if pretreatment with the noradrenergic neurotoxin 6-hydroxydopamine (6-OHDA) would also block the microwave attenuation of EtOH-induced hypothermia.

Ninety-six male and female Long-Evans rats were injected with 6-OHDA or vehicle (1 mg/kg, s.c.) at four days of age. At 60 days of age (mean body weight = 242.6 ± 24.2 g) rats were tested in a 2 X 2 X 2 design comparing pretreatment (6-OHDA/vehicle), microwave or sham irradiation (0.3 W/kg, 45 min, CW irradiation in circularly polarized waveguides), and ethanol (3.5 g/kg, 20% v/v, i.p.) or saline injection. Colonic temperature was monitored before and after irradiation and at 20 min intervals for 2 hr. Panel A of the figure demonstrates that microwave irradiation significantly attenuated the EtOH-induced hypothermia of vehicle treated rats. Panel B illustrates that neonatal pretreatment with 6-OHDA attenuated this microwave effect. No differences were noted on the basis of the sex of the animals. Analysis of central and peripheral tissues indicates nearly complete depletion of NE in 6-OHDA treated animals. The results support our hypothesis of a microwave interaction with noradrenergic systems. Supported by the U.S. Air Force School of Aerospace Medicine.



170.10 THE SITE OF THE ANTIEMETIC ACTION OF METOCLOPRAMIDE ON GASTRIC IRRITATION INDUCED EMESIS IN THE FERRET. J. D. Naylor* and G. E. Lucier (SPON: A. G. M. Bulloch). Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Metoclopramide is an antiemetic which is currently used to alleviate emesis due to antineoplastic drugs. The site of the antiemetic action of metoclopramide has not been clearly defined, although central and peripheral locations have been suggested. This drug is thought to block apomorphine induced emesis centrally by binding dopamine receptors in the chemoreceptor trigger zone (CTZ). Metoclopramide also blocks the visceromotor correlates of emesis, gastric relaxation and reverse peristalsis, and coordinates the aborad peristaltic activity of the gastroduodenum. This action on the efferent limb of the emetic reflex is dependent on the intramural release of acetylcholine (ACh) and possibly the antagonism of the intrinsic non-adrenergic non-cholinergic (NANC) inhibitory neurotransmitter. The purpose of this study was to identify the site of action of metoclopramide during electrical and gastric irritation induced emesis in the ferret.

Adult male ferrets, (0.7 to 1.3 kg) were anesthetized and maintained with a halothane/oxygen mixture. Emesis was elicited by electrical stimulation of the supradiaphragmatic vagal communicating branch as previously reported (Naylor and Lucier, IUPS Proc. 26:P261.12, 1986) and by intragastric administration of hypertonic saline (1.5 M). Each emetic episode was documented by recording tongue, diaphragm and abdominal muscle EMG, thoracic venous pressure and arterial pressure.

In order to show that electrical stimulation and gastric irritation were dependent on a common vagal afferent pathway, the right and left cervical vagal trunks were cooled. This resulted in failure to produce emesis with either stimuli. Following control elicitation of emesis by both electrical and gastrointestinal stimuli, metoclopramide (5 mg/kg IV) blocked vomiting produced by hypertonic saline but failed to have any effect on electrically induced emesis. The persistence of the response to electrical stimulation eliminates the possibility that metoclopramide is acting centrally at a medullary vomiting center, or indirectly through dopamine antagonism in the CTZ. The selective blockade of vomiting due to gastric irritation suggests that metoclopramide exerts this antiemetic effect by acting at the level of the gut, on the afferent limb of the emetic reflex. (Supported by the Alberta Cancer Board)

170.11 SPECIFIC D-1 AND D-2 DOPAMINE AGONISTS AND ANTAGONISTS HAVE SYNERGISTIC EFFECTS IN THE RAT 6-OHDA CIRCLING MODEL. C. Rouillard and P.J. Bédard, Lab. Neurobiology, Laval Univ., Québec, CANADA, G1J 1Z4.

The possibility of a functional interaction between D-1 and D-2 dopamine receptor subtypes has arisen from the recent use of highly selective D-1 and D-2 agonists and antagonists in biochemical, physiological and behavioral studies. In the present study, we have studied the effects of specific D-1 and D-2 agonists (SKF 38393 (2.5 mg/kg i.p.) and RU 24213 (30 mg/kg i.p.)) alone and the synergistic effects of a combination of half-doses of both drugs on circling behavior in rats with a unilateral 6-OHDA lesion of the nigrostriatal pathway. We have also studied the ability of SCH 23390 and sulpiride to antagonize these synergistic effects. Finally, we have investigated the ability of these specific antagonists to block the circling induced by two non-specific dopamine agonists (apomorphine and amphetamine). The results show that some animals responded to only one of the selective dopamine agonists alone, some responded to both but all responded to apomorphine and to the combination of half-doses of SKF 38393 and RU 24213. A powerful synergistic effect was demonstrated when specific dopamine agonists were given in combination. The circling response was greatly enhanced. The increase was 300% when compared to the circling response to SKF 38393 alone and 376% when compared to that of RU 24213 alone. Sulpiride (100 mg/kg i.p.) almost completely blocked the circling response (decrease of 78%) induced by the combination of these drugs. However, the D-1 antagonist SCH 23390 even at a dose of 3.0 mg/kg i.p. was totally inefficient. The blocking capacity of SCH 23390 and sulpiride was highly diminished in the apomorphine-induced circling model compared to the amphetamine-induced circling model where normosensitive dopamine receptors are involved. Amphetamine-induced ipsiversive circling was almost completely suppressed by prior administration of sulpiride (100 mg/kg, decrease of 91%) or SCH 23390 (3 mg/kg, decrease of 96%) while the same doses of antagonists were unable to block more than 65% of the apomorphine-induced contraversive circling. However, when we combined D-1 and D-2 antagonists, a more powerful blockade of the action of apomorphine was achieved (decrease of 83%). These data suggest an heterogeneous responsiveness to D-1, D-2, D-1 plus D-2 and mixed D-1/D-2 dopamine agonists and a strong synergistic effect of the combination of SKF 38393 and RU 24213. In our study, only the D-2 antagonist sulpiride was a potent blocker of that synergy. Denervation also induced some changes in the ability of specific dopamine antagonists to block the behavioral response after the administration of non-specific dopamine agonists. (Supported by MRC and Parkinson Foundation of Canada).

170.12 DIFFERENTIATION OF THE DIRECTLY ELICITED BEHAVIORAL EFFECTS OF TWO HALOPERIDOL-SENSITIVE SIGMA SITE LIGANDS, 1,3-DI-ORTHO-TOLYL-GUANIDINE (DTG) AND (+)3-(3-HYDROXY-PHENYL) N-(1-PROPYL) PIPERIDINE ((+)3-PPP). W.D. Essman[1]*, W. Koek[2]*, J.H. Woods[1,2], E. Weber[3]. Depts. of Psychology[1] and Pharmacology[2], Univ. Michigan, Ann Arbor, Michigan, 48109 and Dept. of Biochemistry[3], Oregon Health Sciences University, Portland, Oregon, 97201.

Two classes of phencyclidine (PCP) binding sites have been proposed; a PCP site associated with an NMDA-antagonist binding site, and a "sigma" site, characterized by high affinity binding of certain neuroleptics (e.g., haloperidol) and two new drugs, (+)3-PPP and DTG (Weber et al., 1986, Proc. Nat'l. Acad. Sci. USA 83: 8784-8788). The purpose of the present study was to evaluate the directly elicited behavioral effects of the two purported selective haloperidol-sensitive sigma ligands, DTG and (+)3-PPP, in rats. Upon intraperitoneal administration, (+)3-PPP produced a dose-dependent increase in elicited behaviors, e.g., increased locomotion (10.0 mg/kg), rearing (18.0 mg/kg), sniffing (18.0 mg/kg), and locomotion with the head held close to the floor (56.0 mg/kg), with clonic convulsions and death produced by doses between 100.0 and 320.0 mg/kg. Increased locomotion, rearing, and sniffing were elicited following administration of 10.0-32.0 µg of (+)3- PPP directly into the lateral ventricle; doses up to 560.0 µg icv did not produce the clonic convulsions and death observed after high doses administered ip. As reported previously, ip injections of DTG did not induce a PCP-like behavioral syndrome (Woods et al., 1987, Fed. Proc. 46: 951); 10.0 mg/kg elicited a still posture with the head resting directly on the floor of the observation chamber, while 32.0 mg/kg induced labored breathing, gaping, and death. DTG administered icv induced a distinct set of behaviors; 32.0 µg elicited an increase in forelimb extension and backward-walking and/or circling (pivoting backward around one hind limb). The percentage of rats displaying these effects increased dose-dependently to 100% at a dose of 100 µg. A dose of 320.0 µg icv induced gaping, loss of righting, and falling, but not death. It is not known what mechanisms or pharmacological factors mediate the different behavioral syndromes observed after administration of these two haloperidol-sensitive sigma ligands. Supported in part by USPHS Grant DA-00154.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM II

171.1 SOMATIC AFFERENT TERMINATION PATTERNS IN THE CAT INFERIOR OLIVE. H.H. Molinari and R.N. Sluyters*. Department of Anatomy, Albany Medical College, Albany, N.Y. 12208.

The gracile nucleus and lumbosacral spinal cord relay most of the somatic information from the hindlimb that reaches the inferior olive. These somatic afferents terminate in three distinct olivary subdivisions, the rostral dorsal accessory olive (rDAO), caudal dorsal accessory olive (cDAO), and caudal medial accessory olive (cMAO). A recent ultrastructural study (Molinari, 1987) demonstrated that in rDAO, gracile axons terminate primarily in dendritic thickets. The present study addressed the question of whether all somatic afferents to the different parts of the inferior olive terminate in a similar fashion.

Gracile terminals in cMAO and spinal cord terminals in rDAO and cDAO were labeled, as in Molinari (1987), by anterograde transport of wheat germ agglutinin-horseradish peroxidase and visualized with tetramethylbenzidine. The tissue was prepared for EM using a modification of Lemann et al. (1985). The postsynaptic elements receiving input from the labeled somatic afferents were classified as isolated, if they did not directly contact any other dendritic element, or part of a complex, if they did. The complexes were called dendritic thickets if the majority of postsynaptic elements were dendritic shafts (Sotelo et al., 1974); and called spine clusters, if the majority were spines.

Gracile and spinal cord terminals in all parts of the inferior olive contained round synaptic vesicles and formed asymmetric synapses and were presumably excitatory. In rDAO, the majority of spinal cord axons, like gracile axons, terminated in dendritic thickets. Synapses in dendritic thickets were seven times more common than were those on spine clusters. The majority of somatic afferents in cDAO and cMAO also terminated in complexes. In marked contrast, however, in these two regions synapses in spine clusters were as common as synapses within dendritic thickets. Thus, the dendritic thicket is the major target of somatic afferents in rDAO but not in either cDAO or cMAO.

Serial reconstructions of the spine clusters that received somatic input revealed that these spines arose from one or two dendritic shafts which were themselves generally part of a dendritic thicket. It is therefore possible that spine clusters develop from dendritic thickets and that the major difference between somatic afferents in rDAO and in cDAO and cMAO is the proximity of their synapses to the spines that arise from groups of olivary dendrites.

Supported by NSF Grant BNS-8506475.

171.2 **ORGANIZATION OF OLIVARY PROJECTIONS TO THE ANTERIOR LOBE OF THE CEREBELLUM IN THE RAT** S.A. Azizi, A. J. Painchaud, and D. J. Woodward *University of Texas Health Science Center at Dallas, Dallas Texas 75235*

As part of an ongoing study of the organization of the olivo-cerbellar system, in this series of experiments we have determined the detailed projections of the olivary neurons to the anterior lobe of the cerebellum in the rat. Small quantities (.01 to .2 µl) of *HRP-WGA* were pressure-injected into discrete areas of the anterior lobe of the cerebellar cortex in 38 rats. The sensitive chromogen tetramethyl benzidine was used to visualize the labeled neurons.

In previous studies we have divided the inferior olivary complex into seven lamellae (*JCN 1987*). Results of the present investigation demonstrated that three of these lamellae project to three distinct sagittal zones in the anterior lobe of the cerebellum. Clusters of cells located in the horizontal lamella (groups *a* and *b*) of the medial accessory olive project to a sagittal zone in the midvermal region of lobules I - V. Cells that constitute the dorsal fold of the dorsal accessory olive (caudal region of *DAO*) project to a sagittal zone located laterally to the above. Cells located in the ventral fold of the dorsal accessory olive project to a third sagittal zone in the cerebellum. In addition, a small group of neurons in the principal olive projects to a lateral zone of lobules IV and V, and a few scattered neurons in nucleus beta and *c* project to lobule I.

A microorganization was noted within the lamellae. Cells located in the lateral aspect of the horizontal lamella (group *a*) project to lobule III, while neurons located in medial aspect of this lamella (group *b*) project to lobules I and II. Similarly, neurons in the caudal lateral regions of the dorsal fold project to lobule III, while the more medial and rostral neurons project to I and II.

In summary, we have studied the organization of the olivary prjections to the anterior lobe of the cerebellum. Three slabs of olivary cells (lamellae) project to three sagittal zones in the anterior lobe of the cerebellum. The horizontal lamella of the *MAO* projects to a midvermal sagittal zone, while the two lamellae that form the *DAO* project to two sagittal zones located more laterally. A microorganization was discovered such that small clusters of cells within each lamella project to small patches within each sagittal zone These patches collectively form the sagittal strips.

Supported by DA-2338 and Biological Humanics Foundation

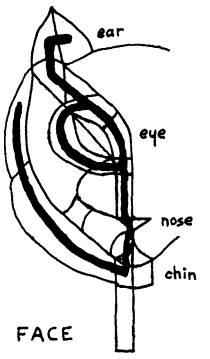
171.3 THE INFLUENCE OF GRANULE CELLS ON PURKINJE CELLS IN TACTILE REGIONS OF THE RAT CEREBELLAR CORTEX: AN IN VITRO INTRACELLULAR AND IN VIVO MULTI-SINGLE UNIT EXTRACELLULAR APPROACH. M. Rao, B. Rasnow, M. Nelson, and J. Bower. Divisions of Biology and Physics, Caltech, Pasadena, CA 91125.

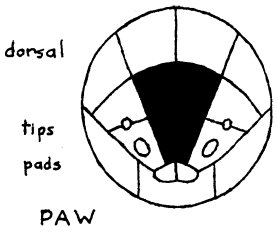
The overall objective of this is to understand input output relationships within cerebellar cortex. Specifically, tactile, mossy fiber inputs to the granule cell layers of the cerebellar hemispheres of the rat are organized in a patchy somatotopic fashion (Shambes et al., BBE 15: 94, 1978). Examination of the output of Purkinje cells (and thus the cortex) overlying these patchy projection patterns has revealed two somewhat surprising results (Bower and Woolston, J. Neurophysiol. 49: 745, 1983): first, no parallel fiber beam-like pattern of activity is evoked following activation of restricted granule cell layer loci, instead activated Purkinje cells are only found directly over active regions of granule cell layer; and second, in activated cells, short duration (5 msec) tactile stimuli result in rate changes in simple spike activity lasting hundreds of msecs.

We have pursued both results using two different physiological techniques. First, we have developed multi-single unit extracellular recording methods to sample activity in numerous Purkinje cells and their underlying granule cell layer locations at the same time. These techniques have allowed us to use tactile stimuli to restrictively activate small regions of the granule cell layer and explore subtle influences of parallel fibers on distant Purkinje cells. Second, we have used intracellular in vitro brain slice techniques to examine in detail the influence of granule cell layer activation of Purkinje cells. For example, using a slice cut parallel to the plane of the Purkinje cell dendrite and multiple stimulating electrodes positioned under the cell, we have found that brief (100 μsec) activation of the granule cell layer evokes a prolonged (200-300 msec) plateau potential that is graded with respect to stimulus intensity, and sums with stimuli delivered simultaneously at different underlying granule cell layer locations. The additional fact that this potential often induces prolonged increases in simple spike activity suggests that it may underlie the long duration responses of Purkinje cells to short tactile stimulation seen in vivo. Supported by NS 22205 and the Joseph Drown Foundation.

171.4 AGGREGATION OF COMPARTMENTS TO FORM CAT CLIMBING FIBER TACTILE RECEPTIVE FIELDS. Gin McCollum and Lee T. Robertson, Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, 1120 NW 20th Ave., Portland, Oregon 97212.

In sets of cells with intersecting receptive fields, the composition of the receptive fields is related both to the type of information they can encode and to the ensembles of cells that work together to encode it. Receptive fields are unions of compartments, the smallest units of skin surface that can be resolved by groups of climbing fibers in the anterior lobe. Analysis of 291 receptive fields shows that both face and paw receptive fields are usually unions of compartments that are neighbors on the skin. However, two neighboring toe tips or toe pads can constitute a receptive field, even though they are separated by intervening skin. On the other hand, two compartments which are neighbors on the skin can fail to behave as contiguous in receptive fields. For example, face receptive fields do not cross the jawline. Rather, they form lines of compartments, as indicated by the heavy line: from nose to ear above the eye, around the eye, and under the jawline. Receptive fields along the nosebridge and the jawline intersect at the chin. Paw receptive fields form medial-to-lateral lines among toe tips and toe pads, but in general their shape is more rectangular: when more than one toe is included, the same surfaces are included in all toes, as in the paw receptive field shown.





Cells with intersecting receptive fields are activated when the intersection of their receptive fields is stimulated. Groups of cells with few pairwise intersections can be separated according to the area of the skin they encode. This is true of cells with face receptive fields. On the other hand, cells with paw receptive fields are not easily separated into groups; information from all areas of the paw must be processed together.

171.5 FEATURES OF SOMATOTOPIC ORDER IN THE REPRESENTATION OF CUTANEOUS MECHANORECEPTOR INPUTS IN THE CEREBELLUM. K. L. Johanson and J. T. Wall. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Ascending circuits from mechanoreceptors in the skin to primary somatosensory cortex have been extensively studied, and are known to be somatotopically organized. The somatotopy of other circuits which process tactile information has received less attention. The present study characterized the somatotopic organization of low threshold cutaneous inputs to the cerebellum in folium 9a of the uvula in rats.

Normal adult rats were anesthetized with ketamine hydrochloride. The caudal cerebellum was exposed, covered with silicone fluid, and photographed. A tungsten microelectrode was angled perpendicular to the folium surface and multiple unit receptive fields to tactile stimuli were defined over a systematic array of penetrations. As indicated by depth readings and by locations of microlesions made during recordings, cutaneous responses were recorded from the granule cell layer.

Cutaneous mechanoreceptor inputs to folium 9a are characterized by the following features. (1) The receptive field location of neurons at any given recording site remains relatively constant throughout the depth of the granule cell layer. (2) Receptive fields are primarily located on the head, and involve the vibrissae pad, upper lip, caudal face, nose, scalp, and neck. Occasional forearm and forepaw receptive fields are also encountered. (3) All receptive fields are ipsilateral. The midline of the folium represents the junction between the two head representations. (4) Receptive fields are relatively large; e.g., vibrissae fields typically involve convergence of several vibrissae onto neurons at a single recording site. (5) Although there is some "between animal" and "between side" variability, each hemifolium consistently has a large mediolaterally oriented strip representing the vibrissae, which is adjacent to two relatively large, but split representations of the upper lip. (6) Within the representation of the vibrissae, inputs from each row diverge widely across the folium. Vibrissae in rows D and E activate neurons at more penetrations than vibrissae in other rows.

These results indicate that folium 9a contains an organized map of the head. The multi-vibrissae receptive fields, wide divergence of inputs from each vibrissae row, and split upper lip representation indicate this map is not as tightly somatotopic as the primary somatosensory cortical map of the comparable skin area. (Supported by Grant NS21105).

171.6 **INTERACTION OF VISUAL AND AUDITORY RESPONSES IN RAT CEREBELLAR PARAFLOCCULUS** Brian N. Maddux, S.A. Azizi, and D.J. Woodward *Dept of Cell Biology and Anatomy, University of Texas Health Science Center at Dallas. Dallas, Texas 75235.*

As part of ongoing studies of sensory inputs to the cerebellum, we have previously demonstrated anatomic connections from regions in secondary visual and auditory cortices via dorsolateral pons to the paraflocculus. In addition, we have demonstrated that parafloccular neurons are responsive to both physiologic auditory and visual stimuli. The goal of the current study was to understand the nature of temporal and spatial interactions among signals incoming to the cerebellar cortex via these sensory modalities. In the initial phase of this project, we report results of interactions of responses to pure tones with electrical stimulation in visual cortex.

Long-Evans female rats were anesthetized with halothane in oxygen. Bipolar concentric tungsten stimulating electrodes were implanted in area 18 of visual cortex. Unit recording in paraflocculus was accomplished with glass micropipettes and conventional unit recording apparatus. Real time computer software (UnitLab) was used for collection and analysis of data. Suprathreshold electrical stimuli consisted of double cathodal pulses (0.1 ms duration spaced 2 ms apart, intensities of 100-500 microamperes), and auditory stimuli were 10 kHz tones (200 ms duration at 85 dB). Preliminary results were obtained from 15 units which responded to electrical and/or auditory stimuli. Each unit recorded was subjected systematically to electrical, auditory, and combined stimulation.

Inspection of post-stimulus time histograms (PSTHs) revealed that the majority of cells responded to electrical stimulation with an excitatory peak at about 8-12 ms, followed by an inhibitory trough of variable duration. The peak of excitation ranged from 40% to 2300% over background activity. Each unit was also tested with auditory stimulation. Two units exhibited a relatively large (65% and 116%) sustained increase in activity during the tone. In four other cells, slight (15-20%) increases were recorded during the entire tone. The remainder of cells did not respond to auditory stimuli.

In the combined stimulus protocol, the electrical stimulus was delivered up to 50 ms after the onset of the tone. In seven cells, the excitatory response to electrical stimulation was accentuated in the presence of the tone. Two of these seven cells had not exhibited any response to auditory stimuli alone, and four others had shown only weak (15-20%) response to the tone. Inhibitory responses to electrical stimulation were slightly reduced in three cells during the tone, two of which displayed enhanced excitatory response. A reduction of both excitatory and inhibitory responses was seen in one cell.

In these studies, the alteration in the activity of a cell in the cerebellar cortex to a stimulus is dependent both on parameters of that stimulus and on the convergence of other inputs. It is clear that nonlinear summation of excitatory and inhibitory influences can occur. Also, significant interactions occur between inputs subthreshold for causing direct changes in Purkinje cell activity. We have particular interest in such subthreshold phenomena in view of recent suggestions that auditory processing in cerebellum may trigger, after a delay, learned motor responses which involve cerebellar circuitry. *Supported by grant DA-02338 to DJW and the Biological Humanics Foundation.*

171.7 CLIMBING FIBER REPRESENTATION OF CRUS I OF THE CAT CEREBELLUM. Stephen A. Elias* and Lee T. Robertson. Neurological Sciences Institute, Portland, OR 97209

This study examines the climbing fiber organization of crus I as part of a long term project to determine the representation of the body surface in the cerebellar cortex. This region of the cat cerebellar cortex has been shown using electrical stimulation, to receive both mossy fiber and climbing fiber input from the face and forelimb. Welker and associates have reported that in the rat, the mossy fiber face representation is restricted to a buried folium of crus I and organized in a patchy fashion. However, little information is available on the corresponding climbing fiber representation to tactile stimulation.

The data were obtained from 7 cats anesthetized with sodium pentobarbital. Individual Purkinje cells were isolated with extracellular recording. The climbing fiber responses were elicited by cutaneous (i.e., light mechanical taps with a force of less than 2.0g) and deep stimulation of the body surface. The receptive fields were delineated with von Frey hairs. Reconstructions of the electrode tracks and locations of the isolated Purkinje cells were made from histological sections.

In crus I, 15% (36/209) of the isolated Purkinje cells had climbing fiber responses evoked by tactile stimulation. The proportion of responsive units was similar for both crus Ia and crus Ip. Of the responsive units, 50% represented the forelimb, 11% the hindlimb, 11% the trunk, and 8% the face. The remaining 20% of the responsive units had split receptive fields, either trunk/forelimb, trunk/hindlimb, trunk/face or forelimb/hindlimb. About 65% of the receptive fields were classified as deep.

Climbing fiber responses representing the body surface were located mainly near the medial border with the vermal lobule VII and the border of crus IIp. Some of the responsive units were arranged in patches with similar receptive fields and modalities. Most of the unresponsive units were in the rostral half of crus I, and appear to be a continuation of the unresponsive area previously reported in crus IIa. In contrast to previous reports, no distinct face region for the climbing fiber system in crus I was observed.

Crus I contains a climbing fiber representation from a variety of body areas, most of which required stimulus forces higher than required for cutaneous activation. Nearly all of the responsive climbing fibers were located in the caudal part of crus I, with little or no body representation in the rostral region. (Supported by NIH grant NS 18242)

171.8 **IDENTIFICATION OF GRANULAR LAYER UNITS IN CEREBELLAR CORTEX.** P.L.E. van Kan*, W.G. Kruberg* and J.C. Houk. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

A previous report described the information content of presumed mossy fiber afferents as determined by single unit recording in the intermediate cerebellar cortex of alert monkeys (Van Kan, Houk & Gibson, Soc Neurosci Abstr 12: 578, 1986). To interpret information flow along the mossy fiber-granule cell-parallel fiber-Purkinje cell path, granular layer neural elements must be identified by adequate and reliable criteria. In the present study we have evaluated the applicability of a method that could be valuable for the identification of mossy fiber afferents in conscious animals. This method is based on the waveshapes of extracellularly recorded action potentials and has been used previously to identify granular layer neural elements in the turtle cerebellum (Walsh, Houk and Mugnaini, J. Neurophysiol. 37: 30, 1974).

10 Cats were anaesthetized with sodium pentobarbital. An array of 3 stimulating electrodes was inserted in the inferior cerebellar peduncle (ICP) for orthodromic activation of mossy fiber afferents. Stimuli consisted of single 50 μs pulses applied between different combinations of electrode pairs. Unitary potentials were recorded with tungsten microelectrodes (1.0-2.5 MΩ) in the anterior lobe of the intermediate cerebellar cortex. Determining the boundaries of the granular layer was aided by the characteristic features of the simple and complex action potentials of Purkinje cells and the shape of the field potential generated by ICP stimulation. Recording sites of single units were histologically verified by electrolytic lesions made at selected sites along each penetration.

Granular layer units were classified on the basis of waveshape. The most frequently encountered types were biphasic (positive/negative) or triphasic (positive/negative/positive) potentials that lacked an IS-SD break. They were of small amplitude (200-300 μV) and had a fast rise time (less than 100 μs). Many of these units were briskly activated from peripheral receptive fields. A subset was asynaptically activated by ICP stimulation. Orthodromic latencies of these units did not vary by more than 0.2 ms over a wide range of stimulus intensities. We attribute these potentials to mossy fiber afferents. Less frequently, we recorded units with larger amplitude waveshapes (1.0-1.5 mV) and more regular firing rates. These units were not activated by ICP stimulation and often receptive fields were not found. We attribute the latter potentials to Golgi cells.

Classification on the basis of waveshape in combination with multiple tests to associate these waveshapes with specific neural structures is potentially of great value because this method can be used to identify all mossy fiber afferents, regardless of their origin. This technique is especially promising for identification of mossy fiber afferents in chronic recording studies since identification under these conditions presents a dilemma for the experimenter: conclusive identification requires chronically implanted electrodes that, over the extended recording periods required, destroy afferent pathways and thus are threatening to the functional integrity of the cerebellum.

171.9 SIMULTANEOUS RECORDINGS FROM PURKINJE CELLS OF DIFFERENT FOLIA IN THE RAT CEREBELLUM AND THEIR RELATION TO MOVEMENT. M. Fukuda*, T. Yamamoto* and R. Llinás (SPON: J. Ransohoff). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Presently our multiple electrode recording technique has been increased from 64 (Yamamoto, Fukuda & Llinás: Soc. Nsci. Abst., 1986) to 96 simultaneously recorded Purkinje cells from various folia, in rats anesthetized with ketamine (100 mg/kg initial dose and 30 mg/kg every 2 hrs). The recordings include Crus 1 bilaterally, Crus 2 bilaterally, and Crus 2 plus a vermal folium. Climbing-fiber evoked complex spike crosscorrelations could be determined for any particular neuron with respect to any of the other 95 cells. As in previous reports, it was found that spontaneous activation of Purkinje cells via the climbing fiber system occurs in rostrocaudal bands in a close to simultaneous fashion (a time interval of less than one millisecond). A study of the onset of spontaneous firing within this rostrocaudal band of activity suggests that the inferior olive generates such patterns by a very rapid electrotonic conduction within the nucleus. However, in the mediolateral direction the degree of electrotonic coupling is variable due to synaptic shunting. Thus, only a mean value for electrotonic spread can be evaluated. This "mean conduction spread" may be expressed as an average conduction velocity of 25-50 cm/sec in the cerebellar cortex. In agreement with the above, mechanical stimulation of the perioral area induced climbing fiber activation in Crus 2 that may spread in either the mediolateral or lateromedial direction. This suggests that sensory input can serve as a pacemaker for electrotonic spread among inferior olivary neurons and that, as indicated above, the actual direction of electrotonic spread between cells in the mediolateral direction in the cortex may vary from one moment to the next, probably reflecting on-going electrical activity at the olive. Finally, the onset of spontaneous movement of the mouth and lips correlates well with the onset of synchronous climbing fiber activation. Recordings from Crus 2 indicate that the onset of movement, whether spontaneous or generated by harmaline, is preceded by a synchronous climbing fiber volley of the order of 5 to 10 msec. [Supported by NS-13742 from NINCDS.]

171.10 **COMPARISON OF DISCHARGE PATTERNS BETWEEN CEREBELLAR AND MOTOR CORTICAL NEURONS DURING WHOLE-ARM REACHING: ONSET LATENCIES, DIRECTIONAL PROPERTIES AND RESPONSES TO LOADS.** Pierre A. Fortier, John F. Kalaska and Allan M. Smith. Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Université de Montréal, Montréal, Québec, Canada, H3C-3J7.

Cerebellar and motor cortex neurons were recorded from monkeys trained to make whole-arm reaching movements from a common central starting position toward 8 radially oriented targets spaced at 45 degree intervals. The time of first change in firing frequency was calculated for neurons showing a significant activity change during the reaction time. The results of this analysis showed that there was no significant difference among the onset latencies of Purkinje cells, interpositus neurons, dentate units, and motor cortex cells. In general, the onset latencies were 132 ± 85 ms (mean ± SD) prior to the onset of movement in the cerebellar population and 120 ± 37 ms in the population of motor cortex cells. The direction related activities formed bell-shaped curves for both the motor cortical and cerebellar populations. However, statistical analysis of their directionality (sector width) revealed that the motor cortex discharge was more narrowly tuned for movement direction. The spontaneous discharge of the cerebellar population was 3 times greater than that of the motor cortex population. Since the sector width analysis takes into account the spontaneous discharge level, we recalculated the sector widths based on only the changes in activity during movement rather than absolute discharge frequencies. This correction reduced the difference between the motor cortex and cerebellar populations, but the motor cortex was still more directional ($p < 0.05$). The effects of loads pulling the arm in any of 8 directions were tested on the discharge frequency of 222 motor cortex cells and 58 cerebellar units. The loads produced relatively similar changes in absolute discharge frequency in the two structures: 26.4 ± 9.8 spikes/sec in the motor cortex and 36.6 ± 15.7 spikes/sec in the cerebellum. However, when expressed as a ratio of the no-load discharge rate of each cell, the relative load effect was much weaker in the cerebellum (load ratio 2.4 ± 1.6) than in the motor cortex (4.6 ± 6.8), due to the higher cerebellar spontaneous firing rates. In conclusion, the time course of activation of cerebellar and and motor cortex cells was very similar prior to movement onset. Cerebellar cells appeared less directional and less sensitive to loads than motor cortex, but this was partly due to their higher overall spontaneous discharge rates.

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171.11 CORRELATION BETWEEN PURKINJE CELL SIMPLE SPIKE ACTIVITY AND MOVEMENT KINEMATICS DURING CLOSED-LOOP, VISUALLY GUIDED MULTI-JOINT MOVEMENT WITH ALTERED GAIN AND DELAY FEEDBACK. D.C. Tam, T.J. Ebner, and C.K. Knox. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455.

The present study investigated the role of Purkinje cells in the feedback control of visually guided voluntary arm movements. Two Rhesus monkeys were trained to perform movement tasks requiring moving a two-joint manipulandum in a horizontal plane. The monkey was required to position a cross-shaped cursor in an initial box, and then move to a second target box displayed on a horizontal video monitor. Cursor position with respect to the manipulandum (hand) position was adjusted by a gain and/or an addition of a time delay to elicit corrective hand movements. The movements were unconstrained point-to-point movements without tracking requirements on the trajectory kinematics. Also the altered feedback conditions were randomly presented, preventing adaptation to the movement task. Two orthogonally placed targets were presented in random order to prevent anticipation. Various combinations of gain factors of 0.8, 1.0, and 1.2, and time delays of 0, 50, and 100 msec were tested.

Chronic extracellular recordings of 124 identified Purkinje cells in the intermediate zone of the anterior lobe showed that the simple spike discharge was modulated with movement in at least one movement direction (97/124). In 92% of the cells simple spike discharge increased (89/97) and 16% decreased (16/97) at the initial phase (onset) of movement for at least one movement direction. The patterns of discharge from the histograms aligned on either movement or target onset were sometimes different for movements orthogonal to each other, either with a latency difference between the onsets of discharge or with different discharge profiles. Following the initial change in simple spike firing, the firing pattern sometimes reversed sign (from an increase to decrease or vice versa) (19/97) in one movement direction. Spike triggered averaging techniques demonstrated that the simple spike discharge was correlated with the hand velocity (and/or acceleration) throughout the movement in the two-dimensional movement space. The correlation profiles to a target were similar independent of the feedback gain or time delays imposed, that is, independent of the corrective movements. Furthermore, the correlation profiles were similar for orthogonal movements except for a time lag in some cases. The patterns of EMG activity of arm and shoulder muscles were coupled to the onset of simple spike activity only. These results suggest the discharge of this population of Purkinje cells is correlated with hand kinematics independent of the imposed feedback alterations. Supported by NIH grant NS-18338.

171.12 ACTIVITY OF CEREBELLAR-RECEIVING THALAMIC NEURONS IN THE AWAKE MONKEY. M.E. Anderson and R.S. Turner* Depts. of Physiology and Biophysics and Rehab. Med. and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195.

Cerebellar information destined for the cerebral cortex relays in the thalamus. Although past studies have described the activity of thalamic neurons during active arm movements, the particular system in which they participated was not identified.

In three awake monkeys, we have identified 65 thalamic neurons that were shown to be excited at short latencies by stimuli applied through electrodes implanted in the contralateral cerebellar nuclei. These cerebellar-receiving (CR) thalamic neurons were further characterized by (1) their response during spontaneous active movements or passive joint manipulation, (2) their discharge pattern as the animal held the arm stationary prior to a visual target cue, and (3) their discharge during a visually-triggered arm reaching task.

The discharge of 28 of the CR neurons changed with respect to active or passive movement of specific body parts (driven cells). Eighteen of these were "arm-related" neurons, and of these, half were driven by manipulation or movement around the shoulder. Four cells changed their firing during movement of the hip or lower leg, and the discharge of the rest was related to eye or head movements.

CR cells tend to have relatively low, irregular "resting" discharge rates, usually less than 20 spikes/sec, in the absence of limb movements. In our sample, "driven cells" with changes in discharge related to proximal limb joints (shoulder, hip) had the highest "resting" rates, with the lowest variability.

Fifty-six of the CR cells, including all of the "arm-related" neurons, showed a task-related change in discharge during the arm-reaching task. Of these, 43 had an initial increase in discharge, and 13, an initial decrease. Of the 28 "driven cells" with task-related changes in discharge, 7 out of 8 of those with initial decreases in firing were driven by movements around the proximal joints (shoulder, hip) or by active eye movements. Of the "arm-related neurons, the earliest changes in firing occurred in "wrist" neurons (180 msec prior to movement initiation), followed by those related to the elbow and then the shoulder.

Thalamic neurons that receive excitatory input from the cerebellum can be identified, and their task-related changes in discharge can be related to parameters of the task and to the internal organization of muscles during task performance.

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BIOLOGICAL RHYTHMS IV

172.1 DIURNALLY INCREASED CELL FIRING IN THE ARCUATE AREA OF THE HYPOTHALAMIC SLICE DUE TO ESTRADIOL AND PROGESTERONE. R.R. Yeoman, A.J. Jenkins, L.E. White, J.N. Miller. Departments of Obstetrics-Gynecology, Neurology, Anesthesia, University of South Alabama, Mobile, Alabama 36688.

Previous studies have demonstrated a predictable afternoon luteinizing hormone rise in the ovariectomized estradiol-primed rat. We set out to record the firing rate of arcuate neurons in vitro to test the hypothesis that their electrical activity is responsive to estradiol (E) and progesterone (P) treatment and correlates in time with luteinizing hormone levels normally observed in vivo.

Female Sprague Dawley rats were raised in a 12:12 light dark cycle with lights on from 0500 to 1700h. At 27-29 days of age the animals were ovariectomized and implanted with capsules of E or cholesterol crystals. Two days later they were sacrificed, the hypothalamus sliced at 400 um and placed in a chamber perfused with oxygenated artificial cerebral spinal fluid alone or plus P (2ng/ml). Single neurons were recorded consecutively for 5 minute sampling times as the microelectrode was advanced during the experiments. Adequate E priming was confirmed by examination of uterine fluid content. In 10 experiments with E pretreatment alone (N=250 cells), units recorded from 1100-1500h had a slow discharge rate with hourly mean ranging from 0.7 to 0.86 spikes/sec. However, in the next hour, a significant increase in firing rate was observed (1.96 ± 0.19 spikes/sec, $P < 0.01$). In 5 additional experiments the rats were sacrificed 2 hours later, and 40 cells were recorded from 1400h through 1700h. An increase in firing rate was also noted after 1500h, suggesting a diurnal mechanism rather than a technical artifact responsible for the timing of activity increase. In 7 steroid control experiments with cholesterol capsules electrical activity was higher than with E before 1500h and did not have a diurnal pattern.

P was added to the superfusate at noon in 8 other experiments with E pretreatment (N=250 cells). A significant increase in firing rate was found from 1300 to 1400, compared to levels before P (0.8 ± 0.1 vs 1.7 ± 0.2 spikes/sec, $P < 0.025$) and compared to firing levels at the same time of day with estradiol pretreatment alone ($P < 0.01$). Activity peaked from 1400 to 1500h at 2.7 ± 0.3 spikes/sec, still earlier than an increase observed with E pretreatment alone. These changes correlated to the time of day that luteinizing hormone rises are seen in the intact animals. We conclude that the arcuate area appears to contain an intrinsic clock mechanism which is revealed by E pretreatment and modulated by P administration. This electrical activity most likely represents interneurons which may project to luteinizing hormone releasing hormone axonal endings and stimulate release of this hormone into the portal circulation.

172.2 **TWO MECHANISMS OF PHOTOENDOCRINE TRANSDUCTION IN CULTURED CHICK PINEAL CELLS**

M. Zatz and D.A. Mullen* Lab. of Cell Biology, NIMH, Bethesda, MD 20892.

Primary cultures of chick pineal cells, maintained under constant red light, display a circadian rhythm of production and release of the hormone melatonin. Single 4 hour pulses of white light caused an acute fall in melatonin release, independent of the phase of the melatonin cycle. These effects indicate a pathway from the photopigment to the melatonin synthesising system. Such light pulses also caused phase-dependent phase shifts in the melatonin rhythm. These latter effects indicate a pathway from the photopigment to the pacemaker generating the melatonin rhythm. Norepinephrine, acting through an α_2-adrenoceptor, also caused phase-independent acute falls in melatonin release but, unlike light, did not cause phase shifts.

Pertussis toxin, which can block the function of certain GTP-binding ('G') proteins, including G_i and transducin, blocked the acute effects of light or norepinephrine on melatonin release. It did not, however, block the ability of light to cause phase-dependent phase shifts. These results suggest the involvement of pertussis toxin-sensitive G proteins in the acute regulation of melatonin, but not in the pathway from the photopigment to the pacemaker. They imply the existence of two mechanistic pathways in the regulation of melatonin production by light.

172.3 **VOLTAGE-DEPENDENT CALCIUM CHANNELS REGULATE MELATONIN OUTPUT FROM CHICK PINEAL CELLS**

N. L. Harrison*, J. L. Barker and M. Zatz (SPON: T.G.Smith Jr.)
Lab. of Neurophysiology, NINCDS, NIH and Lab. of Cell Biology, NIMH, Bethesda, MD 20892.

Chick pineal cells in primary culture display a circadian rhythm of melatonin production and release. The nocturnal increase in melatonin output is suppressed both by inorganic calcium channel blockers (Co or Mn) and by dihydropyridine antagonists; conversely, melatonin output is enhanced by the calcium channel 'agonist' BAY K 8644. These data suggest the existence of 'L-type' Ca channels in the plasma membrane of these cells. Electrophysiological recordings were made at 22°C from individual chick pineal cells after 2-3 days in culture, using the whole-cell patch-clamp technique. In initial studies, the pipette solution contained 145mM K gluconate, 0.1mM $CaCl_2$ and 1.1 EGTA; the extracellular medium was a HEPES-buffered physiological saline containing 1.5mM Ca and 1mM Mg. The cells are of high input resistance (>1 Gohm), and do not generate TTX-sensitive action potentials. No transient outward current was observed, but all cells exhibit a TEA-sensitive sustained outward current. In the presence of TEA, presumed Ca-dependent action potentials are evoked by depolarizing current injection.

To isolate Ca currents, cells were bathed in a K-free solution containing 10mM Ca (or 20mM Ba) and 1mM Mg. The pipette solution contained 110mM N-methyl D-glucamine (NMG) methanesulphonate and 10mM NMG hydrochloride, plus 5mM BAPTA, 5mM ATP, 20mM phosphocreatine and 20U/ml creatine kinase. Cells were voltage-clamped at potentials between -100 and -40mV. From a holding potential of -100mV, a biphasic inward current was activated by steps to potentials positive to -40mV. The peak inward current during a step to 0mV was often 2-5 times greater than the steady-state inward current, indicating the existence of a rapidly-inactivating component. Both components of inward current declined at test potentials positive to +10mV and were abolished by 2mM Co. When the holding potential was -40mV, only a sustained inward current was activated during depolarizing commands. This was enhanced by BAY K 8644 and blocked by nifedipine. The inactivating current was insensitive to dihydropyridines. These two Ca currents are similar to those previously designated L-type (sustained) and N-type (transient) by Nowycky, Fox and Tsien (*Nature*, 316, 440-443, 1985). The above results indicate that voltage-dependent Ca channels are important in controlling the entry of Ca into, and the regulation of melatonin output from, chick pineal cells.

172.4 FMRF-AMIDE AND SEROTONIN HAVE OPPOSITE MEMBRANE EFFECTS ON CIRCADIAN PACEMAKER NEURONS IN Bulla. Jon. W. Jacklet. Dept. Biology, Neurobiology Research Center, SUNY Albany, Albany, NY 12222.

The pacemaker neurons in the basal retina of the eye of Bulla are responsible for generation of the circadian rhythm of autogenous CAP (compound action potential) activity recorded from the ON (optic nerve) of an isolated eye. It has been shown (Jacklet et al., J. Neurobiology, in press) that FMRF-amide immunoreactive efferent axons of brain neurons terminate in the retinal neuropil adjacent to the pacemaker neurons and in position to form synaptic contacts with the pacemaker neuron dendrites. Exogenous FMRF-amide suppresses CAP Activity, suggesting that the efferent axons are capable of suppressing CAP activity also. Now we show by intracellular recording from retinal pacemaker neurons as recording are also made from the ON that FMRF-amide hyperpolarizes the membrane potential and decreases the membrane resistance, suggesting a K conductance increase. The membrane potential of ca. -65 mV hyperpolarizes to -75 mV and membrane resistance decreases to 75% in 1 μM FMRF-amide as autogenous and light-evoked action potentials (ca. 80 mV) and 1:1 correlated ON Caps are suppressed. After FMRF-amide removal, the CAP rate and amplitude increase briefly as the membrane potential depolarizes, action potentials occur and membrane resistance increases, before returning to normal. Serotonin (1 μM) depolarizes the membrane potential and increases the membrane resistance resulting in a burst of action potentials and ON CAPs, suggesting a K conductance decrease. The serotonin effect is mimicked by 1 mM dibutyryl cyclic AMP. FMRF-amide reverses the effects of serotonin. CAP excitability and amplitude increase in serotonin and decrease in FMRF-amide apparently as a consequence of the membrane resistance changes. An increase in membrane resistance should increase the electrotonic coupling among the pacemaker neurons by shunting a greater proportion of current through the electrotonic junctions among the pacemaker neurons and decrease in membrane resistance should have the opposite effect. Changes in CAP amplitude should reflect changes in the number of pacemaker neurons contributing an action potential to each CAP. FMRF-amide and serotonin may produce these effects by acting on the s potassium channel. Supported by NSF BNS 8510626.

172.5 **DEPOLARIZING AND HYPERPOLARIZING IONIC TREATMENTS GENERATE PHASE SHIFTS IN BULLA OCULAR PACEMAKER THROUGH A CALCIUM DEPENDENT MECHANISM.** **G.D. Block and S.B.S. Khalsa. Department of Biology, University of Virginia, Charlottesville, VA 22901.**

The eye of the marine mollusc Bulla gouldiana contains a circadian pacemaker. Previous studies in our laboratory have implicated membrane potential and a calcium flux in the entrainment process since injection of depolarizing current into basal retinal neurons (BRNs) generates phase advances or delays and lowering extracellular calcium (10^{-7}M) blocks phase delays to light pulses (McMahon & Block, Neurosci. Abstr. 11:1138; 12:596).

The present experiments were undertaken to test the generality of these results with other treatments which alter membrane potential and to explore further the role of a calcium flux in phase shifting. We first tested whether elevated potassium pulses (50mM), a treatment which depolarizes BRNs, can phase shift the pacemaker. Eyes were removed from Bulla, recorded for one cycle, treated, and any resultant phase shift calculated as the phase difference in the time of midrise on the second cycle following the experimental treatment. We found that three hr pulses of Hi-K^+ at CT 13-16 generated a phase delay of -1.3 hr (±1.2 = 95% Confidence Interval, N=8) and a phase advance of +1.0 hr(±0.8, N=7) at CT 21-24. Two hr Hi-K pulses likewise generated significant shifts: -1.1 hr (±0.8, N=7) at CT 14-16 and +0.7 hr(±0.4, N=4) at CT 22-24. Similar to light-induced phase shifts, Hi-K^+ phase shifts were blocked in EGTA seawater ($Ca^{++}=10^{-7}$M). Hi-K^+ + EGTA at CT 13-16 resulted in a 0.0 hr shift (±0.5, N=6). Three hr EGTA pulses alone at this phase did not generate phase shifts (0.1hr ±0.5, N=3).

Lowered Na^+ solution (12mM), a hyperpolarizing agent for BRNs, also generated advances and delays but at different phases than depolarizing treatments. Lo-Na^+ pulses at CT 21-1 produced a phase delay of -1hr (±0.6, N=7), an advance of +0.7 hr (±0.5, N=8) at CT 8-11, and no phase shift between CT 10 - 13 (+0.2hr ±0.7, N=3). If hyperpolarizing treatments phase shift by reducing a Ca^{++} flux, EGTA pulses should mimic the action of Lo-Na^+. This appears to be the case since EGTA pulses generated phase delays during the late subjective night/early subjective day (CT 21-3, -1.2 hr ±1.0, N=5) and phase advances during the late subjective day (CT 8-14, +0.6hr ±0.5, N=4).

These results confirm prior observations that changes in membrane potential can phase shift the ocular pacemaker, provide new evidence that phase shifts to depolarizing treatments other than light are blocked by EGTA, and lend support to the proposition that depolarizing phase shifts are effected through increases in a calcium flux and hyperpolarizing phase shifts through a decreased flux. Supported by NS 15264, RCDA NS 00714

172.6 LIGHT-INDUCED PHASE SHIFTS OF THE BULLA OCULAR CIRCADIAN PACEMAKER ARE NOT BLOCKED BY CALMODULIN ANTAGONISTS. S.B.S. KHALSA and G.D. BLOCK. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA., 22901.

A population of retinal cells in the eye of the mollusc Bulla gouldiana functions as a competent in vitro circadian pacemaker. The circadian rhythm of compound action potentials recorded from the optic nerve can be phase shifted by light pulses. A light pulse (22 lux) applied at circadian time (CT) 13-16 yields a phase delay of 1.7 hr. (n = 9, 95% C.I. = 0.5), whereas a light pulse applied from CT 21-24 yields a phase advance of 1.1 hr. (n = 9, 95% C.I. = 0.7 hr.).

McMahon & Block (Neurosci. Abstr. 12, pg. 596, 1986) have shown that light-induced phase shifts can be blocked by reducing the extracellular calcium concentration, suggesting that a transmembrane calcium flux may be essential in the phase shifting mechanism. Since calcium is known to often serve as a second messenger via calmodulin, we have evaluated the hypothesis that calmodulin is directly involved in the mechanism of light-induced phase shifts by applying calmodulin antagonists in an attempt to block these shifts.

Light pulses were applied at CT 13-16 in the presence of calmidazolium, a highly specific calmodulin antagonist, at 10 uM with 0.1% DMSO. The observed shifts were not significantly different from shifts to light alone for delaying pulses (1.9 hr., n = 5, 95% C.I. = 1.0) or advancing pulses (0.7 hr., n = 6, 95% C.I. = 0.5). Calmidazolium alone, applied at the same or similar phases, was ineffective in perturbing the phase of the rhythm. Higher concentrations of calmidazolium were toxic and resulted in fewer expressed cycles, nevertheless, with 100 uM calmidazolium a typical phase delay was present on the first cycle of activity following the pulse (1.3 hr., n = 2). Furthermore, 10 uM calmidazolium was equally ineffective at blocking light-induced phase shifts at CT 13-16 when the light intensity was reduced to < 1 lux (low light + calmidazolium phase delay = 1.4 hr., n = 2; low light phase delay = 1.0 hr., n = 4, 95% C.I. = 0.9).

Trifluoperazine applied with light at CT 13-16 at 10 uM in 0.1% DMSO, against a control of trifluoperazine alone, was also ineffective at blocking light-induced phase shifts (2.2 hr., n = 4, 95% C.I. = 1.5). Variable results were obtained with the antagonist W7, although it appeared that phase shifts were not affected.

These data suggest that calmodulin is not involved in the light-induced phase-shifting mechanism of the Bulla pacemaker, at least not under our experimental conditions.

(Supported by NIH NS15264 and NS00714).

172.7 **CIRCADIAN RHYTHM REGULATION AND TWO DIMENSIONAL GEL ANALYSIS OF PROTEIN PHOSPHORYLATION IN THE APLYSIA EYE.** R. Zwartjes* and A. Eskin. Biol. Dept., Univ. of Houston, Houston, TX 77004.

Serotonin (5-HT) shifts the phase of the circadian rhythm of compound action potentials recorded from the isolated eye of Aplysia. 5-HT appears to act on the pacemaker through activation of adenylate cyclase and the resultant intracellular increase in cyclic adenosine-3',5'-monophosphate (cAMP). Forskolin, an activator of adenylate cyclase, mimics the effect of 5-HT on the rhythm.

In other systems the primary effect of cAMP is to activate cAMP-dependent protein kinase. Thus it is likely that the pathway through which 5-HT acts on the pacemaker includes the phosphorylation of at least one protein. To explore that possibility we have used two dimensional polyacrylamide gel electrophoresis (2D-PAGE) to examine the phosphoproteins of the eye.

One eye from each animal was placed into an experimental group, the other into a control group. Eyes were kept in darkness and incubated in $^{32}P_i$ for 16 hours before treatment. Shorter incubations resulted in variable incorporation of label. After treatment the eyes were rinsed and homogenized, then loaded on urea-acrylamide isoelectric focusing gels. The IEF gels were then run on SDS-PAGE gels which were subsequently autoradiographed. About 80 endogenously phosphorylated proteins are discernible on each gel.

Incorporation of ^{32}P into one protein consistently increased after 25 minute and 6 hour treatments with 5-HT ($5x10^{-6}$M) when eyes were treated at the phase when 5-HT produces its largest advance phase shift. The protein has an apparent molecular weight of 57,000 Daltons and a pI of 7.2. An increase in incorporation into this protein is also seen with a six hour treatment of forskolin ($1x10^{-6}$M).

As a test of the specificity of phosphorylation of the 57kD protein, eyes were treated with phorbol 12-myristate 13-acetate (TPA) ($1x10^{-6}$M) for six hours. TPA, an activator of protein kinase C, does not appear to shift the rhythm at the above phase. The phosphorylation of the 57kD protein was not changed by TPA.

From this evidence, the 57kD protein is worthy of further study as a component of the pathway through which 5-HT shifts the rhythm.

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172.8 CHRONIC CLORGYLINE ALTERS FORMAL PROPERTIES OF THE CIRCADIAN PACEMAKER IN SYRIAN HAMSTERS W.C. Duncan*, P.G. Sokolove, L. Tamarkin*, and T.A. Wehr*(SPON:M. Altstein)Clinical Psychobiology Branch NIMH Bethesda MD 20892 and the Department of Biological Sciences, University of Maryland Baltimore County, Catonsville MD 21228

Clorgyline(CLG), a type A monoamine oxidase inhibitor (MAOI) exhibiting antidepressant properties in humans was evaluated with respect to its capacity to alter a) the intrinsic period of wheel running (Experiment 1) and b) the phase-shift response to brief light pulses (Experiment 2). In Experiment 1, male hamsters, 13-16 wks old with ad lib access to food, water and running-wheels were housed in LD14.5:9.5 for 1 week and then DD for the next 8 weeks. After 3-4 weeks in DD, animals were implanted s.c. with Alzet minipumps (Model 2002) containing clorgyline (2 mg $kg^{-1} \cdot day^{-1}$; N=17) or saline (N=12); at two week intervals, empty pumps were replaced with fresh pumps. Periodogram analysis of wheel-running activity indicated clorgyline increased the intrinsic period of wheel-running compared to clorgyline pretreatment ($p<.005$) or compared to saline treatment ($p<.05$). This change in period was not associated with a change in phase due to the implantation of the clorgyline mini-pumps since extrapolation of activity onsets following treatment back to activity onsets prior to treatment did not indicate a significant change in phase. Chronic treatment with this MAOI increased the amount of wheel-running activity late in the active phase. In a similar experiment, we observed that bilateral enucleation did not prevent chronic clorgyline's capacity to increase the circadian period of wheel-running.

In Experiment 2, group-housed hamsters were pretreated for four weeks with clorgyline using Alzet minipumps. Animals were then placed in isolated cages containing running wheels. Following 7 days in LD 14.5:9.5, hamsters were 1) released into DD for seven days, 2) on day 8 received a 15' pulse of 250-300L fluorescent light and 3)monitored for the next 2 weeks in DD. Phase change was measured by calculating the linear regression to activity onsets prior to, and following the light pulse, and determining the phase change between the two regressions on the day after the pulse. Phase responses were pooled into twelve groups according to the phase of the light pulse relative to the onset of running activity. Two-way ANOVA indicated a significant treatment (CLG vs saline) by circadian phase interaction ($p< .03$). Compared to saline, CLG significantly increased the magnitude of phase delays at CT 14 ($p< .03$) and CT 16 ($p<.04$). CLG affected the phase($p< .032$) but not the slope of the crossover between phase delays and advances. We conclude that in the Syrian hamster clorgyline a) increases the period of the circadian pacemaker, b) this effect does not require an intact retina, and c) alters the response of the circadian pacemaker to brief light pulses. These results support the hypothesis that chemical antidepressants alter formal properties of the mammalian circadian pacemaker.

172.9 SUPRACHIASMATIC NUCLEI SEROTONERGIC FIBERS DO NOT MEDIATE THE EFFECT OF MONOAMINE OXIDASE INHIBITORS ON HAMSTER CIRCADIAN RHYTHMS J.S. Kruse Center for Brain Research, University of Rochester School of Medicine, Rochester, New York 14642.

LY51641, a type A monoamine oxidase inhibitor (MAO-I), delays the onset of wheel running in hamsters entrained to a light-dark cycle. These delays in entrainment of circadian rhythms are similar to results in other laboratories with a variety of antidepressant drugs, including tricyclics, MAO-Is, and lithium (Wirz-Justice 1983). All of these drugs disrupt serotonin neurotransmission. Chronic, peripheral administration of LY516141 inhibits serotonin catabolism in the suprachiasmatic nuclei (SCN), as measured by HPLC with electrochemical detection. The following experiment was conducted to determine whether the serotonergic raphe-SCN pathway mediates the delay induced by LY51641 in the circadian rhythm of wheel running.

Male golden hamsters weighing 100-120 grams were kept in L:D 14:10 with constant access to running wheels. After two weeks of baseline running, the serotonergic neurotoxin, 5,7-dihydroxytryptamine, was injected stereotaxically (bilaterally, 0.3 µl, 10 mg/ml) with the needle aimed at the dorsocaudal end ofthe SCN. Running wheel data were collected for two weeks following surgery; all hamsters remained entrained to the L:D cycle. Osmotic minipumps were then implanted subcutaneously to deliver 10 mg/kg/day LY51641 for two weeks. All hamsters (15/15) demonstrated a delay in running wheel onset during drug treatment. The delay (34 +/- 14 minutes) was comparable to the delay (44 +/- 19 minutes) induced by identical LY51641 treatment in hamsters that had not received stereotaxic injections of the toxin. Immunocytochemistry for serotonin (ABC method) was used to ascertain the accuracy and specificity of neurotoxic lesions. Since hamsters with no detectable serotonin in the SCN region still delayed the onset of their wheel running in response to LY51641, serotonergic fibers are not essential for this drug to effect the circadian clock.

172.10 THE EFFECTS OF THYROPARATHYRIODECTOMY (TPX) AND CALCIUM DIETS (Ca) ON ACTIVITY RHYTHMS AND LEVELS IN MALE AND FEMALE RATS. J. Schull*, J. Cooper*, L. Hillivirta*, K. Fitzgerald*, D. McEachron, J. Ruckdeschel*, D. Schumacher*, D. Stanger*, and J. Walker*, Dept. of Psychology, Haverford College, Haverford, PA 19041

Considerable clinical research has been reported linking affective disorders with abnormal circadian rhythms and with thyroid and parathyroid disfunction. The question remains as to whether these 2 biological phemonena, both of which are linked to affective disease, are somehow linked to each other.

To investigate this question, fourty-eight rats were divided into 4 equal groups: 1. male controls; 2. female controls; 3. male TPXs; and 4. female TPXs. Each animal was placed into an individual running wheel cage where activity was monitored by microswitches attached to a computer. There were 4 phases to the experiment: entrainment (LD 12/12), free-run, free-run on normal dietary Ca, and free-run on a low Ca diet. After the initial free-run period, 1/2 the animals in each group were placed on the low Ca diet while the other half were fed the normal diet. After 10 days, these conditions were reversed and continued for a second 10 days. Period (tau) estimates were determined on computer-generated activity plots by blind rating.

In the free-run phase, females displayed significantly shorter taus than males ($p<0.01$), and TPXs showed shorter taus compared to controls ($p<0.01$). There was an interaction between sex and surgery-female TPXs displayed shorter taus than did male TPXs ($p<0.03$).

Also in the free-run phase, females were significantly more active than males ($p<0.01$) and TPXs were more active than controls ($p<0.01$). Again, there was a significant interaction-male TPXs displayed a great-er increase in activity over controls than did female TPXs ($p<0.01$).

The low Ca diet reduced blood calcium in TPXs by 40% but no significant effects on activity were observed. An apparent shortening of tau with low Ca in female TPXs is presently being investigated.

In summary, thyroid/parathyroid function does appear linked to circadian rhythms and this linkage is influenced by an animal's sex.

172.11 CIRCADIAN RHYTHMS AND ESCAPE LEARNING IN RATS EXPOSED TO SHOCK. K.T. Stewart*, A.M. Rosenwasser, J.R. Volpicelli*, H. Hauser*, and N.T. Adler. Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

Although disturbances in biological rhythmicity have been implicated in the affective disorders, their role has not been established. We have attempted to develop a paradigm based on a well-known animal model of depression in order to explore the relationship between behavioral pathology and changes in circadian rhythmicity.

It has been established that 60-80% of rats given inescapable, but not escapable shock, exhibit several motivational, associative, physiological, and neurochemical deficits which are similar to those seen in many depressed patients. In addition, these animals fail to learn to escape shock even when it is possible to do so. This phenomenon has been described as "learned helplessness", and it can be reversed by the administration of anti-depressant drugs. We employed this animal model in order to examine the relationship between pathological behavior and changes in circadian period.

Free-running circadian rhythmicity was monitored in wheel-running cages for several weeks before and after four sessions of inescapable footshock administered halfway through the animals' "subjective day". We reported last year that several rats showed increases in the free-running period of wheel-running rhythms following shock.

We now report on the relationship between period change and escape learning. We conducted several studies showing that animals which did not show impaired escape learning when tested with escapable shock exhibited increases in period. On the other hand, those animals which did show impairments in escape learning showed no change in the period of activity rhythms.

Thus, inescapable shock resulted in lengthening of period only in animals which did not exhibit deficits in escape learning. These results suggest that changes in the circadian system may be a mechanism involved in adaptive responses to environmental stressors. Others have shown that animals may alter the temporal pattern of behavior in response to stressors such as the presence of a dominant conspecific. It has also been demonstrated that administration of anti-depressant medications and lithium results in increases in free-running period of circadian rhythms; these effects on the circadian system may underlie their therapeutic efficacy.

172.12 EFFECTS OF COMPLETE AND PARTIAL SCN LESIONS ON ULTRADIAN AND CIRCADIAN LOCOMOTOR ACTIVITY RHYTHMS IN LEW/ZTM RATS. F. Wollnik and F.W. Turek. Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Both circadian and ultradian rhythms in locomotor activity have been observed in laboratory rats. However, while the neural pacemaker involved in the regulation of the circadian activity rhythm (as well as many other rodent circadian rhythms) appears to be located in the suprachiasmatic nuclei (SCN), the neural basis for the generation of behavioral ultradian rhythms remains unclear. Complete and partial lesions of the SCN were used to determine if the same neural substitute may underlie both circadian and ultradian rhythms in the wheel-running activity of LEW/Ztm rats. This inbred strain of laboratory rats exhibits very precise and reproducible ultradian rhythms with periods of 4 and 4.8 h in locomotor activity (Büttner & Wollnik, Behav. Genet., 14:137, 1984) as well as in heart rate and body temperature.

Male and female rats of this strain were maintained under light-dark entrainment (LD 12:12) and under free-running conditions (blinded animals). They received complete or partial SCN lesions between days 35 and 50 after initially being housed in running-wheel cages. Wheel-running activity was recorded for a total of 90 days using an on-line microcomputer system. To detect rhythmic components, activity records were subject to chi-square periodogram and harmonic spectral analyses.

While sham lesions had little or no effect on the wheel-running activity pattern, complete SCN lesions resulted in a complete loss of ultradian and circadian rhythms in both male and female animals. The absence of ultradian and circadian rhythms was observed in blinded, free-running animals as well as in those maintained under LD 12:12 conditions. Under both free-running or entrained conditions, ultradian and circadian rhythms were still present after partial SCN lesions, with their amplitude dependent on the size of the lesion. Periodogram analysis for any given animal revealed that the period of the ultradian rhythm was always a submultiple of the entrained or free-running circadian period. Furthermore, a close relationship between ultradian and circadian rhythms was suggested by a high correlation between the amplitudes of their spectral estimates.

The present results indicate that the SCN contributes to the control of both ultradian and circadian rhythms in wheel-running behavior of LEW/Ztm rats. However, it is not clear if ultradian rhythms are caused by an ultradian pacemaker within the SCN or if they are the result of desynchronization among a population of circadian oscillators. Further examination of the LEW/Ztm strain might be useful in developing a better understanding of the origin and function of behavioral ultradian rhythms.

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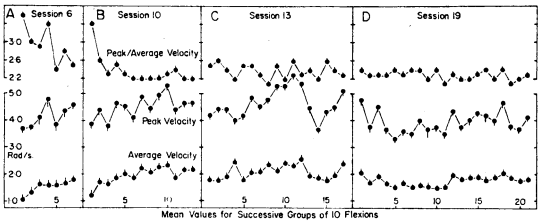
LEARNING AND MEMORY: PHYSIOLOGY I

173.1 PERMANENT LEARNING OF TEMPORARY MOVEMENT ADAPTATIONS BY MONKEYS. V.B. Brooks and S. Watts*. Dept. Physiology, Univ. of Western Ontario, London, Ont. N6A 5C1, Canada.

Adaptations of "continuous" elbow movements (single-peaked velocity profiles) were studied during motor learning of two monkeys. The animals were rewarded for what they did, namely to carry out a step-tracking task with an elbow movement followed by prolonged holding of the arm in the target; but not for how they did it, that is, for any particular mode of movement execution. Rewarding task-related behavior (what to do) by making holding periods long in relation to move-times, so that shortening move-times could not increase rewards significantly. Yet, both animals developed the use of continuous, i.e. programmed, movements with short move-times when they began to understand the behavioral task requirements [cf Brooks, Kennedy, Ross 1983, Physiol Behav 31: 561-563].

Two kinematic movement adaptations of continuous movements were noted, namely increases of (1) peak velocities and (2) average velocities such that their ratio remained steady or decreased. These non-rewarded, adaptive changes tend to optimize movement performance, although our data could not indicate which parameter was being optimized because most continuous movements were not time-symmetrical [cf. Hogan 1984, J Neurosci 4: 2745-2754].

The adaptations occurred again and again in successive daily training sessions, covering the same range of values. Only after about 2 weeks, when the animals approached their best performance proficiencies, were the adaptations learned, that is incorporated into movement programs that were remembered from one session to the next. The change from nonremembered adaptation to remembered learning occurred in one, transitional session [Brooks, Watts 1987 Exp Brain Res. in press]. These simple kinematic observations present new opportunities for future studies of neural actions in memory formation of behaviorally active animals. (MRC of Canada)



173.2 ASSOCIATIVE LTP OF SELECTIVE SPINAL REFLEXES. R.G. Durkovic. Department of Physiology, SUNY Health Science Center, Syracuse, NY 13210.

Potentiation of certain spinal reflexes has been shown to develop as a consequence of cutaneous nerve stimulation presented in an associative conditioning paradigm (Neurosci. Lett. 39:155). The purpose of the present study was to examine the persistence of this conditioned reflex facilitation. Changes in the magnitude of reflexes evoked in semitendinosus (ST), tibialis anterior (TA) and extensor digitorum longus (EDL) muscles of the spinal cat were examined. The CS was 10 Hz electrical stimulation of the saphenous nerve for 1.5 sec (random intertrial intervals averaged 3 min) for both experimental and control animals. The US was 30 Hz stimulation of the superficial peroneal nerve for 0.5 sec. Nerve recordings assured constant afferent inputs which involved all A-alpha and A-delta fibers of both cutaneous nerves. Experimental animals received 30 paired trials of CS and US with a 1.0 sec interstimulus interval. Control animals received the same stimuli, but the US was delivered in the middle of the CS intertrial interval. Following acquisition all animals received 30 additional CS alone trials at 5 min intervals. Low intensity probe CS alone trials, subthreshold for A-delta cutaneous fibers, were presented intermittently throughout the experiment in order to monitor excitability of the "A-alpha spinal circuitry".

During acquisition the response to the CS increased over trials for ST and TA muscles in experimental animals, while those of controls decreased. Little change in EDL responses was observed in either experimental or controls. During the subsequent 2.5 hr (CS alone) period differences between experimental and control animals' ST and TA muscle responses were maintained while the EDL responses of the two groups did not differ. The probe CS responses of the two groups did not differ for any of the muscles. The results show that the classically conditioned reflex potentiation is long-lasting and specific to certain flexor muscle reflexes. Furthermore, changes in cutaneous primary afferent output and generalized increases in motoneuron excitability may be eliminated as mechanisms underlying the associative LTP effects. This spinal cat preparation exhibits the major characteristics of intact mammalian learning and memory behavior, is representative of a complete behaving system (peripheral afferents, central processor, motor efferents), and has the potential for definition of the neural circuitry and the causal events underlying the response plasticity.

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173.3 The coding of spatial information by neurons in the primate hippocampal formation.

J. Feigenbaum*,P.M.B.Cahusac*, and E.T. Rolls. University of Oxford, Dept. of Experimental Psychology, South Parks Road, Oxford OX1 3UD, England.

Damage to the primate hippocampus is known to disrupt the learning of some spatial tasks (see Gaffan, 1987). In order to analyse how spatial information might be processed in the hippocampus we have analysed the activity of 245 single neurons in the hippocampal formation (hippocampus and adjacent gyrus) in the rhesus monkey performing spatial tasks. These tasks included object and place memory tasks, for the performance of which the hippocampus is required (Rolls, 1987). 26.7% of the neurons had spatial response properties, in that they responded when stimuli were in one position but not in another on the test screen or in the laboratory. (These 'space' cells are different from the 'place' cells described in the rat by O'Keefe (1983) and others, which fire when the animal is in a particular place in the room.) For 6% of neurons, the responses remained in the same position relative to the monkey's body axis when the screen was moved or the monkey was rotated or moved to a different position in the laboratory. These neurons thus represented space in egocentric coordinates. For 17% of neurons, the responses remained in the same position on the screen or in the room when the monkey was rotated or moved to a different position in the laboratory. These neurons thus represented space in allocentric coordinates. These results provide evidence that in addition to neurons with egocentric spatial fields, which have also been found in other parts of the brain, there are neurons in the primate hippocampal formation which encode space in allocentric coordinates.

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O'Keefe,J. (1983) Spatial Memory within and without the hippocampal system. Ch. 20, in Neurobiology of the Hippocampus, ed. Seifert, London, Academic Press

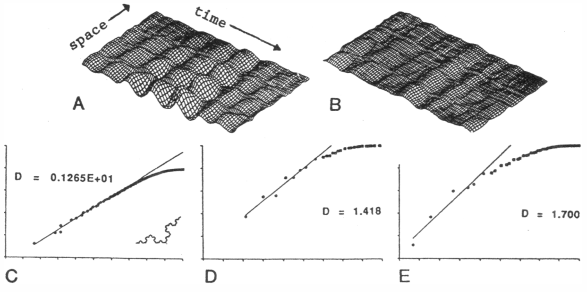
173.4 **FRACTAL DIMENSIONALITY-INCREASE OF THE 40-80 Hz SURFACE POTENTIALS ON THE RABBIT OLFACTORY BULB FOLLOWING PRESENTATION OF A NOVEL ODOR.** **J.E. Skinner, B. Saltzberg*, J.E. Cunningham*, W. Burton*, C.E. Landisman*, and M. Mitra*. Neurophysiology Section, Neurology Department & Neuroscience Program, Baylor College of Medicine, Houston, TX 77030 (Spon. P. Kellaway)**

As in the neocortex, an event-related slow potential (ERSP) is evoked on the surface of the olfactory bulb by presenting a novel odor. Concurrent with the ERSP are spatial (Gray, Freeman & Skinner, Behav.Neurosci.,1986) as well as temporal changes in the bulbar surface activity (Fig. 1A,B). These changes in the two domains are rapid, suggesting a fractal process. Babloyantz & Destexhe (Proc. Natl. Acad. Sci., 1986) described a new method for determining fractal dimensionality of synchronous EEG potentials; they found that when a human subject shifted from waking to a less attentive state (e.g., drowsiness or petit-mal absance), the fractal dimensionality coefficient reduced.

We modified their method and estimated the dimensionality coefficient, in the time domain, by the slope of: (x-axis) log R/Ro (normalized range of differences between zero-crossing intervals) vs (y-axis) accumulated log N/No (accumulative sum of normalized number of cases). First we determined that our method is valid by calculating the dimensionality for a known fractal function (Koch-triangle function); our value (Fig 1C) was the same as that calculated by another method (D = 0.126) by Mandelbrodt (Fractal Geometries of Nature, 1986). Then, we found that the dimensionality increased, from 1.4 during the 1/3-sec of quiet wakefulness just before a novel odor (Fig 1D) to 1.7 during the more attentive 1/3-sec after the odor (Fig 1E).

An explanation for bulbar fractal dimensionality changes is that the underlying molecular activities are themselves fractal. Hess & Markus (TIBS, 1987) demonstrated fractal processes for some biochemical reactions and Liebovitch et al. (Biophys. J., 1987) showed that single-channel ion-kinetics are best modeled by fractal functions.

Our finding (i.e., that an increase in processing state is associated with an increase in fractal dimensionality) is consistent with that of Babloyantz and Destexhe. Thus the two studies together demonstrate that fractal dimensionality may be a useful measure along a spectrum of cognitive processing-states in a cortical or model-cortical system.



173.5 SELECTIVE PICTORIAL INFORMATION IS RETAINED BY NEURONS IN THE VENTRAL TEMPORAL CORTEX OF THE MONKEY DURING THE DELAY PERIOD OF A MATCHING-TO-SAMPLE TASK. Y. Miyashita*, K. Cho* and K. Mori* (SPON: M. Ito) Dept. Physiol., Fac. Medicine, Tokyo Univ., Tokyo, Japan.

Visual delayed matching-to-sample (DMS) tasks have been used widely for testing memory functions in behavioral studies. However, single-unit recordings have been unsuccessful in revealing the neuronal correlates for the retention of visual memory during the delay period of the task, except for the sustained neuronal discharge reported in a delayed color-matching task (Fuster & Jervey, 1982). No neurons have been known to retain shape information during such a delay. We report here that neurons in the ventral temporal cortex of monkeys (Macaca fuscata) exhibit sustained activity during the delay period of a visual DMS task, and that the activity is highly selective to pictorial or configurational information contained in cue signals.

In a trial of our DMS task, sample and match stimuli were successively presented on a video monitor, each for 0.2sec with an interval of 16sec. The stimuli were newly selected for every trial among 100 computer-generated colored fractal patterns and 100 pseudo-colored images of scenery. The monkey was to choose a "go" or "no-go" response, according to a match or non-match combination.

Of the 144 single neurons recorded in the anterior ventral temporal cortex (areas TE and 35), 95 neurons showed an increased or decreased discharge frequency during the delay period, compared to that during the inter-trial period. In 77 of these 95 cells, discharge frequency during the delay was selective to certain cue stimuli, while in the remaining cells, there was no such selectivity. A substantial number of the 77 cells showed especially high selectivity; only a few pictures (sometimes just one) produced strong excitation or inhibition during the delay, whereas other pictures were ineffective. The optimal picture differed from cell to cell. Since the stimulus-selective discharge during the delay was not necessarily preceded by firing changes during the sample period, it did not simply represent sensory afterdischarge.

To further analyze stimulus-selective properties of the delay discharge, 1) stimulus size was reduced by half, 2) stimulus was rotated by 90 degrees in a clockwise direction, 3) colored stimulus was transformed into monochrome by referring to a pseudo-color look-up table, and 4) stimulus position was changed on the video monitor (a 0.2sec stimulus presentation time is short enough to exclude a contribution of saccadic eye movement). These manipulations did not alter stimulus-selectivity during the delay in many of the tested neurons. Thus, it is most likely that the discharge during the delay reflects a mnemonic process which is related to identification or extraction of configurational information in the picture. We propose a hypothesis that these neuronal activities are associated with a pictorial working memory.

173.6 DIRECTION CONTINGENT FIRING OF HIPPOCAMPAL PLACE CELLS C.R.Breese*, R.E.Hampson*, and S.A.Deadwyler. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

Hippocampal place cells tend to fire rapidly when a freely-moving rat enters a particular portion of its environment. Theoretical attempts to explain the firing patterns of these cells include: location in absolute vs relative space; distance from the center of the place field; and difference between the present and future location of the animal (demonstrated by shifting the cell firing along the time and location dimension; Muller & Kubie, Soc. Neurosci. Abs. 141.8, 1986). Place cell firing was analyzed with respect to the above theories in an attempt to explain the nature of cell firing within the place field, and its possible relation to exploratory behavior.

The experimental apparatus consisted of an elevated platform (56 sq cm) with small water cups in the middle and at each corner. Complex spike (CS) cells from CA1 and CA3 hippocampal fields (Christian & Deadwyler, J. Neurophys. 55:331-348, 1986) were isolated and tested for place field firing, as indicated by rotations of prominent sensory stimuli. Results indicated a strong place specificity for all complex spike cells ($CS=6.52\pm1.10$, $p<0.01$), and a correlation of increased cell firing as the animal moved toward the center of the field ($t(260)= -5.89$, $p<0.01$). This trend, however, was nonlinear ($r=-0.34$), indicating that firing rate was not totally dependent on distance to the center of the field. Subsequent analyses revealed that maximum firing occurred when animals entered or exited a particular location (place field) on the platform, suggesting a strong "boundary" of the place field. The increased firing upon entering the place field corresponds to Muller & Kubie's observation of convergence of the place field with the animals' anticipated move to a new location. The observed increase in firing rate upon exiting the field has not been previously reported and indicates that place cells also code for exiting as well as entering the place field. A further analysis of the animals' traversal within a particular place field revealed that the cells tended to fire in relation to specific movement trajectories (vectors) as the animal moved into and out of the place field. Analysis of firing-dependent trajectories indicated that cells in 20 out of 22 fields (all $X^2>20$, $p<0.05$) fired maximally on only two of twelve possible trajectories, indicating traversal patterns into and out of the place field were controlling cell discharge. Thus, within a place field, the traversal pattern of the animal is an important behavioral determinant of place cell firing within the environment.

[Supported by NIDA grants DA 02048, DA 03502, and DA 04011]

173.7 AN ANIMAL MODEL OF ENDOGENOUS EARLY NEGATIVITY AND P300 EVOKED POTENTIAL CORRELATES OF HUMAN SELECTIVE ATTENTION. G.F. Steinfels[1], K. A. Helmstadter*[1], G. P. Albericí*[1], and D. S. Ruchkin[2]; [1]DuPont Pharmaceuticals, Wilmington, DE 19898; [2]Univ. Maryland School of Medicine, Dept. Psyiology, Baltimore, MD.

Human cognitive processing and endogenous components of evoked potentials recorded from human scalp have been studied extensively over the last 20 years. A number of relatively early endogenous negative components and late positive components such as P300 have been linked with specific information processing operations. Using male New Zealand rabbits, the present study examined 2 of these components, P300 and an earlier negative peak, in relation to variation of stimulus probability, stimulus discriminability, and pharmacological manipulations. The recording electrodes were located over the midline region of the frontal, somatosensory, and cingulate cortices referenced to a neck electrode. An "oddball" tone paradigm was used with target and nontarget tones. Training sessions were conducted in the morning and test sessions in the afternoon. Training sessions were comprised of 120 trials with a 0.5 probability for the target tone. During training sessions only, a very mild shock (current adjusted to be 20% below that necessary to evoke a muscle twitch) was applied to the temporalis muscles 750 msec following the target tone. There was no presentation of shock during the test session (400 trials). When target probability was 0.1 during a test, the averaged evoked response displayed an early negative peak at 90 msec followed by a late positive component (P300) at 325 msec. Both the negative and P300 components varied inversely in amplitude with stimulus probability. Similar evoked responses were observed during test sessions if a brief visual flash was substituted for tone during training. A second experiment examined the effects of varying the discriminability between target and nontarget tones. The probability of the target tone was 0.1. Decreasing the frequency separation between the target and nontarget tones decreased the amplitude of the early negativity and P300 elicited by the target tone. Preliminary pharmacological evidence indicates that both the early negative and P300 peaks are sensitive to manipulation of the cholinergic system. Prior studies with animals have demonstrated a P300 analogue which is similar to the human P300 in that the amplitude varies inversely with stimulus probability. The present study supports these data and further adds the finding that variations in discriminability modulate P300 amplitude, which also has been reported for human P300. Recent human studies have identified negative components (N1, N2, Na, Nd) which are associated with different modes of information processing. Latency analysis indicates that the rabbit early negativity appears analogous to N1, however the amplitude variation as a function of stimulus probability is also consistent with an N2 interpretation.

173.8 LEARNING ABILITY OF YOUNG RATS UNAFFECTED BY CHRONIC EXPOSURE TO A STATIC ELECTROMAGNETIC FIELD IN EARLY LIFE. R. Thompson, C-Z. Hong and J. Yu. Department of Physical Medicine and Rehabilitation, University of California, Irvine Medical Center, Orange, CA 92668.

The influence of static magnetic field on cognitive function is still unclear. In this study, infant Sprague-Dawley albino rats were exposed to a static electromagnetic field of 0.0 Tesla (control) or 0.5 Tesla (experimental) for 15 min daily over the first 14 postnatal days. Following a one-month rest period, the experimental (13 males and 10 females) and control (11 males and 14 females) rats were trained on four successive reversals of a position habit in a single unit enclosed T-maze adapted for the use of escape-avoidance of mild foot shock as a motive. There was no significant difference in learning ability between the experimental and control groups in terms of total (initial combined with repetitive) errors committed over the four reversal problems. While the females tended to make more errors than the males, this difference was likewise insignificant.

173.9 IN-VIVO EFFECTS OF PAVLOVIAN CONDITIONING ON CURRENTS MEASURED IN NEURONS OF THE MOTOR CORTEX OF AWAKE CATS WITH SINGLE ELECTRODE VOLTAGE CLAMP TECHNIQUES. C.D. Woody, D. Birt and E. Gruen*. Mental Retardation Res. Ctr., Brain Res. Inst., Depts. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024.

Net currents were measured intracellularly in neurons of the pericruciate cortex of awake cats with single electrode voltage clamp techniques described previously (Woody et al., Soc. Neurosci. Abstr., 1985). Holding currents ranged from -70 to -80 mV with command steps of ± 10 to 40 mV. During Pavlovian conditioning, 14 of 16 cells that developed CRs showed decreases in a net outward current that was activated rapidly by depolarization. None of five cells that failed to develop CRs showed such decreases. The CS (click) and US (glabella tap) employed were similar to those used previously to produce associative behavioral conditioning. Cells of this region are necessary and (with their projections) sufficient for the development of this type of conditioning (Woody et al., J. Neurophysiol., 1972, 1974), and changes in their activity have been shown previously to control initiation of the short latency blink CR (Woody and Engel, J. Neurophysiol., 1972; Sakai and Woody, J. Neurophysiol., 1980). The present studies used unit discharge in response to the CS as an index of conditioning. To speed rates of acquisition of the CR, local iontophoretic application of glutamate (GLUT) was given with the US. Rapid conditioning was not produced when saline was substituted for GLUT. Iontophoresis of saline (n = 13 cells) produced small increases in net outward current in seven cells, small decreases in four cells, and no change in two cells. As in previous studies, conditioning depended on the order of CS + US + GLUT presentation as did the development of the changes in activity and conductance. The increase in unit activity in response to the CS resembled that found after behavioral conditioning. The decrease in net outward current after conditioning resembled that found after local application of acetylcholine or cyclic GMP dependent protein kinase (Woody and Gruen, Soc. Neurosci. Abstr., 1986). Changes in other, sustained currents were also seen in some cells. This is the first direct demonstration of a decrease in conductance in a mammalian cell linked to associative conditioning. The finding was predicted by earlier electrophysiologic studies of behavioral conditioning (Woody and Black-Cleworth, J. Neurophysiol., 1973). We suggest that these changes constitute one of the cortical engrams needed for this type of learned behavior. (Supported in part by AFOSR F49620-85-C-0100 and HD 05958.)

173.10 **LONG-TERM TRANSLOCATION OF PROTEIN KINASE C BY *IN VIVO* AND *IN VITRO* CONDITIONING.** B. Bank*, J.J. LoTurco and D.L. Alkon. (SPON: D.L. Chute) Section on Neural Systems, Laboratory of Cellular & Molecular Neurobiology, NIH-NINCDS, Rockville, MD.

Recent work in our laboratory has shed light on neural substrates of associative learning in mammals by employing classical conditioning of the rabbit's eyeblink as a model. Two important biophysical correlates of eyelid conditioning obtained *in vitro* in hippocampal CA1 pyramidal cells are long-term reduction in the amplitude and duration of the slow after-hyperpolarization (AHP) (Disterhoft et al., PNAS, 83: 2733, 1986), and an increase in the summation of synaptic potentials (LoTurco et al., this volume). Both effects are mimicked by bath application of phorbol esters. We hypothesized that long-term activation of protein kinase C (PKC) mediated the biophysical effects of conditioning. Accordingly, we found that 24 h after conditioning there was a redistribution of PKC activity in hippocampal CA1 cells so that in condition animals 63.3% (± 4.1, n=10) of the PKC was membrane-bound versus 42.0% (± 5.0, n=9) in pseudoconditioned animals and 44.7% (± 5.1, n=10) in naive animals ($p<0.01$, ANOVA). Total PKC activity was the same for all groups, implying that conditioning induced a translocation of PKC activity from cytosol to membrane. We proposed that this was due to a concominant activation of pyramidal cells by glutamate induced diacylglycerol (and hence PKC) activation (CS-pathway) and depolarization induced Ca^{++} influx (US-pathway). We tested this proposal by exposing hippocampal slices to either 1 mM glutamate, 15 mM K^+, 1 mM glutamate + 15mM K^+, or normal ACSF. Immediately after 1 h exposure, all three treatments induced PKC translocation, with the glutamate + K^+ group being twice as much as either the glutamate or K^+ alone. One h following treatment, only the glutamate + K^+ group still exhibited PKC translocation. These results support the hypothesis that conditioning causes an alteration in PKC or its membrane anchoring proteins via activation of PKC by diacylglycerol in conjunction with Ca^{++} influx so that it remains stably attached to the membrane.

173.11 **ENHANCED SUMMATION OF SYNAPTIC POTENTIALS: A CORRELATE OF ASSOCIATIVE LEARNING IN RABBIT HIPPOCAMPAL CA1 NEURONS MIMICKED BY KINASE C ACTIVATION.** J. J. LoTurco, D. A. Coulter, B. Bank*, & D. L. Alkon. Laboratory of Cellular and Molecular Neurobiology, NIH-NINCDS, Rockville, MD.

A novel synaptic correlate of associative learning has been identified by studying hippocampal slices that were isolated from rabbits 24 hours following eyeblink classical conditioning training, explicitly unpaired training, or no training. Previous experiments have revealed a biophysical record, reduction in the after-hyperpolarization (Disterhoft et al., PNAS, 83: 2733, 1986), similar to that of *Hermissenda* classical conditioning (Alkon, *Science*, 226: 1037, 1984). Both records involve persistent reductions of calcium dependent potassium currents. We now report that in addition to a reduction in the AHP there is a conditioning-specific enhancement of the summation of synaptic potentials. This synaptic difference, not apparent for single psps, is characterized by increased depolarization during 50 hz, 300 msec stimulation of the Schaffer collaterals in cells from conditioned (10.2±SEM 0.8mV) relative to control (unpaired, 5.2±SEM 0.6mV; naive 4.4±SEM 0.5mV; p<.001) animals. This enhanced summation correlates with AHP reduction and may involve NMDA receptors as it is blocked by bath application of APV (50 µM).

We propose that, in CA1 pyramidal neurons, stable association of kinase C with the plasma membrane leads to an array of learning-related biophysical changes. In support of this proposal, i) classical conditioning induces stable attachment of kinase C to the plasma membrane of cells in area CA1 of the hippocampus (Bank et al., this volume), and ii) phorbol ester (DPBA, 250 nM), a kinase C activator, induces properties in CA1 neurons that parrallel conditioning-specific properties: reduced AHP and enhanced summation of synaptic potentials. Thus, both learning-specific properties may be mechanistically linked and are likely to work in concert to contribute to the functional role of hippocampal CA1 pyramidal neurons in classical conditioning.

173.12 C-KINASE ACTIVATION MEDIATES PROTEASE REGULATION OF VOLTAGE-DEPENDENT K^+ CURRENTS. D.L. Alkon, C. Chen* & P.E. Gallant (SPON: H. Pant). Lab of Cellular and Molecular Neurobiology, NINCDS-NIH, Bethesda, MD 20982.

Activation of C-kinase by phorbol esters such as DPBA (12-deoxyphorbol 13-isobutyrate 20-acetate) depends on translocation of this enzyme from the cytosolic to the membrane compartment. Recent evidence (Bank et al., Nature, 1987) indicates that similar translocation in CA1 hippocampal neurons persists at least one day after classical conditioning of the rabbit. This conditioning-specific translocation can account for biophysical changes measured under the same in vitro (slice) conditions. Conditioning-induced reduction of voltage-dependent K^+ currents (I_A and I_C) underlies both the biophysical changes of CA1 cells as well as long-lasting excitability changes (of Type B cells) causally implicated in classical conditioning of the mollusc Hermissenda (Alkon, Science, 226:1037-1045, 1984). C-kinase activation by DPBA together with Ca^{2+}-loading conditions also reduces Hermissenda I_A and I_C and a protein substrate for C-kinase undergoes conditioning-specific changes in phosphorylation (Neary et al., Nature 293:658-660, 1981). In the present study two microelectrode-voltage-clamp (at -60 mV) of isolated Type B somata was used to measure I_A and I_C. The simple presence of concentrated (250 µg/µl) leupeptin, a protease inhibitor, in the voltage-recording microelectrode (also containing 0.95 MKAc pH 7.4) caused marked reduction at 0 mV of I_A (14.8 ± 7.7 S.D., nA, N=6) and I_C (7.7 ± 4.3, N=6) as compared to values obtained without leupeptin: I_A (62.4 ± 11.7, N=10, p<.001) and I_C (21.0 ± 8.8, N=10, p<.001). By contrast, the simple presence of concentrated trypsin (8 µg/µl), a leupeptin-sensitive protease, in the microelectrode caused enhancement of I_A (84.0 ± 1.7, N=3, p<.005) and I_C (30.0 ± 6.2, N=3, p<.03). After a ten-fold reduction of leupeptin concentration, little current differences were observed with impalement alone, but injection (+2.0 nA, 2 min) caused marked reduction of I_A (Δ: -16.4 ± 6.5, N=7, p<.001) and I_C (Δ: -5.4 ± 6.7, N=7, p<.001). This reduction did not occur in the absence of leupeptin and was greatly reduced by pre-exposing the cells to the phorbol ester DPBA 500 nM: I_A (-5.33 nA, N=3) and I_C (-1.33 nA, N=3). DPBA eliminated I_A and I_C reduction by injection of smaller currents (-1.0 nA, 2') through the leupeptin-containing microelectrode: I_A: -4.5 ± 1.7 nA, N=4 vs. -0.4 nA (with DPBA), N=4; I_C: -2.5 ± 2.6, N=4 vs. -0.12 nA (with DPBA). These results suggest that: 1) endogenous cytoplasmic protease degrades C-kinase in the cytoplasm on an ongoing basis and thereby increases the amplitude of I_A and I_C; and 2) translocation of C-kinase (e.g., with classical conditioning) achieves persistence, at least in part, because of membrane-associated C-kinase's relative invulnerability to degradation by Ca^{2+}-independent, cytoplasmic protease.

173.13 CHANGES IN REGIONAL OXIDATIVE METABOLISM INDUCED BY TACTILE LEARNING AND TACTILE RECOGNITION IN MAN. P.E. Roland, L. Eriksson*, L. Widén* and S. Stone-Elander*. Department of Clinical Neurophysiology, Karolinska Hospital, S-104 01 Stockholm, Sweden

There are two important questions with regard to how the brain stores and retrieves memorized information about the outside world: 1) are the anatomical structures which participate in the storage the same as those participating in the analysis of the sensory information? 2) Are the anatomical structures which store the information the same as those participating in the retrieval of the stored information? We measured the regional cerebral oxidative metabolism (rCMRO2) in normal healthy volunteers in three different stages: rest, tactile learning and tactile recognition. During tactile learning blindfolded subjects had to learn 10 complicated geometrical objects they manipulated in their hand. In tactile recognition these objects were mixed with similar, but previously unknown objects. The frequency of manipulatory movements during tactile recognition was twice that of tactile learning. Tactile recognition with the right hand increased rCMRO2 in six prefrontal cortical areas, bilaterally in the supplementary motor areas, the premotor areas and supplementary sensory areas, in the left primary motor and primary sensory area, in the anterior superior parietal lobule, the secondary somatosensory area, the anterior insula, lingual gyri, hippocampus. basal ganglia, anterior parasagittal cerebellum, and lobus post. cerebelli. Except for the anterior insular cortex, the posterior intermediate prefrontal cortex and the lateral cerebellum - all these structures have in earlier experiments been found to participate in manipulatory movements and analysis of somatosensory information. Tactile learning increased rCMRO2 in the same structures as tactile recognition did. Thus we found no differences in the anatomical structures participating in storage and retrieval. However the rCMRO2 increases in the left premotor cortex, supplementary motor area and left somatosensory hand area were larger during tactile recognition in accordance with the higher frequency of manipulatory movements and higher flux of somatosensory information from the periphery during recognition. Despite this the rCMRO2 was significantly higher in the neocerebellar cortex during tactile learning. The extra rCMRO2 in the posterior lobe appeared during the learning interval but disappared when the stored information about the objects was retrieved during the recognition. This extra energy demand in the posterior lobe could not be related to motor learning, because there were no learning effect on the manipulatory movements. Therefore the metabolic activity in the posterior lobe of cerebellum was attributed to energy demanding processes necessary for the storage of somatosensory information.

174.1 CENTRAL INJECTIONS OF NEUROTENSIN INDUCE POLYDIPSIA IN THE RAT. J.D. Baker*, M.F. Hawkins, and A.A. Baumeister. Department of Psychology, Louisiana State University, Baton Rouge, LA 70803.

Microinjections of neurotensin (NT) into various brain loci have been reported to decrease food intake in food-deprived rats (e.g., Hawkins, M.F. et al., Physiol. & Behav., 36:1, 1986; Hawkins, M.F., Life Sci., 38:2383, 1986). Little is known, however, about the effect of this peptide on water intake. The present study investigated the effects of central injections of NT on water intake in male, Sprague-Dawley rats (280-320g) under 16 hours of water deprivation. Guide cannulae (22 gauge) were implanted unilaterally in the lateral ventricle (LV), and bilaterally in the lateral hypothalamus (LH), preoptic area (POA), and ventral tegmental area (VTA) under Ketamine HCl anesthesia (0.2mg/g, im). After water intake had stabilized (approximately 9 days) the animals were injected with NT (0.1, 1.0, & 5.0µg; Sigma) or saline vehicle and water intake was monitored at 15, 30, 60, 90, and 120 minutes post-injection. A within-subject design was used so that every animal served as its own control. Injection volume was 5.0µl in the LV and 1.0µl in the LH, POA, and VTA. The data were analyzed with an analysis of variance for repeated measures and the Newman-Keuls procedure. NT produced a dose-related increase in drinking when injected into the LV (see table below). This effect was brief, with no statistical differences occurring after 15 minutes post-injection. The peptide had no effect on water intake when injected into the other loci investigated. The data suggest that central NT may be involved in the regulation of water intake and that the LH, POA, and VTA may not mediate this effect.

LV Injection*	15 Minutes Post-Injection Mean	SE	30 Minutes Post-Injection Mean	SE
Saline	8.8	±2.0	1.3	±0.7
0.1µg NT	11.5	±2.2	3.2	±0.2
1.0µg NT	16.5[a,b]	±3.6	3.2	±0.8
5.0µg NT	23.5[a,b]	±2.2	2.8	±1.0

*There were 6 animals in the group. a = p<.05 compared to saline; b = p<.05 compared to all other means

174.2 EFFECT OF OSMOTIC DEHYDRATION ON FOOD INTAKE, GASTRIC FUNCTION AND OXYTOCIN SECRETION IN RATS. L.M. Flanagan*, J.G. Verbalis, and E.M. Stricker. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260

Previous studies in this laboratory have shown that LiCl and CCK, agents known to decrease food intake, each cause a dose-related inhibition of gastric emptying in rats. Because these agents also have been found to stimulate neurohypophyseal secretion of oxytocin (OT), we examined the effect of hypertonic saline, which also stimulates OT secretion, on these other variables.

In the first experiment, rats with indwelling gastric catheters were food-deprived overnight, then allowed to eat chow for one hour the next afternoon. After this meal, gastric pressures were recorded for a 20-min baseline period. Rats then were injected with 2 M NaCl (0.5-2 ml), LiCl (0.75-3.0 mEq/kg) or CCK (0.5-10 ug/kg). Whereas the rats showed large and frequent stomach contractions before the injections, each of the three agents abruptly and markedly reduced gastric motility. In the second experiment, gastric emptying of a 10% glucose load was measured in rats with indwelling catheters, pretreated with 2 ml 2 M NaCl, 3.0 mEq/kg LiCl or 10 ug/kg CCK. Gastric emptying was inhibited by all three agents. The third experiment compared the effects of 2 M NaCl and LiCl on food intake and OT secretion. Rats were maintained on a regimen of two daily feedings, with access to chow but not water for 20 min in the morning and chow with water for 2 hr in the afternoon. On the morning of the test day, they were pretreated with 2 M NaCl or LiCl. Both agents produced a dose-related inhibition of food intake, over the same range of doses that inhibited gastric motility. At the end of the 20-min feeding period, rats were decapitated and trunk blood was collected and assayed for OT. In both groups, the decrease in food intake was significantly correlated with increased plasma levels of OT.

Our results demonstrate that hypertonic saline resembles LiCl and CCK in its effects on food intake, gastric function and OT secretion. Because the latter effects are known to be mediated by oxytocinergic neurons projecting from the paraventricular nucleus (PVN) of the hypothalamus, these data therefore add further support to the notion that changes in gastric motility and pituitary OT secretion reflect activation of PVN pathways that mediate the central inhibition of food intake.

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174.3 INCREASED FEEDING BEHAVIOR AND REDUCED PLASMA LH LEVELS IN RING DOVES FOLLOWING INTRACRANIAL INJECTIONS OF PROLACTIN: DOSE-RESPONSE AND SPECIFICITY PROPERTIES. J.D. Buntin*, R.W. Lea* and G.R. Figge* (SPON: R. Quock). Dept. of Biological Sciences, Univ. of Wisconsin-Milwaukee, Milwaukee WI 53201 and Dept. of Applied Biology, Lancashire Polytechnic, Preston PR1 2TQ England

Food intake is markedly increased and gonadal weight is significantly reduced in male and female ring doves given intracerebroventricular (ICV) injections of ovine prolactin (oPRL; Buntin & Tesch, Horm. Behav., 19:188, 1985). The present study examined the dose-response relationships and hormone specificity characteristics for these centrally mediated effects. Daily food intake was monitored and post-treatment plasma LH levels were measured by RIA in 10 groups (n=8-10/grp) of male doves given 5 daily ICV injections of oPRL at 0.1, 0.5, 1.0, or 2.0 ug/day, turkey PRL (tPRL), turkey growth hormone (tGH), human GH (hGH), or ovine GH (oGH) at 1.0 ug/day, ovine luteinizing hormone (oLH) at 10 ug/day, or saline vehicle (2 ul/day). A significant ($p < .05$), dose-dependent increase in food intake was observed with all doses of oPRL (mean ± sem % increase over baseline levels = 28.4±4.5, 57.2±6.4, 78.3±6.2, & 91.4±2.7 for 0.1, 0.5, 1.0, & 2.0 ug oPRL, respectively; saline = 9.1±5.7). The three GH preparations also increased feeding significantly (56.4±4.7, 60.1±5.6, & 70.1±6.4 for oGH, tGH, and hGH, respectively), but only hGH was as effective as 1.0 ug oPRL. Turkey PRL and oLH failed to stimulate feeding. LH levels were significantly reduced by tPRL (3.6±0.7 ng/ml vs. 6.2±1.1 for saline) and by 0.5, 1.0, & 2.0 ug oPRL (2.3±0.6, 1.2±0.2, & 1.7±0.4 ng/ml, respectively), but not by 0.1 ug oPRL (6.6±0.8). Plasma LH levels were also decreased significantly by the three GH preparations (2.3±0.7, 1.7±0.6, & 0.8±0.1 ng/ml for oGH, tGH, & hGH, respectively). Data from oLH injected birds were inconclusive due to difficulties in blood collection. Testis weight differences between groups generally paralleled differences in LH levels. Crop sac analyses provided no indication of significant systemic effects of ICV injected PRL.

These results suggest that PRL and GH can act centrally to alter feeding behavior and pituitary gonadotropin secretion. Moreover, the dose-response relationship between feeding and oPRL and the hormone specificity characteristics for this response are similar to those observed for LH changes. However, hormones from ovine, turkey, and human sources vary markedly in their effectiveness. Future studies will attempt to identify the nature and location of the binding site(s) responsible for mediating these effects, to determine whether these effects are causally related, and to assess the degree to which PRL or GH promote these changes under normal physiological conditions. (Supported by NIMH grant 41447 and NSF grant DCB 8303026 to JB)

174.4 INSULIN-LIKE GROWTH FACTOR II (IGF-II) IN RAT BRAIN: DISTRIBUTION OF IGF-II NEURONS AND FIBERS AND PEPTIDE CONCENTRATION CHANGES WITH FED STATE. T.J. Lauterio*, L. Marson, M.A. Della-Fera and C.A. Baile Washington University School of Medicine, St. Louis, MO 63110 and University of Kentucky, Lexington, KY 40511.

The peroxidase-antiperoxidase method of immunohistochemical detection was used to localize IGF-II containing neurons and fibers in brain. Colchicine treatment (24-48 hours, 100 µg/rat) was employed to enhance the visualization of IGF-II containing neurons prior to immunohistochemistry. IGF-II fiber staining was conducted in untreated rats. Rats were then anesthetized with equithesin and perfused with isotonic saline followed by 10% paraformaldehyde, in preparation for sectioning. Frozen brain sections (40 µ) were then incubated with a 1:500 dilution of a monoclonal antibody raised against human IGF-II which is cross-reactive with rat IGF-II. Slides were then serially incubated with mouse IgG, PAP (1:50) and diaminobenzedine (15 min) to visualize immunoreactive areas and examined with a light microscope. Prominent staining for IGF-II was seen in the ventral hypothalamus, dorsal to the optic tract. Greatest immunoreactive staining was observed in the median eminence, with dense staining of fiber bundles occurring in the arcuate nucleus and retrochiasmatic areas of the hypothalamus. In a separate study, brain tissue concentrations of IGF-II in fasted rats were compared to IGF-II concentrations in meal fed rats. Animals were acclimated to an overnight fast followed by presentation of food. After rats consistently ate meals of 3 grams or greater within 30 minutes of food presentation, they were randomly assigned fed or fasted treatments and either sacrificed the following morning without a meal (fasted) or sacrificed upon completion of their meal (fed). Brains were immediately removed and frozen on dry ice. Punches were then made from brain slices and sonified tissue was assayed for protein and IGF-II content. Greater tissue concentrations of IGF-II were observed in meal fed rats compared to fasted rats in the suprachiasmatic nucleus, paraventricular nucleus and dorsomedial hypothalamus. The physiological and immunohistochemical data accumulated in these studies suggest a role for the suprachiasmatic region and dorsal hypothalamus in the IGF-II regulation of food intake in the rat. The finding of dense IGF-II fiber staining in the median eminence and arcuate nucleus also support the hypothesis that IGF-II is important in neuromodulation of pituitary function. Supported by NIH grant # 20000 and a grant from the Monsanto Company.

174.5 BEHAVIORAL CHARACTERIZATION OF THE INCREASED FOOD INTAKE BY RATS MICROINJECTED WITH RAT HYPOTHALAMIC GROWTH HORMONE-RELEASING FACTOR (rhGRF) INTO THE SUPRACHIASMATIC NUCLEUS/MEDIAL PREOPTIC AREA (SCN/MPOA). P. Dickson, and F.J.Vaccarino. University of Toronto, Canada, M5S 1A1.

Intracerebroventricular injections of rhGRF have been shown to cause a significant increase in food intake in food deprived and sated rats. This effect is believed to be independent of the growth hormone releasing properties, since further work has shown the feeding effect when rhGRF is microinjected into the SCN/MPOA. The present study was designed to replicate the SCN/MPOA finding, and to determine how this feeding effect is behaviorally expressed.

Food (Purina Rat Chow) and water intake of adult male Wistar rats with cannula implants aimed at the SCN/MPOA was measured for a 90 min period on 6 consecutive days following 90 min exposure to fresh food. On subsequent test days, consumption was measured for the 90 min period following an intracranial rhGRF injection. Observations of the feeding behavior profile were also carried out. The following doses were tested in random order: 0.0, 0.01, 0.1, and 1.0 picomoles. rhGRF was dissolved in a 0.1% ascorbic acid vehicle and administered in a 0.5 ul volume over 1 min. A minimum of 2 no-treatment days separated drug tests.

The results demonstrate that microinjections of rhGRF into the SCN/MPOA significantly increased food intake in sated rats. Behavioral observations suggest that at lower doses the rhGRF effect is expressed through significantly longer meals. At the highest dose the increased intake is condensed into normal meal lengths, resulting in a faster rate of eating. Since both water intake and latency to onset of eating are unaffected, it is suggested that rhGRF has a specific effect on maintenance of eating behavior, rather than on initiation of food intake.

This research was supported by NSERC grant U0443 to FJV. We thank Drs. J. Rivier and W. Vale for their generous gift of rhGRF.

174.6 VTA MICROINFUSIONS OF (S)-CHOLECYSTOKININ OCTAPEPTIDE POTENTIATE AMPHETAMINE CONDITIONED PLACE PREFERENCES. H.O. Pettit and K. Mueller, Department of Psychology, Texas Christian University, Fort Worth, Texas 76129.

Cholecystokinin (CCK) and dopamine (DA) have been shown to coexist in both cell body and terminal areas of an A10 mesolimbic pathway which projects from the ventral tegmental area (VTA) to the nucleus accumbens (N ACC). Autoradiographical studies report extensive CCK binding sites in the N ACC, but not in the VTA. However, iontophoresis of CCK into the VTA results in activation and/or deactivation of DA firing rates and bursting activity (depending upon dose). CCK could have neuromodulatory effects upon VTA-DA neurons. In two studies, behavioral effects from VTA infusions of CCK were examined in the conditioned place preference (CPP) paradigm. In the CPP paradigm the effects of a drug are paired with one environment and effects of the drug vehicle are paired with another environment. If a drug has reinforcing properties, then animals, when given a choice, will spend more time in a drug-paired than in a vehicle-paired environment. In the first experiment, 40 rats received permanent cannula implants aimed at the VTA. Rats were conditioned to associate the effects of CCK infusions into the VTA (0.0, 0.04, 0.4 and 4.0 ng/cannula) with one novel environment, and the effects of a CCK saline vehicle with another environment. Significant CPPs were not produced by any dose tested. Although some tendency towards reinforcing effects was observed, the effects were not significant. Thus, quantifiable reinforcing effects were not induced by the three CCK doses which were infused into the VTA. In the second experiment, 50 animals received bilateral cannula implants aimed at the VTA. In four groups of subjects (N=10/group) the effects of amphetamine (1.0mg/kg, S.C.) combined with effects of VTA infusions of CCK (0.0, 0.04, 0.4 or 4.0 ng/cannula) were examined in the CPP paradigm. The 10 remaining animals received amphetamine vehicle and CCK vehicle as a procedural control. Amphetamine CPPs were potentiated by CCK infusions. Furthermore, the effects were dose-dependent and linear in form. Results demonstrate that CCK infusions in the VTA can potentiate the reinforcing effects of amphetamine. A possible peptide neuromodulatory role in drug-abuse, and VTA mechanisms of action for CCK are implicated.

174.7 VASOPRESSIN (VP) IMMUNOREACTIVITY IN THE HYPOTHALAMUS OF CASTRATED, TESTOSTERONE-TREATED DOMINANT HAMSTERS AS COMPARED TO THEIR CASTRATED, UNTREATED SUBORDINATE PARTNERS. C.F. Ferris and J.K. George* (SPON: P. Grigg). Department of Physiology, University of Massachusetts Medical Center, Worcester MA 01605.

The Golden hamster communicates dominance status by rubbing specialized scent glands located on its flanks against objects in the environment. This behavior, called flank marking, is dependent upon VP-sensitive neurons in the anterior hypothalamus. The present study was undertaken to determine dominant/subordinate (D/S) behavior in pairs of castrated hamsters when one member of the pair was treated with testosterone proprionate (TP) while its partner was untreated, and to subsequently compare the VP immunoreactivity in the hypothalamus of these D/S hamsters. Male hamsters (n=20) were anesthetized, castrated and implanted with silastic capsules (2.2 cm) containing TP or with empty capsules. Animals were individually housed and maintained on a 14:10, L:D cycle for 3 wks. On the 4th wk TP-treated hamsters were paired with untreated animals for a 15 min period in a neutral cage. The same animals were paired for 5 consecutive days of testing. During each of the 5 test periods the number of attacks, bites, retreats and flank marks made by each hamster was scored. A hamster was considered dominant if it had a higher aggressive index (attacks, plus bites, minus retreats) than its partner in three out of the five test periods. Nine out of 10 pairs of hamsters established D/S relationships. In each of the 9 cases the TP-treated hamster was dominant over its untreated partner. The TP-treated hamsters had a significantly higher ($p<0.001$) mean aggression index (25.3±5) and mean frequency of flank marks (21.4±5) than their subordinate partners (3.0±2 and 3.3±1, respectively). The brains from three of the nine pairs were fixed, sectioned (50μm) on a vibratome and prepared for ICC using PAP with DAB. The anti-VP (Immunonuclear) was used at a dilution of 1:1000, and preabsorption controls showed the antibody to be specific for VP. The number of VP-immunoreactive perikarya in the nucleus circularis was significantly greater ($p<0.05$) in the dominant hamsters (64±15) than in their subordinate partners (15±5). The number of VP neurons in the bed nucleus of the stria terminalis (21±2 vs 23±1), paraventricular nucleus (210±28 vs 202±22) supraoptic nucleus (1105±121 vs 1184±78) were not significantly different between D/S hamsters. It should be noted that the VP neurons of nucleus circularis are located in an area of the anterior hypothalamus known to be involved in the organization and expression of flank marking behavior. Hence the pronounced difference in flank marking behavior between D/S hamsters may be correlated to the difference in VP-immunoreactivity in this site of the hypothalamus. (This work was supported by NIH grant NS-23557)

174.8 INJECTIONS OF CCK-8 INTO THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS INHIBITS LORDOSIS BEHAVIOR IN THE FEMALE RAT. A.M. Babcock,* G.J. Bloch,* R.A. Gorski and P.E. Micevych (SPON: M. Hines). Dept. of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

We have reported that i.p. injections of CCK-8 will stimulate and inhibit lordosis behavior in female rats (Physiol. Behav., 39: 217, '87). Since the tissue content and binding sites of CCK-8 within the ventromedial nucleus of the hypothalamus (VMH), a region critical for the expression of lordosis, are sexually dimorphic and sensitive to the steroid environment, we investigated the effects of CCK-8 on lordosis behavior following injection into the VMH. Two weeks following ovariectomy, unilateral guide cannulae were implanted into the VMH of 30 adult female Long-Evans rats. One week following surgery, each rat was injected with 5 μg estradiol benzoate and tested 53 hrs later during the dark phase of the photoperiod. Ten minutes prior to testing, 0.5 μl injections of CSF (vehicle) or CCK-8 (5, 50, 100 ng) into the VMN were made. Lordosis behavior, expressed as lordosis quotient (LQ), was assessed by recording the number of lordotic responses to 10 mounts by a stimulus male. Receptivity increases with repeated steroid administration; therefore rats were retested using the identical procedure every 10-12 days for a total of 5 behavioral tests with treatment groups randomly assigned for each test. Data were evaluated by ANOVA and Newman-Keuls post hoc tests.

Injections of CCK-8 failed to alter LQ in tests 1-4 (CSF-LQs were 17, 58, 48, 88, respectively); however, CCK-8 significantly inhibited LQ in test 5 (CSF-LQ was 95). Post hoc analysis revealed a significant inhibition of LQ, as compared to CSF controls, for the 5, 50, 100 ng doses in test 5 (CCK-LQs 40, 58, 23, respectively). In our previous study, peripheral injections of CCK-8 facilitated LQ when receptivity was low and attenuated LQ when receptivity was high. CCK-8 injections into the VMH inhibited LQ when receptivity was high; however, these VMH injections of CCK-8 did not facilitate LQ when receptivity was low. The present experiment indicates that VMH CCK-8 may participate in the CNS regulation of lordosis behavior. The inability of VMH injections of CCK-8 to significantly increase lordosis behavior suggests that CCK-8 may interact with some other CNS region to produce a facilitation of lordosis.

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174.9 EFFECT OF 5α-DIHYDROTESTOSTERONE ON THE FACILITATION OF FEMININE SEXUAL BEHAVIOR BY NEUROACTIVE PEPTIDES. Erskine, M.S. & E. Kornberg*. Dept. Biology, Boston University, Boston, MA 02215.

In female rats, the 5α-reduced androgen, 5α-dihydrotestotserone (DHT), exerts strong inhibitory effects on the display of estradiol (E_2)-induced sexual behavior (lordosis) and gonadotropin release. In the past several years, we have attempted to determine the mechanism(s) by which DHT is having its behavioral effects; evidence suggests that DHT does not prevent the priming action of E_2 in inducing hypothalamic cytoplasmic progestin receptors, nor can large doses of estradiol benzoate (EB) overcome its inhibitory effects. The present experiments were carried out to determine whether DHT acts by preventing the known ability of LHRH to facilitate lordosis. Naloxone (Nal) and Substance P (Sub P), two peptides known to increase LHRH activity in brain, were also tested. Seven days after ovariectomy and 3-4 days after implantation of intracerebral guide cannulae aimed at the lateral ventricle, two injections (pulses) of E_2 (1 μg/injection s.c. in 10% ethanol given 8 hrs apart) were given/day for three consecutive days. Experimental substances were given on the following day through internal infusion cannulae in 4 μl over 1 min and the behavioral response observed. Females were tested for lordosis with sexually active males approximately 30 min prior to (pretest), and at 30, 60, 90, and 180 min after infusion. Seven days after the initial E_2 injection, another 3 days of pulsed E_2 began, with DHT (2.5 mg/rat, s.c.) being given at the time of the first E_2 injection on day 3. As before, infusion and testing was carried out on the day following the third day of E_2 injections. All animals received the same infusate on this second test as on the first. After the E2-only injections, animals receiving LHRH (500 ng) and Nal (1 μg) showed significant increases in lordosis by 30 min after infusion and remained highly receptive until 180 min. Lordosis did not increase in Sal-treated animals. Unexpectedly, Sub P (1 μg) induced no increase in lordosis over levels seen in vehicle (Veh, 0.01 N acetic saline) control levels. DHT treatment in the second week had no effect on pretest levels of lordosis; however, this androgen completely prevented the stimulatory actions of LHRH and Nal seen the previous week. DHT did not reduce the level of receptivity seen in Sub P or Veh animals. These data are inconsistent with previous findings in which LHRH was able to reinstate receptivity in EB + DHT-treated mice (Luttge, 1977). The present results demonstrate that DHT does not inhibit lordosis by preventing the facilitatory effects of LHRH, and raise the question of whether DHT acts in a specific manner on E_2- or E_2 + progesterone-induced sexual receptivity or whether this steroid acts through a more general mechanism to inhibit behavior. Support: HD 21802 to MSE.

174.10 THE EFFECTS OF MEDIAL PREOPTIC AREA INJECTIONS OF SUBSTANCE P AND CHOLECYSTOKININ ON MALE RAT SEXUAL BEHAVIOR. W.A. Dornan*, C.W. Malsbury (SPON: G.Carre). Department of Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

The critical importance of the medial preoptic-anterior hypothalamic continuum (MPOA-AH) for male rat sexual behavior has been clearly demonstrated in studies using a variety of experimental approaches. Recently, the MPOA-AH has been shown to contain numerous neuropeptides. At this point, however, little is known about the role of peptidergic afferents/efferents of the MPOA-AH in the regulation of male sexual behavior. Two peptides found within the MPOA-AH that are perhaps most relevant are substance P (SP) and cholecystokinin octapeptide (CCK-8). For example, both CCK-8 and SP concentrations within certain areas of the CNS previously implicated in male rat sexual behavior have been shown to be dependent on gonadal hormones. This suggests that SP and CCK-8 may play a role in the neural regulation of male rat sexual behavior. The following series of experiments was designed to explore this possibility.

Sexually experienced Sprague Dawley male rats each received a pair of stereotaxically implanted stainless steel guide cannulae aimed at either the MPOA-AH, lateral ventricles (LV), or the dorsal caudate/putamen (CP). Each animal received two post-operative tests with a receptive female. On the second test, different groups of males were injected bilaterally with either SP (10 ng, 100 ng, or 200 ng/0.3 ul per cannula), CCK-8 (same doses), or the acidified saline vehicle into the MPOA-AH. Two additional groups were bilaterally injected with SP (10 ng/0.3 ul per cannula) into the LV or CP.

Injections of all three doses of SP into the MPOA-AH significantly reduced the latency to initiate copulation, while the two lower doses also reduced ejaculation latencies. In contrast to the MPOA-AH, bilateral injections of SP into the CP had no appreciable effect on male copulatory behavior. Injections of SP into the LV significantly reduced the number of intromissions to ejaculation and the post ejaculatory interval. Interestingly, a reduction in the number of intromissions prior to ejaculation was not observed following the same (10 ng) dose of SP injected into the MPOA-AH. CCK-8 injections into the MPOA-AH had no significant effect on any of the parameters of copulatory behavior, although there was a non-significant trend for reduced latencies following the larger doses of CCK-8 compared to the saline controls.

174.11 ARE GASTROENTEROPANCREATIC PEPTIDES INVOLVED IN REM SLEEP MODULATION? O. Prospéro-García,* R. Pérez-Montfort,* M.T.Pacheco* and R. Drucker-Colín. Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM. Apartado Postal 70-600, México, D.F. 04510. México.

Recently, we have shown that the intraventricular (IVT) injection of either cerebrospinal fluid of sleep deprived cats (CSF SD), Vasoactive Intestinal Polypeptide (VIP) and Cholecystokinin (CCK-8) induces REM sleep in the parachlorophenylalanine (PCPA) pre-treated insomniac cats. These results additionally showed that while CSF SD and VIP increased the frequency of REM epochs, CCK-8 increased their duration. On the basis of such observations, these experiments were designed to determine the effects of IVT administration of CSF SD incubated with antibodies antiVIP on PCPA-insomniac cats, in order to test whether the antibodies can inhibit CSF SD hypnogenic properties. We additionally tested the effects of combining VIP and CCK-8 . A complementary study was done in order to determine whether VIP is able to restore REM sleep in Basal Forebrain Area (BFA) lesioned cats. A total of 50 cats were used in this study. Ten of these cats were used as donors of CSF and 40 were recipients of either CSF or other substances. All cats were implanted with a stainless steel cannula into the 4th ventricle. Recipients cats were additionally implanted with electrodes for standard recording of the sleep-wake cycle. Seven days after surgery, donors were sleep deprived during 24 h using the water tank method. The total volume of CSF was divided into 2 parts. One of them was incubated with antibodies antiVIP during 12 h (CSF VIPab), the other portion was not treated (CSF SD). Thirty PCPA pre-treated recipients cats were divided into 6 groups (n=5). Twenty four h after the second injection of PCPA, cats belonging to a given group were IVT injected with either CSF SD, CSF VIPab, VIP (200 ng) CCK-8 (100 ng), VIP + CCK-8 and saline. The last ten cats were BFA electrolytically lesioned and then divided into 2 groups (n=5). Ten days after the lesion, cats belonging to a given group received VIP (200 ng) or saline. In all cases, the injected volume was 100 ul. Results showed that, first antibodies antiVIP were able to inhibit the CSF SD hypnogenic effect. Second, the administration of VIP + CCK-8 had additive effects. Finally, VIP was able to restore REM sleep in the BFA lesioned cats. In conclusion, we can suggest that CSF SD has an antigenically VIP-like substance which is involved in the observed CSF SD hypnogenic properties. We can also suggest that VIP and CCK-8 are sinergistic and that the effect observed in the BFA lesioned cats gives additional evidence supporting VIP's involvement in REM sleep modulation.

174.12 DIFFERENTIAL BEHAVIORAL AND ENDOCRINE EFFECTS PRODUCED BY INTRA-RAPHE ADMINISTRATION OF THE NEUROKININ AGONIST, DiMe-C7, AND THE $GABA_A$ AGONIST, MUSCIMOL. J. Lee*, H. Mitsushio*, J.M. Paris and S.A. Lorens. Behavioral Pharmacology Laboratory (Bldg. 135), Loyola Univ. Med. Ctr., Maywood, IL 60153; and, J. Ritchie and C. Nemeroff. Dept. of Psychiatry, Duke Univ. Sch. of Med., Durham, NC 27710.

We have shown previously (Pharm. Biochem. Behav. 18:407, 1983; Behav. Brain Res. 1987, in press) that intra-raphe injections of muscimol and DiMe-C7 produce dose-dependent increases in the locomotor activity (LMA) of male Sprague-Dawley rats. The increase in LMA induced by DiMe-C7, in contrast to that produced by muscimol, depends on intact midbrain raphe serotonin (5HT) neurons, and is blocked by the dopamine (DA) antagonist, haloperidol (200 μg/kg, i.p.). These observations suggest that two distinct neuronal systems, which originate in the midbrain raphe, play a role in the regulation of behavioral arousal as measured by changes in activity level. One system involves a neurokinin - 5HT - DA circuit, whereas the other involves a $GABA_A$ - non-5HT - non-DA circuit. The objective of the present series of experiments was to determine whether these distinct "arousal modulating systems" are functionally linked to different neuropsychological processes.

A conditioned place preference paradigm was used to determine whether intra-raphe injections of muscimol (25 - 100 ng in 0.5 μl vehicle) and DiMe-C7 (30 ng - 1.0 μg in 0.5 μl vehicle) produce dose-dependent rewarding effects. Neither drug induced consistent or robust alterations in place preference. The data suggested, however, that intra-raphe infusion of muscimol may produce a mildly aversive effect, whereas DiMe-C7 may induce a rewarding effect. This possibility currently is being examined using an intra-raphe drug self-administration paradigm.

In a separate series of experiments, it was demonstrated that intra-raphe muscimol (25 - 100 ng) administration, but not DiMe-C7 injections, dose-dependently elevated plasma ACTH and corticosterone levels. In addition, it was shown that the muscimol induced increases in plasma ACTH and corticosterone levels are not dependent on intact midbrain raphe 5HT cells.

In a third series of experiments (still in progress), evidence has been obtained which indicates that intra-raphe infusion of muscimol, but not DiMe-C7, induces eating and drinking behavior in non-deprived animals.

Overall, the data suggest that two distinct neuronal systems emanating from the midbrain raphe modulate behavioral arousal but differentially affect endocrine and motivational processes. Only one of these systems depends on the integrity of mesencephalic raphe 5HT neurons.

174.13 INHIBITORY EFFECTS OF NEUROTENSIN ON BEHAVIORS INDUCED BY PHYSOSTIGMINE AND A SMALL DOSE OF THE DOPAMINE AGONIST N-PROPYLNORAPOMORPHINE. R. Rivest, M.A. Gagne*, S. St-Pierre* and F. B. Jolicoeur. Department of Psychiatry Faculty of Medicine, University of Sherbrooke, Sherbrook, Quebec, Canada J1H 5N4. We have shown previously that intraventricular (IVT) administration of neurotensin (NT) inhibits both yawning and penile erections induced by a small dose of apomorphine, a drug thought to stimulate dopaminergic presynaptic inhibitory receptors (autoreceptors). (Jolicoeur et al Soc. Neurosci. Abstr. Vol II, Part 1 p. 618, 1985). In order to verify the specificity of these findings we first examined the effects of IVT administration of various doses of NT (0.025-3.75 µg) on the same behaviors elicited by subcutaneous administration of 1.9 µg/kg of N-propylnorapomorphine (NPA), a dopamine (DA) agonist whose binding affinity in subcortical limbic structures has been reported to be markedly decreased by NT (Agnati et al. Acta Physiol. Scand. 119: 459,1983). Both number of yawns and penile erections were found to be significantly decreased by 1.875 µg and totally suppressed by larger doses of the peptide. These dose related effects of NT are identical to those we previously described for apomorphine. In another series of experiments the effects of the same doses of NT on yawning induced by 100 µg/kg of the anticholinesterase, physostigmine, were studied. Interestingly, 0.05 µg IVT injection of NT was sufficient to significantly reduce the number of yawns induced by this drug, pointing to a prominent anticholinergic effect of NT. Since yawning behavior is generally considered to implicate a DA-cholinergic link, our observed inhibitory effects of NT on yawning and penile erections produced by small doses of DA agonists could be attributed to anticholinergic effects of the peptide. However, although yawning induced by the dopamine agonist was significantly decreased by 1.0 mg/kg atropine, doses up to 10 mg/kg of this well-known direct anticholinergic agent failed to affect number of penile erections. Together these results suggest that NT exerts both anticholinergic effects and an inhibitory action on dopamine autoreceptors.
Supported by Medical Research Council of Canada (Grant No. DG-284).

174.14 EFFECTS OF DIRECT ADMINISTRATION OF NEUROTENSIN INTO DISCRETE BRAIN REGIONS ON BOTH SPONTANEOUS AND ADTN INDUCED MOTOR ACTIVITY. D. Gaudin*, R. Rivest, A. Drumheller and F.B. Jolicoeur. Department of Psychiatry, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4.
Intraventricular administration of the tridecapeptide neurotensin (NT) has been shown to significantly reduce, in rats, both spontaneous locomotor activity and the strong and persistent hyperactivity produced by intra-accumbens injections of ADTN, a potent and long acting dopamine receptor agonist (F.B. Jolicoeur et al., Handbk. Neurochem. 8:93 1985; Neuropeptides 6:143, 1985). The purpose of the present study was to delineate the neuroanatomical sites where the peptide exerts its prominent inhibitory actions on both spontaneous activity and on the hyperactivity produced by bilateral intra-accumbens administration of 12.5 µg ADTN. Selection of brain regions was based on known output efferents of the accumbens and/or sites where high immunoreactive concentrations of the peptide are found. For each region, NT (0.0 to 3 µg) was administered bilaterally in a volume of 0.5 µl NaCl.

Results indicate that none of the doses of NT significantly affected spontaneous locomotor activity when injected into either substantia nigra, accumbens or striatum. However, injections of 0.375 and 1.5 µg NT into the central amygdaloid nucleus and substantia innominata, respectively, significantly attenuated spontaneous activity of animals. Injection of the peptide into the striatum was also ineffective in reducing ADTN induced hyperactivity. On the other hand, hyperactivity was markedly reduced with bilateral injection of NT into the central amygdaloid nucleus (0.187 µg), substantia innominata (0.375 µg), nucleus accumbens (1.8 µg) and substantia nigra (3.0 µg). These data indicate that certain brain regions are more sensitive to the inhibitory action of NT on motor activity. Furthermore, our data demonstrate that the peptide's actions are more prominent on activity stimulated by pharmacological activation of the mesolimbic dopaminergic pathway. In order to further characterize the influence of NT on activity, we are presently examining the effects of the peptide administered into the globus pallidus, stria terminalis and the "mesencephalic locomotor regions". Supported by MRCC Grant No. DG-284.

174.15 SPINAL EXCITATORY MODULATION OF THE STARTLE REFLEX BY SUBSTANCE P AND SEROTONIN (5-HT$_{1A}$) RECEPTORS. D.S. Simmons*, J.H. Kehne, and M. Davis (*SPON: T. Baker), Dept. Psychiat., Yale Univ. Sch. Med., 34 Park St., New Haven CT 06508

In the ventral horn of the spinal cord, the neuropeptide substance P is colocalized with serotonin (5-HT) in descending terminals (Gilbert et al., 1982; Neurosci., 7, 69-87). Furthermore, both 5-HT and substance P exert excitatory neuromodulation on motor neurons (White, 1985; Brain Res., 335, 63-70). Previous studies indicated that spinal 5-HT receptors exert an excitatory modulation on startle reflexes elicited by auditory stimuli (Davis et al., 1986, Psychopharm. Bull., 22, 837-843). The first purpose of the present study was to further characterize the 5-HT receptor subtype that mediates this excitatory modulation of the startle reflex and to assess its possible linkage to pertussis toxin sensitive G-proteins. The second purpose was to assess the role of spinal cord substance P in the modulation of the startle reflex, and to evaluate some possible interactions between substance P and descending 5-HT neurons.

Previous work demonstrated that intrathecal infusion of the selective 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) increased startle following systemic or intrathecal, but not following supraspinal infusion (Davis et al., ibid.). In the present study, the excitatory effect of intrathecal infusion of 25 µg 8-OH-DPAT was blocked by 200 µg of the 5-HT$_1$ antagonist pindolol. Intrathecal pindolol did not attenuate the excitation produced by intrathecal infusion of the phosphodiesterase inhibitor rolipram (25 µg). Finally, pretreatment with pertussis toxin (2 µg; 2-3 days before testing) which inactivates G$_i$ as well as other membrane-bound G proteins, failed to attenuate the excitatory effect of 25 µg of intrathecally administered 8-OH-DPAT.

Intrathecal infusion of the stable substance P analogue (pGlu^{5}MePhe^{8}Sar^9) substance P (5-11) ("DiMe-C7"; 6.25 - 50 µg) produced a dose-related increase in startle amplitude. In contrast to 8-OH-DPAT, the excitatory effect of 25 µg DiMe-C7 was not blocked by intrathecal pindolol (200 µg). The excitatory effect of intrathecal infusion of 8-OH-DPAT or DiMe-C7 was not attenuated by destruction of spinal 5-HT terminals by prior pretreatment with the 5-HT neurotoxin 5,6-dihydroxytryptamine (3 x 20 µg, injections 24 hr apart; testing 3 wks after final infusion).

These data indicate that: (1) Serotonin exerts an excitatory modulation of the startle reflex through spinal 5-HT$_{1A}$ receptors that are not linked to pertussis-toxin sensitive G-proteins; and (2) Stimulation of spinal substance P or 5-HT$_{1A}$ receptors increases startle amplitude by a mechanism that does not involve intact 5-HT terminals in the cord.

174.16 DECREASED MONONUCLEAR LEUKOCYTE TSH SECRETION IN PATIENTS WITH MAJOR DEPRESSION. D. Harbour*, A. Anderson*, J. Farrington*, A. Wassef*, E. Smith* and W.J. Meyer, III* (SPON: R. M. Rose). Dept. of Psychiatry & Behavioral Sciences, The University of Texas Medical Branch, Galveston, TX 77550.

A subgroup of individuals with major depressive disorder have an impaired thyrotropin (TSH) response to thyrotropin releasing hormone (TRH) (Loosen, Psychoneuroendocrinology 10:237, 1985). The possible causal relationship and mechanism of this "blunted" TSH response in depression is unknown. Numerous, recent studies have characterized similarities and interactions between the immune and neuroendocrine systems. The immune system both produces (Smith et al, PNAS 80:6010, 1983) and responds to TSH (Kruger et al, J Immunol 137: 197, 1986). Our studies indicate that the major cell type producing ir-TSH is the T lymphocyte. We utilized a peripheral blood leukocyte system to compare immunoreactive (ir)-TSH responsiveness in 11 adult patients (2M, 9F) with Research Diagnostic Criteria for major depressive disorder to that of 10 normal controls. All subjects had normal baseline serum TSH concentrations. Isolated mononuclear leukocytes were treated in vitro with 0.5 µg/ml Staphlococcal enterotoxin A (SEA), 50 µg/ml TRH or no stimulant. After incubation, the cells were monitored for ir-TSH production by indirect immunofluorescence and reverse hemolytic plaque assay using antisera to TSH-β. The culture supernates were analyzed by TSH-RIA. SEA and TRH treated cell cultures from depressed individuals had significantly fewer immunofluorescent positive cells as well as significantly fewer number and size of plaques than did similarly treated normal leukocytes. The increase in supernatant ir-TSH was significantly less in TRH treated cultures from depressed patients as compared to normals ($P < .05$). These results suggest that examination of leukocyte TSH production may reflect the state of neuroendocrine function and thus be a useful marker for major depressive disorder. In addition, the leukocyte system may serve as a tool to elucidate the mechanism of TSH hyporesponsiveness to TRH observed in patients with major depression.

175.1 MODIFICATION OF A MECHANORECEPTOR CHANNEL PRODUCES HABITUATION IN THE PROTOZOAN, STENTOR. D.C. WOOD. Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

The contractile protozoan, Stentor coeruleus, habituates during repetition of the mechanical stimulus used to elicit the initial contractions. This decrement in response probability is highly correlated with a reduction in receptor potential amplitude while the amplitude of the action potentials, which trigger the contractions, does not change.

Three lines of evidence indicate this receptor potential reduction is due to an alteration in the voltage-dependence of the mechanoreceptor Ca++ channel conductance:

1) The I-V plots of mechanoreceptor currents elicited from cells before and after habituation have a voltage-dependent region between -60 and -20 mV and a linear voltage-independent region between -20 and 20 mV with a reversal potential near 20 mV. The slope of the linear region did not change as a result of habituation indicating that the maximum mechanoreceptor channel conductance and hence the total number of mechanoreceptor channels was the same in control and habituated cells. The mechanoreceptor reversal potential was changed by only a few millivolts, from 21.6 to 17.2 mV, leaving the driving force term for the mechanoreceptor current at resting potential relatively unchanged. On the other hand, the voltage dependence of the mechanoreceptor channel conductance became significantly steeper after habituation (9.6 mV/e-fold change) compared with its value before habituation (12.6 mV/e-fold change).

2) The amplitude of receptor potentials elicited from cells depolarized by current pulses habituated to only a very limited degree while receptor potentials elicited at resting potential underwent marked habituation.

3) Radioactively labeled (+)-tubocurarine (TC*) binds selectively to that conformation of the mechanoreceptor channel which predominates at resting and hyperpolarized potentials and binds only weakly to that conformation which predominates at depolarized potentials (Wood, 1985). TC* binding was significantly increased in habituated animals relative to controls when both were at normal resting potential, but this difference disappeared as both types of cells were depolarized by increased extracellular concentrations of K+. Thus, the voltage-dependent characteristic of TC* binding was effected by the habituation process.

Taken collectively, these data suggest that the voltage gate of the mechanoreceptor channel has a larger charge after habituation than before it.

175.2 PLASTICITY IN THE SHORTENING REFLEX OF THE SEMI-INTACT LEECH: BEHAVIORAL AND CELLULAR ANALYSIS. J.MAZER*, N.BOULIS*, C.SAHLEY, BIOLOGY DEPT, YALE UNIVERSITY, CT.

Habituation, sensitization, and classical conditioning have been demonstrated in the leech, Hirudo medicinalis (Lockery et al, 1985; Henderson & Strong, 1972; Sahley & Ready, 1985). To begin the cellular analysis we developed a semi-intact preparation that allows simultaneous behavioral and physiological observations. We show here that this preparation exhibits plasticity similar to that of the intact leech. Also, this preparation was used to study the role of the fast conducting system (FCS) in plasticity.

FCS activity elicited by tactile stimulation is often accompanied by whole body shortening (Magni & Pellegrino, 1978), and FCS activity mimics behavioral changes seen during simple learning (Belardetti et al, 1982). To further study the role of the FCS in reflex plasticity, we simultaneously recorded behavioral and FCS changes during habituation, sensitization, and dishabituation of the shortening reflex in a semi-intact preparation consisting of the first 9 segments and 3 exposed ganglia. FCS activity was recorded extracellularly. Shortening was measured through a force transducer attached to the sucker. Stimuli were low voltage shocks delivered to the anterior portion of the leech's back.

In Exp 1 behavioral and FCS changes were recorded during 20 repeated stimulus presentations at a 2 min ISI, and for 4 trials following dishabituating stimuli. Behavioral decrement as a result of the repeated stimulus (habituation) and a recovery from this decrement following the dishabituating stimulus were observed. In contrast, spiking showed no significant change. In Exp 2 identical protocols were used as in Exp 1, except that three ISIs were tested (10 sec, 45 sec, 5 min). Spiking showed a decrement over trials, but did not correlate with behavioral changes. Behavior showed significant decrement at the 45 sec and 5 min but not the 10 sec ISI. In Exp 3 presentation of a noxious stimulus prior to habituation training (sensitization) was studied. No increase in responding was observed, but subsequent habituation was prevented. The same stimulus, though not increasing initial responding, produced an increase from a habituated baseline.

The semi-intact preparation is, thus, a suitable means of studying the cellular basis of behavioral plasticity. Further, changes in FCS activity during training are not a simple source of the behavioral plasticity seen in whole body shortening.

Support: NIMH 5RD1-MH37902, Sloan BR2486, Whitehall to C.L.S.

175.3 DISHABITUATION AND SENSITIZATION OF THE TOUCH-ELICITED SHORTENING REFLEX IN THE LEECH, *HIRUDO MEDICINALIS*: THE FACILITATORY STIMULUS MODIFIES T AND P SENSORY NEURONS. C. L. SAHLEY, (SPON: C. STEVENS), DEPT. OF BIOLOGY, YALE UNIVERSITY, NEW HAVEN, CT.

The touch elicited shortening response of the leech can be modified by several types of learning including habituation, dishabituation, sensitization, and classical conditioning. We have recently begun to analyze the cellular and molecular changes mediating these learned changes in behavior. Since the same noxious stimulus, shock to the skin for the intact animal or shock to the connective for the semi-intact preparation, can be used to produce dishabituation and sensitization of the reflex as well as be used as the unconditioned stimulus (US) in conditioning experiments, we began our cellular analysis by examining the effects of this facilitating stimulus on identified cells within the neural circuit underlying the shortening reflex.

The touch sensitive (T) and pressure sensitive (P) mechanosensory cells are activated by the tactile stimulus used in our experiments and make monosynaptic connections on to the longitudinal (L) motorneuron. Contraction of the L motorneuron results in whole body shortening. Any changes in the intrinsic characteristics of the sensory cells such as increases or decreases in the width of the action potentials or increases or decreases in the level of excitability could be reflected as an enhancement or decrement in the reflex. For this reason we have looked at the effects of the facilitating stimulus on the T and P sensory cells.

Using semi-intact preparations or a chain of isolated ganglia we compared the effect of the facilitatory stimulus on the response of the T and P cells to a threshold intracellular current injection. The responce to three pulses was measured prior to the delivery of the facilitating stimulus and again following the stimulus at intervals of 2 min, 5min, 10 min, and 20 min. The results indicated that T and P cells were affected differently by the facilitating stimulus. T cells showed an increase in action potential duration while P cells changed from giving a single spike to firing multiple spikes to the test stimulus. Both of these results are consistent with the enhancement of the reflex seen in intact leeches following presentation of the facilitating stimulus.

Support: NIMH 5RD1-MH37902, Sloan BR2486, Whitehall to C.L.S.

175.4 FOOD AVERSION CONDITIONING OF A CARNIVOROUS LEECH. T. Karrer and C. L. Sahley, Psychology Dept, Yale University, New Haven, CT. 06520

Although the leech has proven useful in the study of the cellular basis of many behaviors including swimming (Brodfuehrer & Friesen, 1986) and feeding (Lent & Dickinson, 1984), it has rarely been the focus of classical conditioning studies (Henderson & Strong, 1972). For the most part leech studies have concentrated on the shortening reflex and employed only aversive reinforcers. Our goal is to expand our understanding of learning in the leech by studying both aversive and appetitive learning.

Feeding is modified by learning in a number invertebrates, including *Pleurobranchaea* (Mpitsos & Davis, 1973), *Limax* (Sahley et al, 1981), *Achatina* (Croll & Chase, 1980), *Lymnaea* (Audesirk et al, 1982), and *Drosophila* (Tully & Quinn, 1985). The study of feeding behavior is advantageous since it can be modified with both appetitive and aversive reinforcers (Sahley et al, 1982; Tempel et al, 1983). We have chosen to study plasticity of feeding in the carnivorous leech, *Haemopis marmorata*. Our initial studies employed a food avoidance protocol to test whether this leech could learn to associate a food taste with the aversive taste of quinidine, such that the leech would decrease its preference for the food paired with quinidine.

We employed discriminative training, in which there were two CS's: A CS+, which consisted of a food paired with a bitter chemical (US); and a CS-, a different, but equally attractive food, which was not paired. Counterbalanced groups of leeches were run simultaneously: A Chic+ group, which received CS+ trials of paired chicken (CS) + quinidine (US), and CS- trials of liver alone; and a Liv+ group, which received CS+ trials of paired liver (CS) + quinidine (US), and CS- trials of chicken alone. Training was conducted in randomly ordered CS+/CS- trial pairs, whose within-pair ITI = 20 min. Between each trial pair was a 1 hr interval. Tests were conducted either 1-3 hr after the last training trial or test, or 1-3 days later. Testing consisted of simultaneous presentation of both CSs, without the US; the leeches' preferences were tabulated.

The results suggested that learning had occurred. Comparing responses to a food that had been paired with quinidine to responses to that same food when it had been unpaired revealed that the food was chosen more often when it was unpaired than when it was paired. In other words, the leeches preferentially avoided the paired food.

Support: NIMH 5RD1-MH37902, Sloan BR2486, and Whitehall to C.L.S.

175.5 ROTATION-INDUCED OPIOID ANALGESIA IN THE TERRESTRIAL SNAIL, CEPAEA NEMORALIS. F.S. Tepperman, M. Kavaliers and K.-P. Ossenkopp, Div. of Oral Biology and Dept. of Psychology, University of Western Ontario, London, Ontario, Canada.

There is evidence to suggest that endogenous opioids are involved in the regulation of the aversive behavioral and physiological responses of both vertebrates and invertebrates. Administration of morphine to the terrestrial gastropod mollusc, Cepaea nemoralis, elicits a naloxone-reversible increase in the latency of the avoidance behavior to a warmed surface. This response, which is analogous to the morphine-induced analgesic responses of mammals, appears as an increased latency in the lifting of the anterior portion of the fully extended foot from a warmed surface. In mammals, exposure to a variety of stressful stimuli has been shown to induce endogenous opioid-mediated analgesic responses that are analagous to the responses observed following administration of morphine. In the present study, we describe the effects of a commonly used laboratory stress, body rotation, on the aversive thermal responses of Cepaea.

Various groups of snails were held in moist, sealed containers and exposed to either continous (79 rpm) or intermittent (15, 30 sec, on,off) body rotation for varying periods of time (5-30 min). At specific times (0-60 min) after rotation, the latency of the foot-lifting response to a surface held at 40°C was determined for individual Cepaea. Snails exposed to either continuous or intermittent rotation were analgesic, displaying significantly greater thermal response latencies than sham-rotated or control animals. Pre-treatment with low doses of naloxone (1.0 mg/kg) blocked both the continuous and intermittent rotation-induced analgesia, while having no evident effects on the responses of control snails. These opioid-mediated analgesic responses were evident up to 60 min after continuous rotation, with longer rotation periods eliciting greater durations of analgesia. Intermittent rotation produced a relatively prolonged analgesia, whose duration was also dependent on the temporal parameters of the rotation. These results suggest that the stress of body rotation leads to the activation of endogenous opioid systems in Cepaea and the display of opioid-mediated analgesic responses that are similar to those observed in mammals.

175.6 LONG-TERM MEMORY OF LEARNING THAT FOOD IS INEDIBLE IN APLYSIA IS UNAFFECTED BY GUT DENERVATION. M. Schwarz* and A.J. Susswein, Dept. Life Sciences, Bar Ilan Univ., Ramat Gan, 52 100 ISRAEL.

Feeding in Aplysia is modulated by an associative learning task resembling operant conditioning (Susswein et al., J. Neurosci. 6: 1513-1527). When feeding is paired with internal stimuli arising from failure to consume food, animals learn that food is inedible, and stop responding. Successful feeding is a positive reinforcer. Memory of training is retained for at least 48 hours, as shown by decrease in time to retrain animals. Gut stimuli are necessary for producing reinforcement of feeding, as shown by an inability to learn when the esophageal nerves innervating the gut are bilaterally sectioned (Schwarz & Susswein, J. Neurosci. 6: 1528-1536).

The present study investigated whether memory of training is retained for longer than 48 hours, and whether gut stimuli are necessary for retention of training. We found that memory is retained for at least 3 weeks, and that denervation of the gut **after** learning does not affect retention of the learned change.

In experiments testing retention of memory for 3 weeks, Aplysia were fed the seaweed Ulva wrapped in plastic net. Animals responded to netted food, but were unable to consume it. As training proceded, animals made fewer successful feeding responses, and eventually ceased responding to food. Twenty-four hours later, Aplysia were retrained. Fewer successful feeding responses occurred, and time to stop responding was 48.0% ± 8.0 (SEM) of that on the previous day. Three weeks later animals were trained again to determine if memory was retained. Time to stop responding was 69% ± 11.7 (SEM) of that on the first training session. Thus, responsiveness was higher than during the second session, but still significantly less than during the first session.

We also tested memory a week following a single training session. Time to stop responding was 51% ± 7.6 (SEM) of that on the first session, indicating that memory is virtually intact from 1 to 7 days after training.

In experiments testing whether gut innervation is necessary for retention, as it is for the original training, Aplysia were trained as described above. One day after training animals were anesthetized, and the esophageal nerves innervating the gut were bilaterally sectioned. One week later animals were trained a second time in a blind procedure, along with naive, sham-operated and previously-trained unoperated animals as controls. All three groups showed similar decreases in time to stop responding upon retraining (Unoperated: 51% ± 7.6 (SEM); Sham Operated: 40.6 ± 10.8 (SEM); Esophageal Nerves Cut: 43% ± 5.8 (SEM)). These data show that gut inputs are **not** necessary for retention of learning, and that retention is due to changes occurring in the central nervous system.

175.7 MOLLUSCAN OLFACTORY INTERNEURONS: MEMBRANE RESPONSES AND CALCIUM TRANSIENTS A. Gelperin, D. W. Tank, J. A. Connor, and J. Flores*. Molecular Biophysics Research Department, AT&T Bell Laboratories, Murray Hill, NJ 07974.

The procerebrum (PC) of the terrestrial slug *Limax maximus* contains half a million interneurons which receive direct axonal projections from the superior and inferior noses. The extensive neuropil of the PC contains a dense matrix of connections between the intrinsic interneurons, input fibers from the noses and modulatory fibers containing serotonin, dopamine, SCP_B and FMRFamide. As part of an ongoing study of olfactory processing in the *Limax* PC, we have studied the membrane responses and intracellular calcium levels of isolated PC neurons in culture.

Procerebral lobes were isolated and completely freed of connective tissue by dissection. After a 1 hour incubation in Sigma type IX protease at 35°C, lobes were mechanically dissociated by stirring at 4°C for 1 hour. A 0.25 ml volume of cell suspension was plated onto a polylysine coated surface. After allowing 2 hours for cells to adhere to the substrate, additional medium was added. Cells were grown in *Limax* saline supplemented with 15 mg/ml bovine serum albumin (BSA). BSA was necessary to obtain extensive neurite outgrowth. Cells were studied after 1 - 8 days in culture. For studies of intracellular calcium levels cells were loaded with fura2/AM indicator by incubation for 20 min in saline containing 5 uM fura2 that had been dissolved in DMSO. Indicator readings were obtained by either dual wavelength (340/380 nm excitation) differential imaging (Connor, Proc. Natl. Acad. Sci. USA, 83:6179, 1987) or single photodiodes monitoring the intensified images of PC neurons. Membrane currents were monitored with whole-cell patch recording. Spontaneous action potentials were measured by loose and tight seal cell-attached patch recording.

Most isolated PC neurons display spontaneous action potentials and under whole cell voltage clamp display fast inward and slow outward currents which might underlie action potential production. Cells showed both regular and irregular bursting. Cultured PC neurons also display spontaneous fluctuations of intracellular calcium as determined from fluorescence measurements.

Isolated cells were challenged with 5X potassium saline and modulatory neurotransmitters known to be present in the PC lobe. Depolarization with 5X saline resulted in rapid but transient increases in intracellular calcium. Work is continuing on recordings aimed at measuring synaptic interactions between PC neurons in culture and immunocytochemical determination of additional neurotransmitters operating in the PC circuit.

175.8 IN APLYSIA OSMOREGULATION AND ITS NEURAL CORRELATES ARE AGE-SENSITIVE. T.L. Skinner* and B. Peretz. Dept. Physiology and Biophysics, Univ. of Kentucky, Lexington, KY 40536.

Osmoregulation in Aplysia is affected by age. When exposed to 90% artificial seawater (90%-ASW), old animals (244 days old) take signigicantly longer than mature animals (173 days old) to: 1) reestablish their original weight, 2) reestablish their original hemolymph osmolality and, 3) increase their metabolic activity. Yet, when exposed to 100%-ASW after being volume and osmotically stressed internally by injections of distilled water, old and mature animals reestablish their control weight and hemolymph osmolality at the same rate. The results suggest that the non-neural mechanisms involved in water balance are unaffected by age. This study examines the age effects in the neural pathway mediating osmoregulatory behavior (ORB) in response to 90%-ASW.

First, the effect of lesioning the osphradium was examined in 12 mature animals to determine the contribution of the osphradium to the ORB in response to 90%-ASW. The time course of osmoregulation in osphradial-lesioned mature animals was significantly slower than that of sham-lesioned mature animals, $F(9,90)=6.2$, $p<0.03$, but not significantly different from that of non-lesioned old animals, $F(9,126)=0.47$, $p<0.5$. The osphradium then is necessary for the mature response to 90%-ASW. The age effect seen in the ORB may be due to age-related changes in the osphradium.

R_{15} possibly mediates water balance (Kupfermann, I. and K.R. Wiess, J. Gen. Physiol., 67:113, 1976; Bablanian, G.N. and S.N. Triestman, J. Comp. Physiol. B, 155:297, 1985). Exposure of the osphradium to hypo-osmotic seawater evokes an hyperpolarization (hyperpol.) in R_{15} and inhibits it (Stinnakre, J. and L. Tauc, J. Exp. Biol., 51:347, 1969). Second, when the osphradium from mature preparations (n=3) was exposed to 80%-ASW, an hyperpol. of 21.4±6.8 mv was evoked in R_{15} which inhibited its bursting; but, in old ones (n=3) an hyperpol. of only 3.2±3.1 mv was evoked in R_{15} which did not change its bursting. Possibly, R_{15}'s decreased inhibition during old animal's exposure to 90%-ASW contributes to the age-dependent slowing of the ORB. Single pulses to the branchial nerve evoked in mature R_{15}s an hyperpol. of 24.6±8.1 mv while in old R_{15}s it evoked an hyperpol. of 13.1±3.4 mv. The input resistance of old R_{15}s is nearly half that of mature R_{15}s, i.e. 5.1±0.7 vs 9.3±1.2 M , which can explain the difference in the amplitude of the evoked hyperpol. But, the decreased hyperpol. of old R_{15}s to osphradial stimulation is not explained by this resistance change, because the hyperpol. is nearly 7x less than that in mature R_{15}s. Two sites in the ORB pathway in response to hypo-osmotic stress are affected by age: sensory activity and neuroeffector responsiveness.

175.9 THE APLYSIA GILL WITHDRAWAL REFLEX AND ITS NEURAL CORRELATES ARE AGE DEPENDENT. J.L. Ulmer* and B. Peretz (SPON: L. Boyarsky). Dept. Physiology and Biophysics, Univ. of Kentucky, Lexington, KY 40536.

Peretz et al. (P.N.A.S., 81:4232, 1984) showed that in old Aplysia there was a significant decrease of motor neuronal efficacy for L_7, but not for LDG_1. The present study was done to determine if L_7's age-sensitivity affected the gill withdrawal reflex, (GWR).

In preparations from 19 mature (152 days old) and 24 old animals (235 days old) with the CNS innervation to the gill, mantle, and siphon left intact, activity of motor neurons in the abdominal ganglion was recorded during the GWR, which was elicited by tactile stimuli of 0.2 to 5 g/mm^2 to the siphon. The GWR amplitude was significantly reduced in old as compared to mature animals, $F(1,32)=11.7$, $p<0.002$. The relationship between GWR amplitude and the range of stimulus intensities was significantly uncoupled in old animals as compared to that in mature ones, $F(4,128)=8.8$, $p<0.001$. To determine the neurons responsible for the GWR age-sensitivity, the contribution of each motor neuron was determined by comparing the GWR amplitude, with a neuron hyperpolarized, to that when it was allowed to spike. L_7 mediated on the average 56% of the GWR in the mature group (cf. Kupfermann, I., et al., J. Neurophysiol., 37:998,1974), but only 13% in the old group, $F(1,13)=12$, $p<0.005$. LDG_1's average contribution of 40% in mature animals was reduced to 2% in the old group, $F(1,10)=5.4$, $p<0.05$. Lg and LDG_2 mediated an average of 35% of the GWR in both age groups. The contributions of the 4 neurons being > 100% in the mature group shows that their effects are not simply additive. In old animals, L_7's and LDG_1's reduced role in the GWR may result partially from decreased effectiveness of afferent inputs to them, because fewer spikes were evoked in both old L_7s and old LDG_1s as compared to the mature groups, $F(1,15)=15.8$, $p<0.001$ and $F(1,13)=7.1$, $p<0.025$ respectively. Also, the relationship between the number of spikes evoked in old L_7s and stimulus intensity was reduced compared to that in mature L_7s, $F(4,60)=6.5$ $p<0.001$; this finding appears primarily a result of L_7's age-sensitivity. Unlike the reduced spiking evoked in LDG_1 by siphon stimulation, spiking evoked during gill respiratory movements was virtually unchanged with age. Hence, in addition to L_7's motor neuronal efficacy being reduced, the afferent input initiating the GWR appears to be age-sensitive, thus compromising both L_7's and LDG_1's ability to mediate the GWR.

175.10 PROPERTIES AND CHARACTERISTICS OF GILL GANGLION CELLS IN APLYSIA CALIFORNICA. Elaine Colebrook and Ken Lukowiak. Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Utilizing anatomical, electrophysiological, pharmacological and immunohistochemical techniques, various properties of the gill ganglion (gg) of Aplysia californica have been identified as a first step towards determining the role that this ganglion may play in the mediation of gill behavior.

Studies with LY have shown the cells to be monopolar, with many of their axons coarsing towards the gill. The axons of other neurons remain intrinsic to the ganglion. Dense bushy dendrites are seen directed towards the abdominal ganglion.

The neurons vary in their electrophysiological properties. Some cells are coupled, coupling ratios from 0.1 to 0.9 have been observed between different pairs of cells. Use of the semi-intact gill, siphon, mantle preparation has revealed that depolarization of some gg cells can induce gill withdrawals; suggesting that certain of these cells have similar properties to the gill motor neurons in the abdominal ganglion. Bursts of activity in the cells correlate with spontaneous gill withdrawals. Such bursting is evident even in the absence of the abdominal ganglion. This indicates a role for the gg cells in respiratory pumping movements of the gill. Hyperpolarizing responses are induced in certain cells by stimulation of the pedal nerves and tapping the gill or siphon causes depolarizing responses. Hence a likely role in defensive gill reflexes and, possibly, their plasticity.

Low concentrations of 5-HT (10^{-6}) inhibit the activity of spontaneously active gg cells. Higher concentrations of 5-HT (10^{-4}-10^{-5}) increase the rate of firing in spontaneously active cells. Bath application of SCP_B (10^{-5}-10^{-6}) induces regular bursting activity in gg cells. These effects are apparent with and without an intact branchial nerve, indicating a direct effect of these neurotransmitters on the gg cells.

5-HT antisera revealed fibres within the branchial nerve which pass by the gg. No cell bodies within the gg were revealed with the 5-HT antisera. There was no SCP_B immunoreactivity in or around the gg; suggesting that the excitatory effect of SCP_B may be mediated via the blood.

These findings together suggest that the heterogeneous neurones of the gill ganglion play a role in the mediation of gill behavior, and probably its plasticity.

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175.11 TIME COURSE OF STRUCTURAL CHANGES AT IDENTIFIED SENSORY NEURON SYNAPSES DURING LONG-TERM SENSITIZATION IN APLYSIA. C.H. Bailey and M. Chen. Ctr. for Neurobiol. & Behav., Dept. of Anat. and Cell Biol., Neurol., & Psychiat., Columbia Univ., P&S, and NYS Psychiat. Instit., N.Y, NY 10032.

We have used the gill- and siphon-withdrawal reflex of Aplysia californica to explore the morphological basis of the synaptic plasticity that underlies simple forms of learning and memory. In earlier studies we described two classes of structural changes at identified sensory neuron synapses that occur following long-term sensitization. These structural alterations occur at different levels of biological organization and include increases in the number, size, and vesicle complement of active zones as well as an overall increase in the total number of synaptic varicosities per sensory neuron. In the present study we have begun to examine which of these anatomical changes might be necessary for the maintenance of long-term sensitization by exploring the time course over which they occur and in particular, their duration relative to the persistence of the memory assessed behaviorally.

Toward this end we have quantitated changes in the total number of varicosities of single horseradish peroxidase (HRP)-labeled sensory neurons taken from long-term sensitized and control animals at different intervals following training. Experimental animals were trained for long-term sensitization after the 4-day protocol of Pinsker et al. (1973); control animals were carried under identical conditions but without training. Experimental (N=15) and control (N=8) animals were then examined for retention of sensitization at 1-2 days, 1 week, or 3 weeks following the end of training. A single sensory neuron from each animal was intrasomatically pressure-injected with HRP, incubated for 2 hrs., fixed, histochemically processed and embedded in EPON. The neuropil arbor of each cell was completely reconstructed using serial 20 μm slab-thick sections and the total number of HRP-labeled varicosities for each sensory neuron was counted through a blind procedure. A total of 23 sensory neurons were analyzed in this fashion.

As reported in an earlier study, we have found that long-term sensitized animals examined within 48 hrs following the completion of training demonstrate an increase in the total number of varicosities per sensory neuron (2841 ± 372 S.E.M., N=6) compared to control animals (1521 ± 66 S.E.M., N=3). This increase persists unchanged for at least 1 week (3066 ± 554 S.E.M., N=4 vs. 1356 ± 154 S.E.M., N=3) and is only partially reversed at the end of 3 weeks (1878 ± 246 S.E.M., N=5 vs. 1232 ± 211 S.E.M., N=2). The duration of these changes in varicosity number parallels the behavioral time course for the retention of long-term sensitization. These similarities suggest that the timekeeping steps for a long-term memory trace may, in part, be specified by morphological change and strengthen the notion that alterations in the number of sensory neuron synapses may play a role in the maintenance of long-term sensitization.

175.12 INVOLVEMENT OF SEROTONIN IN SENSITIZATION OF APLYSIA GILL-WITHDRAWAL REFLEX. A.M. Dyke*, S.L. Mackey*, D.L. Glanzman and R.D. Hawkins (SPON: M. Segal). HHMI & Columbia P&S, NY, NY 10032.

Several lines of evidence suggest that serotonin (5-HT) plays a role in sensitization of the Aplysia gill-withdrawal reflex. 5-HT and tail shock both produce behavioral sensitization due in part to presynaptic facilitation of the siphon sensory neurons. Injecting animals with the serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) reduces the facilitation produced by tail shock (Glanzman et al., 1986). Immunohistochemical studies (Kistler et al., 1985) indicate there are serotonergic endings on the sensory neurons. Finally, we have identified a pair of serotonergic neurons in the cerebral ganglia (LCB1 and RCB1), stimulation of which produces presynaptic facilitation of the sensory neurons (Hawkins, 1986; Mackey et al., 1986). Here we provide evidence that the CB1 neurons participate in behavioral sensitization of gill withdrawal. In addition, we report that treatment with 5,7-DHT reduces this behavioral sensitization.

To assess the role of the CB1 neurons in sensitization, we recorded their response to cutaneous stimulation in a semi-intact preparation. They respond with a phasic increase in firing to moderate tactile stimulation anywhere on the body surface, and respond with a very prolonged (in some cases greater than 15 minutes) increase in firing to noxious stimuli such as those used to produce behavioral sensitization. Intracellular stimulation of CB1 neurons with approximately this physiological pattern produces reliable broadening of action potentials in the siphon sensory neurons. We also injected CB1 neurons with Lucifer yellow and found that they 1) have 5-HT-like immunoreactivity, and 2) send axons to both cerebral-pleural connectives. The morphological appearance of these axons is consistent with serotonergic axons which Longley and Longley (1986) showed extend to the abdominal ganglion and branch extensively in the neuropile. These results support the idea that the CB1 neurons participate in mediating behavioral sensitization of gill withdrawal. Since their facilitatory effects are modest, however, other serotonergic cells may also participate in sensitization.

To assess the role of all serotonergic facilitatory neurons in behavioral sensitization, we injected Aplysia with 2 g/kg 5,7-DHT and then tested sensitization of gill withdrawal using a semi-intact preparation. The 5,7-DHT treatment reduced behavioral sensitization by over 90% with either tail shock (N=10, U=17.5, $p<.02$) or mantle shock (N=15, U=61, $p<.05$) as the sensitizing stimulus. In addition to sensitizing the evoked contractions, tail and mantle shocks produced an increase in the frequency of spontaneous contractions of the gill; this effect was also significantly reduced by 5,7-DHT treatment. There were no significant differences between the 5,7-DHT-treated and control preparations in either the pre-shock test scores or the direct responses to the shock.

Together, the results of these two sets of experiments provide additional evidence for the involvement of 5-HT in behavioral sensitization of the Aplysia gill-withdrawal reflex.

175.13 THE HELIX ASPERSA "LOVE DART" INJECTS A DIGITIFORM GLAND FACTOR(S) THAT SYNCHRONIZES MATING BEHAVIOUR. S.A. Adamo and R. Chase. Dept. Biology, McGill Univ., Montreal, Quebec, H3A 1B1, Canada.

In Helix aspersa, a simultaneously hermaphroditic terrestrial snail, copulation follows after eversion of the genital apparatus, which occurs during a courtship period lasting 45-125 min. We have identified 7 stages of genital eversion. Stage 5 includes the shooting of a calcareous dart. The dart carries mucous secreted by the digitiform glands into the hemocoel of the mating partner. Copulation follows within 2-20 min. of the second of the reciprocal dart shots.

It was recently reported (Chung, D., J. Exp. Zool. 238: 129-139, 1986) that injections of digitiform gland extract produce some genital eversion in animals that had none prior to injection. We have found that the duration of the response ($p<0.05$) and the percentage of animals responding to digitiform gland ($p<0.05$) is dependent on the extent of the animal's initial eversion. Control snails injected with either dart sac plus dart or with saline showed no increase in eversion at any preinjection eversion stage.

Preliminary results with dartless and digitiform glandless animals show that these animals have a larger range of courtship times than controls. The dartless and digitiform glandless animals differ most from controls when the courting partners are at very different genital eversion stages. In these cases, the time between dart shooting (or attempted dart shooting) and copulation is lengthened, and more pairs fail to reach copulation.

Our interpretation of these results assumes that the successive stages of genital eversion represent increasing levels of arousal and that the two courting snails must reach progressively higher levels of arousal in approximate synchrony for copulation to occur. The snail receiving the first dart is brought closer to the arousal level of the shooting snail. This increases the probability of copulation. Because the effect depends on the animal's initial state of arousal, the digitiform gland secretion material appears to work synergistically with other hormonal or neurohormonal agents.

175.14 SELF-LOATHING AND EVASIVENESS IN NOTASPID SNAILS: POSSIBLE REGULATION OF AVOIDANCE BEHAVIOR BY DEFENSIVE ACID SECRETION. Rhanor Gillette and Rong-Chi Huang. Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.

Notaspidean opisthobranchs are notable for their ability to secrete sulfuric acid from their skin in response to noxious stimuli (Thompson and Colman, J. Moll. Stud. 50, 66). Because of this secretion, and perhaps other factors, these mollusks are rejected as food by most predators, including such markedly tasteless scavengers as crabs, herring gulls and starfish; aversion is specific to the skin, as internal organs are readily accepted. Indeed, skin contact frequently triggers stereotyped avoidance/escape responses in starfish, many species of snails and swimming anemones. Accidental observations arising from a mis-made solution while working on local notaspids at the Isle of Man led to demonstration that acid solutions elicit a stereotyped avoidance behavior similar across 5 species.

Solutions of seawater adjusted to pH 2-3 (10-100 times less acid than animals' defensive secretions) and pipetted slowly onto the oral veil cause stereotypic avoidance behavior consisting of local withdrawal, aversive turns and rapid escape locomotion in specimens of Pleurobranchus membranaceus, Berthella plumula, Berthella californica, Berthellina citrina and Pleurobranchaea californica. Pleurobranchus and occasional Pleurobranchaea showed swimming escape locomotion. Acid solutions were aversive at any part of the body, and caused local withdrawal when applied to isolated Pleurobranchaea skin resting in buffered seawater, suggesting a component of avoidance behavior mediated peripherally. The avoidance behavior of Pleurobranchaea is indistinguishable from that often shown to conspecifics, and that observed in associatively food-avoidance trained animals.

These data show that avoidance behavior in these species is intimately tied to defensive acid secretion, and they suggest further ties. The importance and potential contribution of acid secretion to protection from cannabalism and learned food-avoidance behavior in Pleurobranchaea is under investigation.

The hospitality of the University of Liverpool Marine Station at Port Erin, I. O. M., is gratefully acknowledged.

175.15 SHORT-TERM AND LONG-TERM CHANGES IN IDENTIFIED B-PHOTORECEPTORS OF HERMISSENDA PRODUCED BY LIGHT AND SEROTONIN. J. Forrester* and T. Crow (SPON: J. Horn). Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Positive phototactic behavior in Hermissenda undergoes a long-term reduction after repeated pairings of light with rotation. Associated with conditioning of Hermissenda are intrinsic changes in the primary sensory neurons (type B-photoreceptors) of the pathway mediating the conditioned stimulus (CS). We have been investigating the contribution of the neuromodulator 5-HT to conditioning in Hermissenda. Previous research demonstrated that light responses of Hermissenda B-photoreceptors were enhanced and prolonged by bath application of 5-HT (Crow & Bridge, 1985). Recently, it was shown that 5-HT immunoreactive fibers and varicosities terminate near the optic nerve and in the neuropil near photoreceptor synaptic terminals (Land & Crow, 1985). In addition, the reduction in normal positive phototactic behavior can be mimicked by pairing light with direct application of 5-HT to the exposed circumesophageal nervous system (Crow & Forrester, 1986, PNAS 83:7975). Collectively these results suggest a role for 5-HT in conditioning in Hermissenda. In order to determine if pairing light and 5-HT produces intrinsic changes in sensory neurons we have investigated changes in the light responses of identified B-photoreceptors at different times following the application of light and 5-HT.

Light responses (generator potentials) of surgically isolated medial and lateral B-photoreceptors were examined 1 hr and 24 hrs after one session of light paired with 5-HT. Controls received light and 5-HT unpaired. Both the paired group (N=23) and the unpaired group (N=25) exhibited a significant enhancement in the amplitude of the generator potential evoked by a 2 min light step 1 hr after training as compared to normal controls (N=10). The mean amplitude of the generator potential at the end of the light step was 28.9mV for medial B-photoreceptors from the unpaired group, 27.9mV for medial B-photoreceptors from the paired group, and 24.6mV for normal controls. Overall differences were statistically significant, $p<.05$. The mean amplitude of the generator potential of lateral B-photoreceptors from the paired group at the end of the light step was 27.8mV compared with 28.1mV for the unpaired group and 25.2mV for the normal controls ($p<.05$). The enhanced light responses of medial B-photoreceptors from the paired and unpaired groups, and lateral B-photoreceptors from the unpaired group were short-term. The light responses of isolated medial B-photoreceptors and lateral B-photoreceptors from both the paired (N=23) and unpaired groups (N=23) examined 24 hrs after the light-5-HT session revealed that only the lateral B-photoreceptors exhibited an enhanced response at the end of the light step (paired, $\bar{X}$=29.1mV; unpaired, $\bar{X}$=25.6mV, $p<.05$). These results indicate that paired light and 5-HT produce both short-term and long-term changes that are intrinsic to identified B-photoreceptors. Short-term enhancement of the generator potential is not pairing-specific. Long-term pairing-specific changes in the light response are detected in only the lateral B-photoreceptors and are expressed by changes in adaptation to sustained illumination.

175.16 CHARACTERIZATION OF A SUBSET OF PUTATIVE MOTOR NEURONS IN THE PEDAL GANGLIA OF HERMISSENDA WHOSE LIGHT RESPONSES ARE DUE TO SYNAPTIC INPUT FROM PHOTORECEPTORS. T.M. Hodgson* and T. Crow (SPON: O. Reinmuth). Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Suppression of the normal positive phototatic response in the marine mollusk Hermissenda is produced by a conditioning procedure which pairs stimulation of the visual system and gravity-detecting statocysts. The learning is expressed as a suppression of the initiation of locomotion in the presence of the CS (light). The neural circuitry underlying the conditioning has been well studied for the sensory systems involved in the CS and UCS (vestibular) pathways, but investigation into the neural circuitry underlying the expression of the behavior and its modification by conditioning has just begun.

As an initial step in this investigation intracellular recordings were made from putative motor neurons in the pedal ganglia. The responses of the pedal cells to light were tested with a 10 second light step. Four new types of light-responsive neurons were found. One cell type is an intermittent burster which hyperpolarizes at the onset of light; its return to baseline has a variable time course (from <1 sec up to the duration of the light step). The other three cell types show excitatory responses to light. Two of these are intermittent bursters, one of which shows a transient (<1 sec) burst of impulse activity at light onset followed by an immediate return to baseline activity. The other cell also exhibits this transient, plus a more prolonged (3-5 sec) increase in impulse activity. During spontaneous hyperpolarization or with the injection of extrinsic hyperpolarizing current to eliminate action potentials, the light response appears as an EPSP at light onset, accompanied by a slight depolarization whose time course extends 1-2 seconds past the duration of the light step. The third cell type with an excitatory light response shows considerable synaptic activity between action potentials and produces a transient burst of impulse activity at light onset followed by an immediate return to baseline activity.

To determine if the light responses were intrinsic to the pedal neurons or due to synaptic input from the photoreceptors, the cells' light responses were recorded before and after one of two manipulations. The first procedure involved cutting the optic nerve to isolate the photoreceptors from the rest of the nervous system. The second procedure was a bath application of the Ca^{2+} blocker $NiCl_2$ to a final concentration of 20 mM to block all synaptic activity. The light responses of all the cell types were abolished after either treatment, indicating that the responses were produced by synaptic activity originating in the photoreceptors.

These results show that a subset of putative motor neurons in the pedal ganglia of Hermissenda are light-responsive due to synaptic inputs from the photoreceptors. Further experiments are planned to determine which photoreceptor(s) provide input to these cells, and whether the light responses of these pedal cells can be modified by conditioning.

175.17 ELECTRICAL RESPONSE OF ABALONE LARVAE TO GABA; A SETTLEMENT-INDUCING MOLECULE. L. A. Barlow*. (SPON: M. L. Block). Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

The neurobiology of the veliger larvae of the red abalone, *Haliotis rufescens,* and of the pinto abalone, *H. kamtschatkana* , was investigated by making intracellular recordings from the ciliated cells of the larval swimming organ, the velum. Velar cells had resting potentials between -55 and -65 mV, which were independent of the age of the veliger. While these electrically coupled cells were at rest, the cilia of the velum beat metachronally. Each action potential recorded in a velar cell was correlated with a ciliary arrest. The action potentials of abalone velar cells were slow (lasting 250 msec) and overshooting with a total amplitude of ca 65 mV. Spike shape did not change with respect to veliger age.

The velar action potential was not blocked by .3 or 3 uM tetrodotoxin, but did show a variation in shape when the larva was perfused with low Na+ seawater (Na+ substituted with N-methyl-D-glucamine). Both Co++ and Ni++, when substituted for Ca++, blocked the action potential, indicating that the velar spike was carried primarily by Ca++.

The larvae of both red and pinto abalone are induced to settle and metamorphose when presented with a molecular component of the crustose coralline algae, *Lithothamnium* and *Lithophyllum* . GABA mimics the action of the coralline inducer substance. The electrophysiological response to GABA was used to examine an individual's ability to perceive the settlement cue. Spontaneous electrical activity consisted of random action potentials and slight fluctuations in membrane potential. When GABA was presented to the larva by perfusion, firing frequency in the velar cells increased abruptly. The ability of larvae to respond to GABA was both dose- and larval age-dependent. Precompetent larvae required larger doses of GABA to elicit a response than did older metamorphically competent larvae. Greater concentrations of GABA also induced higher rates of firing. These data suggest that larval abalone become increasingly sensitive to GABA as they approach metamorphic competency.

GABA did not appear to act directly on the velar cells, since no change in input resistance was observed at subthreshold concentrations. It is more likely that velar electrical activity represents integrated motor activity in response to GABA.

Since GABA arrests ciliary beat, and therefore swimming, perhaps this initial physiological and behavioral response is related to the phenomenon of induction of settlement by the GABA-like substance found in the natural inducer, crustose coralline algae.

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175.18 A CLASS OF DROSOPHILA BEHAVIORAL MUTANTS THAT ARE SENSITIVE TO BOTH MECHANICAL VIBRATIONS AND TEMPERATURE CONDITIONS. M.G. Burg and C.-F. Wu, Dept. of Biology, Univ. of Iowa, Iowa City, Ia. 52242.

Several Drosophila behavioral mutations are known to affect basic neurophysiological processes, resulting in paralysis at high temperatures. A class of so called "bang-sensitive" mutations are characterized by paralysis after exposure to mechanical vibrations. These mutations have been mapped to different loci on the X chromosome: tko (technical-knock-out), kdn (knock-down), eas (easily-shocked), bas (bang-sensitive), and bss (bang-senseless) (Ganetzky and Wu, Genetics 100: 597, 1982).

We examined the responses of individual bang-sensitive (b.-s.) mutants to both mechanical vibration and temperature conditions. Surprisingly, all the b.-s. mutations exhibited temperature-induced paralysis. Of the 4 mutants analyzed, bss and eas were paralyzed by low temperatures, whereas bas and tko were paralyzed by high temperatures. In addition, responses of individual flies to mechanical vibrations demonstrated distinct differences between these mutations.

In Drosophila, mutations that alter related functional processes may interact, resulting in more severe phenotypes (Ganetzky and Wu, Ann. Rev. Genet. 20: 13, 1986). Viable b.-s. double mutants exhibited a more severe vibration-induced paralytic phenotype than single mutants, suggesting that a functional interaction may be occurring between mutant gene products. In addition, both individual mutant phenotypes were present in the double mutants, i.e. the bas bss double mutant being paralyzed by high and low temperatures.

Behavioral analysis of b.-s. genetic mosaics containing part mutant and part normal tissue suggests that different regions of the nervous system are responsible for the various mutant phenotypes exhibited. Together, the results from double-mutant and mosaic analyses suggest that the b.-s. mutations may affect different cellular processes. Alternatively, they may affect similar processes in separate discrete regions of the fly's nervous system. Physiological experiments are presently underway to investigate the affected neural regions and the underlying cellular defects. This work was supported by an NIH pre-doctoral training grant GM07091 to MGB and NIH grants NS00675, NS15350, and NS18500 to CFW.

175.19 TACTILE REFLEXES OF COCKROACH LEG. C.A. Meyer* and M.V.S. Siegler*, (SPON R.L. Calabrese). Dept. of Biol., Emory Univ., Atlanta, GA 30322.

Behavioral and developmental studies on several insect species indicate that epidermal cells, cuticular sensory receptors, and associated sensory neurons are specified according to their location on the body surface. In the locust, spatial information from tactile receptors on the surface of a leg is integrated in the CNS to organize avoidance reflexes that differ according to the location of the stimulated receptors (Siegler and Burrows, J.Neurosci., 6, 507, 1986). In the cockroach, excised cuticular structures are regenerated in a way that indicates there is a continuum of positional values along the length of a leg segment and around its circumference (French, Phil.Trans.R.Soc.B, 295, 601, 1981).

The present study was undertaken to test whether in the cockroach, as in the locust, this spatial information might be of some behavioral significance. We report that tactile sensilla on the metathoracic legs of the cockroach Periplaneta americana are organized into receptive fields. Touch to sensilla that lie within different receptive fields elicit different avoidance reflexes of a leg. These fields occur on the different segments of a leg, and are oriented either with respect to the dorsoventral or the anteroposterior axis of the leg. For example, touching the proximal, ventral femur elicits promotion of the coxa, levation of the trochanter, and extension of the tibia, whereas touching the proximal, dorsal femur elicits promotion of the coxa, depression of the trochanter, and flexion of the tibia. Touching sensilla on the anterior ventral ridge of the coxa elicits levation of the trochanter and extension of the tibia, whereas touching sensilla on the posterior surface of the coxa elicits depression of the trochanter and extension of the tibia. (The surfaces of a leg are described as if it is held at a right angle to to the longitudinal axis of the body, with all its joints fully extended (Snodgrass, Smith.Misc.Coll., 82, 1, 1929). Accordingly, the meron of the coxa is on the dorsal surface of the leg, whereas the femoral spines are on the ventral surface of the leg.) Some ten further reflexes are described that are likewise elicited by touching sensilla that are located on different regions of the metathoracic legs.

175.20 FMRFamide PREVENTS HABITUATION OF THE EVOKED GILL WITHDRAWAL RESPONSE IN THE ISOLATED GILL OF APLYSIA. D. Cavthorpe and K. Lukowiak (SPON: P. Hicks). Dept. of Medical Physiology, University of Calgary, Alberta, Canada, T2N4N1.

The evoked gill withdrawal response (GWR) of the isolated gill of Aplysia californica may exhibit simple forms of learning such as habituation or sensitization. The peptide, FMRFamide, when perfused through the isolated gill of Aplysia, elicits spontaneous contractions of the gill. As well, FMRFamide immunofluorescence has been localized to nerve terminals innervating the gill, (Weiss, et al. 1984). This study examined the effects of FMRFamide on the habituation of the evoked GWR.

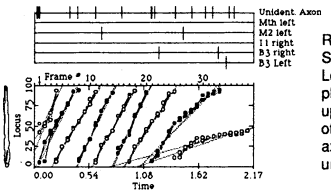
The gill, siphon and mantle were pinned dorsal side down to the base of a Sylgard dish. The abdominal ganglion was removed. The gill was cannulated via the afferent vein and perfused at a rate of 5-10 ml/minute. The gill was attached to a Grass FT03C force transducer by a thread tied to a single pinnule of the gill. Gill movements and tension were recorded on a Grass 79D polygraph. The gill was stimulated with a mechanical tapper. Artificial sea water (ASW; Instant Ocean) was used as perfusate. FMRFamide, (Peninsula), was diluted in ASW to the desired concentration immediately prior to perfusion through the gill. Experiments were commenced once a steady perfusion was attained and the gill was allowed to rest for one hour. In all experiments two control GWR's were recorded with an ISI of 20 minutes. Measurements of increases or decreases in the amplitude of the evoked GWR are expressed as a percent of the second control response preceeding that experimental run.

FMRFamide induced gill contractions with a threshold of 1-3 nM. Perfusion of FMRFamide (10 nM) elicited maximal gill contractions whereupon, washout of FMRFamide was commenced. The GWR was evoked upon return of gill tone to the baseline tension. FMRFamide (10 nM) increased the amplitude of the evoked GWR to 142% (S.E.=20%) of control (n=10). For control habituation experiments, a standard stimulus was delivered with an ISI of 5 minutes. The evoked GWR amplitude was recorded. After ten such stimuli at this rate, the amplitude of the evoked GWR decreased to 38.1% (S.E.= 11.03%) of control (n=10). The viability of the gill was tested with a gill or siphon pinch, which generally resulted in dishabituation of the habituated GWR when evoked immediately following the pinch. For experimental habituation runs, FMRFamide (10 nM) was perfused for approximately 1-3 minutes during each ISI. Once spontaneous contractions began the perfusion of FMRFamide was terminated by switching to control ASW. When the gill became quiescent and the gill tension returned to baseline, the standard tactile stimulus was delivered and the evoked GWR amplitude recorded. This was repeated until ten stimuli had been delivered, as in the control run. When FMRFamide was perfused in this manner habituation of the evoked GWR did not occur.

In these experiments the perfusion of FMRFamide prevented habituation of the evoked GWR and increased the amplitude of the evoked GWR with respect to control. In addition to the induction of spontaneous gill movements, FMRFamide may also be involved in modulation of adaptive gill behaviors.

176.1 **CORRELATION OF SWIMMING BEHAVIOR WITH THE ACTIVITY OF IDENTIFIED GIANT RETICULOSPINAL NEURONS IN THE LAMPREY SPINAL CORD** Joseph Ayers and C. M. Rovainen, Marine Science Center, Northeastern University, East Point, Nahant, MA 01908

The lamprey spinal cord contains the largest number of re-identifiable neurons of any vertebrate, but, their function during normal behavior is unknown. We have developed techniques which allow us to record from reticulospinal axons at two sites in the spinal cord during swimming and then subsequently extract their activity by a signal processing algorithm. Recordings from axons of reticulospinal neurons are obtained from the spinal cords of freely behaving lampreys with silver electrodes inserted into the dorsal meningeal canal. The action potentials of identified neurons exhibit a unique triplet signature (rostral waveform, caudal waveform, inter-electrode latency) which can be distingushed from those of other reticulospinal axons. The spinal cord recordings survive reduction of the preparation to allow intracellular stimulation of identified cell bodies in the brain. The spinal recordings can be triggered off the intracellular spike to allow each triplet signature to be ascribed to a identified neuron. These identified triplets can then be extracted from the *in vivo* recordings by a template matching algorithm which relies on a product-sum correlation of the proximal and distal templates on a point by point basis with the digitized *in vivo* data. Synchronization of the behavioral analysis and electrophysiological recordings is based on a signal common to the two forms of data, a photoelectric LED. The behavioral analysis starts with the first movie frame where the LED is illuminated, and the analog digitization is triggered off the voltage pulse which illuminates the LED. The overall analysis results in a raster plot of identified neuron activity plotted over a graph of the locus of flexion waves as a function of time shown below *(Science 221:1312-1314)*, allowing the behavior to be related directly to the neuron activity. Supported by NSF Grant *BNS8406880*



Reticulospinal Activity during Swimming. In the Lower Panel, the Locus (position) of lateral flexions. plotted as a function of time. The upper raster plot indicates the times of occurrence of activity in different axons. Unident. axon indicates an unidentified neuron

176.2 AGGRESSIVE BEHAVIOR IN SIAMESE FIGHTING FISH, *BETTA SPLENDENS*; NEURAL PATHWAY FOR GILL-COVER ERECTION. D.L. Gorlick, Dept. Biol. Sci., Columbia Univ., New York, N.Y. 10027.

There are no complete descriptions of the neural pathway mediating any aggressive behavior patterns in fishes. The primary goal of this research is to describe the neural pathway controlling gill-cover erection in male *Betta splendens*. Gill-cover erection is a characteristic component of aggressive encounters in *Betta*. It occurs during frontal display and consists of extreme lateral movement of the opercula until they are at right angles to the body axis where they may be held for periods of a few seconds up to a minute or more.

Gill-cover erection is produced by a single muscle, the opercular dilator. This muscle is large in male *Betta* and may be primarily dedicated to the performance of gill-cover erection. HRP injections into the opercular dilator show that the motor neurons that innervate this muscle are located in the anterior medulla in the lateral and medial parts of the motor nucleus of cranial nerve VII. Work to date has concentrated on the lateral part of VII.

Iontophoretic injections of HRP into lateral VII (VII-L) label ipsilateral neurons in the adjacent medial reticular area (Rm), nucleus intermedius octavolateralis (Octavo), and nucleus vestibularis magnocellularis (Magno). Octavo and Magno may process lateral line inputs related to water movements produced by displaying fish. Afferent input to VII-L also comes from ipsilateral and contralateral cells in the anterior pole of the motor nucleus of cranial nerve X. This part of X also contains motor neurons that innervate muscles of the branchiostegal membrane that are involved in production of the other component of aggressive frontal display behavior in *Betta* (Augenbraun & Gorlick, Soc. Neurosci. Abstr., 1987).

Rm receives afferent input from contralateral and ipsilateral Magno, contralateral Rm, and from ipsilateral inferior reticular neurons (Ri) that are located medial to neurons in cranial nerve nuclei IX and X-XI. Magno receives afferent input from contralateral Magno and from ipsilateral Octavo. Magno also sends a strong efferent fiber projection anteriorly in the lateral lemniscus that terminates in the contralateral torus semicircularis. Octavo appears to receive input only from contralateral Octavo.

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176.3 MOTOR NUCLEI RESPONSIBLE FOR BRANCHIOSTEGAL MEMBRANE EXTENSION DURING AGGRESSIVE DISPLAY IN *BETTA SPLENDENS*. E. Augenbraun* and D.L. Gorlick (SPON: Darcy B. Kelley), Dept. Biol. Sci., Columbia Univ., New York, NY 10027.

There are two components of aggressive display behavior in the Siamese fighting fish *Betta splendens:* frontal and lateral. During the frontal display the fish extends a highly developed branchiostegal membrane composed of thin sheets of muscle that run between the branchiostegal rays. Contraction of these muscles causes the branchiostegal membrane to fan out during frontal display. An adjacent pair of muscles, "*X*," also appears to contribute to frontal display as cutting them modifies the shape of the branchiostegal membrane. These muscles originate anteriorly in the angle of the lower jaw and appear to insert distally on the inside of the branchiostegal membrane. Left and right muscles cross at the midline forming an "*X*." Male fish have larger, thicker branchiostegal membranes and larger "*X*"- muscles than do females. The behavior is also sexually dimorphic in that males display more frequently and for longer periods of time.

We have used retrograde tracing with Horseradish peroxidase (HRP) to identify the motor nuclei controlling this behavior. Large neurons in cranial nerve nucleus n.IX and n.X-XI innervate the branchiostegal membrane in both males and females. In males most of the labeled n.X cells are in a prominent medial bulge at the anterior end of the nucleus. Neurons in this area send efferent projections to motor neurons that control gill-cover erection, the other component of frontal diplay in *Betta splendens* (Gorlick, Soc. Neurosci. Abstr., 1987). In females, however, the bulge appears to be less prominent and many labeled cells are found outside the bulge. Motor neurons that innervate the "*X*"- muscle are also located in n.X. However, they appear to be located just posteriorly to those that innervate the branchiostegal membrane.

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176.4 THE NEUROETHOLOGY OF A VISUALLY MEDIATED BEHAVIOR: THE EYE-BAR RESPONSE. M.M. Hagedorn, R.D. Fernald. Institute of Neurosciences, Univ. of Oregon, Eugene, OR 97403

African male cichlid fish, Haplochromis burtoni, respond to threats from male conspecifics at the boundaries of their territories by facing one another and displaying a dark, pigmented eye-bar beneath each eye. Usually, both males possess eye-bars and ultimately, when the loser turns to flee, the eye-bar pales. The eye-bar response is largely a visually mediated behavior related to aggressive interactions between males and is effected by a darkening or paling of chromatophores in the scales below the eyes.

The anatomy and function underlying this behavior was investigated at two levels: 1) the signal input pathway from the retina; 2) the motor output pathway. Since visual information passes primarily to the tectum, tectal efferents were mapped using horseradish peroxidase (HRP). Tectal injections were made in adult males (n=5) by pressure injecting a 20% solution of HRP or a 1% solution of lectin conjugated peroxidase. The animals were allowed to survive for 2-3 days and then prepared for histology. The animals were anesthetized and perfused intracardially with saline followed by a 4% gluteraldehyde solution, post-fixed for 4 hours, cut at 60 μ and reacted with a heavy metal intensified diaminobenzidine reaction. Labelled tectal efferents were found in pretectal nuclei, the anterior nuclei, the posterior commissure, the glomerulus nuclei, the torus longitudinalis, the torus semicircularis, the valvula, the tectobulgar tracts, the vagal lobe, the medio longitudinal fasciculus and the fasciculus medialis tracti optici.

To analyze the function of the motor output, neural lesions were made in the trigeminal nerve. Previous studies have suggested that chromatophore nervous control is part of the sympathetic trigeminal system and that the chromatophores in the eye-bar are neurally controlled by a post-ganglionic nerve which follows the orbit of the eye and travels with a branch of the maxillary nerve along the jaw into the cranium. Stimulation of this post-ganglionic nerve results in a contraction of the chromatophores and a paling of the eye-bar, whereas, nerve lesions result in an expansion of the chromatophoes and a darkening of the eye-bar. Pre-ganglionic nerve lesions were made in the trigeminal nerve in 2 animals. These animals recovered and their behavior monitored for 4 hours, no expansion of the chromatophores or darkening of the eye-bar was observed. This suggests two functional possibilities for the eye-bar pathway; one, the preganglionic nerve is not part of the trigeminal nerve or two, a population of the post-ganglionic fibers are spontaneously active, thus maintaining the chromatophore in a contracted state despite the removal of the pre-ganglionic input. This is the first step in identifying the pathways required for the behaviorally significant eye-bar response.

This work was supported by an NIH training grant # 5 T32 MH 1714803

176.5 A GOLGI STUDY OF THE SONIC MOTOR NUCLEUS OF THE OYSTER TOADFISH, P.J. MOSCA* AND M.L. FINE. Dept. of Biology, Virginia Commonwealth University, Richmond, Virginia 23234

The sonic motor nucleus (SMN) of the oyster toadfish Opsanus tau is sexually dimorphic: females have small neurons and males can have either large or small neurons. Golgi stains (Golgi Kopsch method) were performed on the SMNs of 50 toadfish. Five neuron types (fusiform, rostral, stellate, ventral and caudal) were separated on the basis of position, soma shape and size, and direction and pattern of dendritic branching. All neuron classes were present in both types of males and in females, and sexually dimorphism was manifest as differences in soma size. The ratio of mean area of small cells to large cells ranged from 0.56 to 0.87 for the different types. The SMN was organized into 3 layers with fusiform and rostral neurons forming a chain across the dorsal-dorsolateral surface, stellate cells in the middle and ventral cells below. Caudal neurons were localized in the caudal fifth of the nucleus, often lined up in rows. Fusiform, stellate and caudal neurons had similar soma areas ($\bar{x}$ = 1466 to 1560 um^2 in small-celled SMNs and 1803 to 1812 um^2 in large ones), ventral cells were smaller (1243 and 1789 um^2) and rostral cells smallest (780 and 1379 um^2). Fusiform neurons were ovoid in shape and characteristically sent a large-diameter dendrite ($\bar{x}$ = 9.3 um) toward the center of the SMN and 1-3 thinner dendrites ($\bar{x}$ = 3.8 um) in rostral and caudal directions. They had the smallest number of primary dendrites but the greatest amount of branching, starting within 50 um of the soma. Rostral cells had an average of 3.0 dendrites, which tended to project ventrally and caudally and share a similar branching pattern with fusiform neurons. Stellate cells had the roundest somas and the greatest number of primary dendrites ($\bar{x}$ = 4.8), which were distributed evenly. A small amount of branching was concentrated between 50 and 100 um from the soma. Ventral cells had rounded somas except on the dorsal surface where they were flat or concave between pairs of thick dendrites (4-7 um), which projected dorsolaterally and dorsomedially and exited the SMN. An intermediate amount of branching, was distributed uniformly to 200 um from the soma. Caudal cells were round or ovular in frontal and horizontal sections and more elongate in sagittal sections. Dendrites were polarized in the rostral and caudal directions, and caudal dendrites exited the SMN. A small amount of branching was distributed uniformly to 200 um in horizontal section. Retrograde transport of horseradish peroxidase from the sonic muscles indicated that all cell types were motoneurons. Ventral and caudal cells receive afferent information from higher centers. We suggest that rostral and fusiform neurons synchronize the SMN along its rostrocaudal axis, and that stellate cells provide a bridge between the other cell layers.

Supported by MH38921

176.6 ELECTRIC CUES ELICIT SONIC COURTSHIP IN THE MORMYRID FISH Pollimyrus isidori. J. D. Crawford* and C. D. Hopkins (SPON: L. Nowak). Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

The African electric fish, Pollimyrus isidori, uses both acoustic and electric social signals. The electric repertoire consists of a single highly stereotyped electric organ discharge (EOD; 100-200 µs duration) which can be produced in a wide range of temporal patterns. The acoustic repertoire consists of 5 distinct sounds. *Grunts* and *Growls* are pulsatile sounds with pulse repetition rates of 50 and 25 Hz respectively. The peak power of individual pulses for both sounds is 220 Hz. *Moans* are quasi-sinusoidal with a dominant fundamental at 220 Hz. All three sounds are probably produced through a common mechanism involving the swim bladder. The *Pop* is a single pulse with a broad power spectrum and the *Hoot* is tonal with a variable dominant frequency of about 100 Hz.

Three of the sounds are used during courtship and the other two during aggression. Territorial males concatenate *Grunts*, *Moans* and *Growls* to produce a song when they are approached by egg-bearing females. This sonic courtship can be elicited experimentally by placing a "test" fish, captive within a small perforated container, into the territory of a "resident" male. When a gravid female is introduced in this fashion, the resident sings acoustically for the duration of a 2-4 min trial. When another male is introduced, the resident produces *Hoots*, *Pops* and high frequency bursts of EODs, and relatively few *Grunts*, *Moans* or *Growls*.

An electric dipole was substituted for a real fish in the test container in order to investigate the possibility that electric signals emitted by test fishes determine the resident male's response. Trains of electric stimuli were presented using lists of interpulse intervals stored on a computer to trigger an arbitrary waveform generator loaded with EODs (digitized at a sampling frequency of 1.0 MHz). Males respond vigorously with acoustic courtship when the electric behavior of a conspecific is mimicked by playing-back EODs at species typical intervals. Natural female EODs played-back in a female typical pattern, male EODs in a male typical pattern , male and female EODs played back in a random pattern of species typical intervals, and 5 KHz sinusoidal waveforms presented in regular synthetic patterns all elicit sonic courtship. Electric stimuli are clearly potent releasers for male sonic courtship in Pollimyrus isidori. The basis of male-female discrimination is not yet clear, but the role of the EOD waveform and discharge repetition patterns are currently being investigated.

This research is supported by NIMH grants 5T3215793 (JDC) and MH37972 (CDH).

176.7 WHITE NOISE ANALYSIS PREDICTS TEMPORAL SPIKE PATTERN OF TUBEROUS ELECTRORECEPTORS IN MORMYRID ELECTRIC FISH.
C.D. Hopkins and G.D. Harned*. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York. 14853.

Electric fish from the African family Mormyridae have tuberous Knollenorgan electroreceptors which have been identified as putative *communication sensors* for electric communication. They appear to be used for detecting electric organ discharges from other electric fish and for species- and sex-recognition.

Knollenorgans have a low threshold, are tuned to the power spectrum of the species-specific electric organ discharge (EOD), and are ideally suited for preserving timing information in electric stimuli. Knollenorgans can be studied electrophysiologically by applying electric stimuli through a bridge amplifier to the electroreceptor pore while simultaneously recording spike-like receptor potentials from the receptor organ.

We used Gaussian-distributed white noise, bandwidth limited to 10 kHz, as a stimulus and used the technique of reverse correlation (deBoer and Kuyper, . IEEE Trans. Bio. Med. Eng. 15:169) to determine the linear part of the transfer function of the spike generator in the Knollenorgan. We computed spike-triggered averages of stimulus waveforms from a minimum of 2000 spikes. These reverse averages are the linear part of the system impulse response.

The impulse responses determined in this way were highly consistent from one measurement to the next, over an 18 dB dynamic range of stimulus amplitudes.

Impulse responses were typically highly damped, showing an initial excitatory phase followed by a weaker, longer-lasting inhibitory or refractory phase. There is a very close resemblance between the impulse response of a receptor and the shape of the extracellularly-recorded receptor potential. Low-frequency Knollenorgans had longer duration impulse responses than did high frequency receptors.

The reverse-correlation generated impulse response was subjected to Fourier analysis. The power spectrum of the impulse response is the "gain" of the linear filter. The power spectrum was compared to the frequency tuning curve of the receptor. Best frequencies and quality (Q_{10}) were closely matched for the two methods. The overall shape of the tuning curve, although highly variable from unit to unit, was matched by the shape of the power spectrum.

We measured compound post-stimulus time histograms of spike density in response to a variety of complex stimulus waveforms , including: single period sine waves, repeated or static white noise. and EOD waveforms. We also compared the observed compound PSTH to the predicted output generated by calculating the convolution integral between the stimulus waveform and the impulse response of the system. There is a very close match between the predicted waveform and the observed compound post stimulus time histogram, indicating that the linear model of the receptor accounts for much of the observed output. At high stimulus intensities, the amplitude of observed responses differed from predicted, indicating the presence of amplitude nonlinearities.

The reverse-correlation computed impulse response was used to accurately predict the response times to arbitrary waveforms, like species-specific EODs and other signals.

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176.8 GONADOTROPIN-INDUCED SHIFTS IN THE ELECTROSENSORY SYSTEM OF *STERNOPYGUS*. H. H. Zakon, H.-Y. Yan* and P. Thomas*. Dept. of Zoology and the Marine Sciences Institute, University of Texas, Austin TX, 78712.

The weakly electric fish Sternopygus macrurus emits a sinusoidal electric organ discharge (EOD) from an electric organ in its tail and detects its own EOD with tuberous electroreceptors distributed across its body. The duration of a single electric organ (EO) pulse, the frequency of firing of the EO, and the tuning of electroreceptors are highly coordinated in each individual and are sexually dimorphic. All three of these parameters have been shown to be influenced by the exogenous administration of the androgen hormones testosterone (T) and dihydrotestosterone. Only a single study has observed the effects of an exogenously-applied ovarian hormone, estradiol 17ß (E), on EOD frequency in this species (Meyer, 1983). The effect reported in this study was significant but small. We investigated the effects of gonadotropin on fish of both sexes in order to determine if a more robust effect might be induced in females by the release of endogenous ovarian hormone(s) and to estimate circulating levels of endogenous androgens and estrogens that are sufficient to induce changes in the electrosensory system.

Large juvenile or small adult fish of both sexes were used (~ 25-40 cm length). Blood samples were taken to determine baseline levels of circulating steroids. Baseline measures of EOD frequency and EO pulse duration were taken. Single unit recordings of electroreceptive afferent fibers were made (N = 20 per fish) to determine mean receptor best frequency (the frequency to which a receptor is most sensitive). Fish in the experimental group were then given 3 injections of human chorionic gonadotropin (HCG, 10 units/ gram body weight) spaced 5 days apart. Control groups of both sexes received saline injections. EOD frequency was tracked during this time, blood samples were taken at various intervals, and EO pulse duration and electroreceptor tuning were measured again 3-5 days following the last injection.

HCG caused increases from baseline levels of T (from < 1.0 ng/ml plasma to > 5.0 ng/ml) and 11-ketotestosterone (11-KT, the major androgen in fishes) (<1.0 to > 3.0 ng/ml) in males, and T (< 1.0 to >7.0 ng/ml) and E (<0.5 to > 1.0 ng/ml) in females. When compared to the saline control groups whose EOD frequencies were unchanged, EOD frequency was significantly lowered in HCG-primed males and increased in females ($p < 0.005$, Mann-Whitney U, for both sexes). In addition, preliminary analysis of EO pulse duration and receptor tuning show changes in the expected direction: EO pulse duration of males is increased and that of females is lessened, receptors of males are tuned to lower frequencies, while those of females are tuned to higher frequencies.

These data demonstrate that endogenously-released steroid hormones (in concentrations of a few nanograms/ ml plasma) are capable of effecting changes in the electrosensory system previously reported to occur after administration of exogenous steroids of comparable, or in some cases unknown, concentrations. In addition, HCG induces larger changes in EOD frequency than the exogenous application of E. Whether this is due to a difference in timing of release or average circulating levels of E by HCG and exogenous injection, co-release of other steroids from the ovary by HCG, or direct effects of HCG on the EOD generating circuitry is not known.

Supported by NSF grant # BNS-8606744.

176.9 HORMONE-MEDIATED SHIFTS IN ELECTRORECEPTOR TUNING OCCUR IN FISH WITH MEDULLARY PACEMAKER LESIONS. M. B. Ferrari* and H. H. Zakon (SPON: G. Pollak). Dept. of Zoology, University of Texas at Austin, Austin, TX 78712

The weakly electric gymnotiform Sternopygus macrurus produces and detects a quasi-sinusoidal electric field; this field is generated by an electric organ in the tail and is detected by electroreceptors in the epidermis. The firing frequency of the electric organ is determined by a medullary pacemaker nucleus (PMN) and each fish has its own characteristic frequency, with a species range of 60-200Hz. Sternopygus also exhibits a sexual dimorphism; the males discharge at low frequencies and the females discharge at high frequencies. The discharge frequency of the PMN and frequency tuning of the electroreceptors are correlated such that the fish remains tuned to its characteristic frequency.

Previous studies have demonstrated that androgens, specifically DHT, lower both the frequency of discharge of the PMN and concomitantly lower the best frequency tuning of the electroreceptors. Recent studies have also demonstrated that the fish's electric field does not influence the hormone-mediated tuning shift of electroreceptors. Although it appears that the hormone directly affects electroreceptor tuning, the possibility remains that changes in the firing frequency of the pacemaker nucleus may affect the tuning of the electroreceptors, even though no known anatomical connection between the two has been demonstrated. Therefore, in the present studies we lesioned the PMN to examine if hormone-mediated shifts in electroreceptor tuning depend on PMN firing frequency.

Fish were curarized and recordings were made from electroreceptor afferents (n=20 per fish) to establish the baseline mean best frequency (xBF) of electroreceptor tuning. The PMN was located electrophysiologically with a large-bore (5-10um) micropipette and the nucleus was then lesioned with a pressure injection of $CoCl_2$. In no cases have the fish regained a normal discharge. After lesioning, a small silastic capsule filled with DHT was implanted in the peritoneal cavity. Control fish received empty capsules. Recordings from the same afferent populations two weeks post-implant showed significant decreases in xBF for experimental versus control fish ($p<0.01$, Mann-Whitney). The percentage change in xBF for experimentals ranged from -16.9% to -35.5% (mean= -25.4%) while that of control fish ranged from -2% to -14.2% (mean= -7.1%). Previous results from intact, DHT-implanted fish show a mean change of -23.4% indicating no significant difference between PMN- lesioned fish and normal animals ($p<0.29$, Mann-Whitney).

These results support the idea that hormone-mediated changes in PMN firing frequency and electroreceptor tuning occur independently but concomitantly in order to preserve the fish's ability to remain tuned to its own discharge. Supported by NSF Grant # BNS-8606744.

176.10 A MODEL FOR AGE-RELATED INCREASES IN SENSITIVITY OF ELECTRORECEPTIVE AFFERENTS. D.Y. Sanchez and H.H. Zakon Dept. of Zoology, The University of Texas, Austin TX 78712.

The weakly electric fish Sternopygus possesses in its skin electroreceptors which are specialized for sensing its own electric discharge as well as that of its conspecifics. The receptor cells are organized into clusters within epithelial capsules which are termed tuberous organs. The organs are innervated by afferents from the anterior lateral line nerve. Often, an afferent fiber innervates more than one organ. The afferent and all of the organs which it innervates is called a tuberous unit. These fish add new organs as they grow and tuberous units gradually develop into two distinct sub-populations: one adds organs while the other remains at only one or two organs per axon. Thus, fish relatively small in size show a single unit population of only a few receptor organs per axon, whereas those of relatively larger size show, in addition, a population of units which may have ten or more organs per axon.

In order to investigate how growth-related changes in morphology might affect the physiology of this system, recordings were made from individual electroreceptive afferents. Large sample sizes were collected; more than 30 units per fish. Threshold and best frequency (BF) were measured for each unit and the probability of firing to a stimulus at the unit's BF was calculated at various stimulus intensities. When probability of firing as a function of stimulus intensity (in dB) was plotted, sigmoidal curves resulted that were similar in shape from fish to fish and within individuals. The average threshold value for the most sensitive units in each fish, when plotted against fish length, shows an increase in sensitivity equivalent to about one order of magnitude as fish increase in size from 12 - 42 cm. Unit size increases from about two to over ten organs per axon in fish over this size range. We then designed a model to explain how increased convergence of receptor organs onto afferents might yield an increase in sensitivity.

The synaptic terminals beneath each receptor cell may be capable of generating spikes since, in a closely related fish, they stain for electrogenic membrane (potassium ferrocyanide stain; Saidel, personnal communication). If one assumes that there is a spike initiating zone beneath each receptor cell and that each receptor cell responds linearly with intensity, our model predicts the probability of recording a spike in the axon, P(axn), given an arbitrary probability for a spike under each receptor cell, x.

$$P(axn) = 1 - (1 - x)^{a\,b}$$ where x = probab of spike @ receptor
a = #cells per organ
b = #organs per axon

Plotting P(axn) as a function of log x generates sigmoidal curves which closely match the curves of probability of firing vs. stimulus intensity from actual data. Graphs of these curves demonstrate that increasing the number of organs per axon ten-fold would not affect the shape of the curve but would shift its position on the graph one log unit to lower "x" values (i.e. would make the unit an order of magnitude more sensitive). Thus, this model predicts, as our data demonstrate, that a ten-fold increase in unit size with growth correlates with an increase in sensitivity of approximately one order of magnitude.

176.11 DIVERSITY OF NEURONAL CELL TYPES IN THE ELECTROSENSORY LATERAL LINE LOBE OF STERNOPYGUS. B. Losier* and J. Matsubara, Dept. of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada.

The electrosensory lateral line lobe (ELLL) is an important medullary processing center for electric behaviors such as electrolocation and the jamming avoidance response (JAR). Evidence of the complexity of the ELLL is seen in its composition of 8 laminae, 11 cell types and three somatotopic maps of tuberous electroreceptors. It receives both primary electroreceptive afferents and descending signals from the dorsal praeementialis and lobus caudalis. As such, the ELLL is pivotal in its location within the electroreceptive pathway.

We have undertaken a detailed anatomical and immunocytochemical analysis of the cell types in the ELLL of Sternopygus since this species displays unique behaviors: It is the only wave-species whose electrolocation abilities are unimpaired by electrical noise and, accordingly, does not possess a JAR. Correlated to these differences, physiological studies revealed species-typical response properties of neurons in the ELLL (Matsubara, 1981) as well as the main target of the ELLL, the torus semicircularis (Rose and Heiligenberg, 1986). Using Golgi-Cox, Nissl, cytochrome-oxidase and calbindin immunocytochemistry we observed that the cell types in the ELLL of Sternopygus include the main types previously seen in other species and other cell types unique to Sternopygus (i.e. horizontal cells in the polymorphic layer and spindle cells intermixed between fiber bundles in the plexiform layer). In the deep neuropil layer (DNL), we identified a cell type which is distinguished from the spherical cell by its elongated, tapered soma and extensive dendritic bush. Analysis of perimeter form factor (FF), an index of the similarity between the shape of a cell and a circle, revealed that the cells in the DNL of Sternopygus (FF = 0.51, s.e. 0.02) differed considerably from spherical cells in Apteronotus (FF = 0.84, s.e. 0.01). Immunocytochemical differences exists: in Apteronotus, spherical cells stain intensely with antisera against calbindin, in agreement with previous reports (Maler et al, 1984). Cells in the DNL of Sternopygus and Gymnotus (a pulse-species with EOD frequencies of 50-60Hz) surprisingly, show no immunoreactivity when tested with the same antibody. Finally, levels of cytochrome-oxidase (CO), a long-term metabolic activity marker, were high in the ELLL of Sternopygus and Gymnotus, and less so in Apteronotus. Calbindin immunoreactivity and CO levels, thus, appear inversely related. Our results indicate that the anatomical and chemical make-up of the ELLL cells vary considerably among species and may provide the anatomical bases underlying observed species-typical behavioral differences.

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176.12 BICUCULLINE AFFECTS BOTH TEMPORAL AND RECEPTIVE FIELD PROPERTIES OF PYRAMIDAL CELLS IN THE ELECTROSENSORY LATERAL LINE LOBE. C.A. Shumway and L. Maler, Neurobiology Unit, Scripps Inst. of Oceanography, UCSD, La Jolla,CA 92093 and Dept. of Anatomy, Fac. of Health Sciences, Univ. of Ottawa, Ottawa, Canada K1H 8M5

The electrosensory lateral line lobe (ELL) of weakly electric fish is a laminated hindbrain structure which has been well characterized anatomically (Maler et al.,1981,JCN 195:87-139) and physiologically (see Bastian,1986, in Electroreception,Bullock and Heiligenberg, eds). Primary afferent input from electroreceptors encoding changes in EOD amplitude (P-type units) converges either directly or indirectly (via inhibitory granule cells) onto 2 types of ELL output neurons (basilar and nonbasilar pyramidal cells, respectively). Two physiological transformations that occur at the level of the ELL are 1) Pyramidal cells are phasic in response to a step change in EOD amplitude, showing a 25-100 fold increase in the rate of adaptation relative to the electroreceptors. 2) Pyramidal cells have center-surround receptive fields. Both transformations are mediated by local inhibitory neurons, primarily granule cells and polymorphic cells which are themselves strongly influenced by descending input (Bastian,1986,JN 6:553-562).

We investigated the effect of bicuculline (a GABA antagonist) on the physiological response properties of basilar pyramidal cells. GABAergic synapses are found predominately in 2 main regions of the ELL: 1) in the pyramidal cell layer and 2) in the granule cell and deep neuropil layer (Maler and Mugnaini, 1986,Soc.of Neurosci.Abstr.,312). We selectively iontophoresed bicuculline (1 mM, pH 3.5) in one or the other of these regions. Bicuculline applied to the pyramidal cell layer caused an overall increase in excitation and made the basilar pyramidal cells more tonic (n=10). The mean firing rate increased 68% and the mean time constant of adaptation increased 205% We believe the effect is due primarily to removal of granule cell inhibition of the basilar pyramidal cell. In preliminary experiments, bicuculline applied in the granule cell/deep neuropil layers caused a dramatic inhibition of the basilar pyramidal cell (n=1). Spontaneous activity decreased from 31.3 to 1.3 spikes/sec, and the response to a moving metal object decreased from 20.6 to 5.2 spikes/sec. We interpret this result to be due to removal of inhibition of granule cells (by polymorphic cells) which increases the granule cell inhibition of the basilar pyramidal cell. Thus the phasic properties, the receptive field properties, and the spontaneous activity of the basilar pyramidal neuron are shaped primarily by granule cells. The granule cells' influence on the basilar pyramidal cell is mediated by both excitatory descending input and inhibitory polymorphic cell input.

176.13 CENTRAL TERMINATION OF AFFERENTS FROM MORMYROMAST ELECTRORECEPTORS. C.Bell. Neurol. Sci. Inst., Portland, OR 97209.

Electric fish of the family Mormyridae use mormyromast electroreceptor organs in their skin for active electrolocation. Mormyromast electroreceptor organs have two types of sensory cells (types A and B of Szabo and Wersall, 1970), which are distinct in both morphology and location. The two sensory cells also have separate primary afferent innervation. Afferents from mormyromast electroreceptors terminate centrally within two distinct zones (medial and dorsolateral) of the electrosensory lateral line lobe (ELLL). The present experiments were designed to determine whether the afferents from the two types of sensory cells project to different ELLL zones, and if so, to determine which type projects to which zone.

Horseradish peroxidase (HRP) was iontophoretically injected into each of the two ELLL zones, in separate experimental animals. The injections were made into the termination regions of mormyromast afferents from the skin just anterior to the eye, using evoked potentials to local electrosensory stimuli to locate these regions centrally. The fish were then allowed to survive for 5 to 6 days before being sacrificed and perfused. The skin in front of the eye was removed, reacted for HRP as a whole mount, and embedded in plastic (soft Epon) for sectioning.

Examination of the skin sections showed that afferents from Type A sensory cells project exclusively to the medial zone of ELLL, whereas afferents from the Type B sensory cell project exclusively to the dorsolateral zone of ELLL.

Physiological studies combined with anatomical identification of the termination zone indicate that afferents to the medial zone of ELLL have a higher theshold and respond with a smaller number of spikes to a maximal stimulus than do afferents to the dorsolateral zone. There are some additional small differences between the two afferent types but the threshold difference is the most striking one. This threshold difference suggests that the two sensory cell types may serve to extend the dynamic range of the electrosensory system as a whole, without sacrificing incremental sensitivity.

176.14 A FEW CELLS WITHIN EIGENMANNIA'S DIENCEPHALIC ELECTROSENSORIUS COMPLEX WOULD BE SUFFICIENT TO DISCRIMINATE THE SIGN OF DIFFERENCE FREQUENCIES IN THE JAMMING AVOIDANCE RESPONSE. C H Keller and W Heiligenberg. Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

The gymnotiform electric fish Eigenmannia shifts the frequency of its electric organ discharge (EOD) away from that of a neighboring fish, a behavior called the Jamming Avoidance Response (JAR). Determination of the proper direction in which to shift the EOD is dependent upon the analysis of phase and amplitude modulations contained within the mixed EODs of the two fish. Cell types responsible for processing this information have been found in the medulla and in the torus semicircularis and tectum opticum of the midbrain (for review: Heiligenberg 1986, Ch 20 in Electroreception, Bullock and Heiligenberg eds.). Carr et al. (1981, J Comp Neurol 203:649) demonstrated a projection from the electrosensory torus to a diencephalic area which they named "nucleus electrosensorius". Subsequently, Bastian and Yuthas (1984, J Comp Physiol 154:895) recorded extracellularly from this area and found cells whose response depended upon the sign and magnitude of the frequency difference (Df) between the jamming signal and the animal's own signal. Such "sign-selective" cells are considered important for the control of the JAR.

We here report the results of more extensive recordings from this area and provide intracellular lucifer-yellow fills of sign-selective cells that demonstrate their dendritic morphology. A variety of physiological cell types is found in this area. For at least some of these cells the response is independent of the orientation of the jamming signal with reference to the animal's own signal. Threshold of discrimination of the sign of Df in terms of the relative intensity of the jamming signal approaches that of the behavior in intact fish. In contrast, toral and tectal sign selective cells respond selectively to one particular orientation of the jamming signal and are an order of magnitude less sensitive. This sensorimotor interface is thus characterized by continual convergence to a smaller number of cells whose response properties more closely match those of the intact behavior.

176.15 INDIVIDUAL PREPACEMAKER NEURONS CAN MODULATE THE MEDULLARY PACEMAKER OF THE GYMNOTIFORM ELECTRIC FISH, EIGENMANNIA. M.Kawasaki and W. Heiligenberg. Neurobiology Unit, Scripps Institution of Oceanography, A-002, University of California at San Diego La Jolla, California 92093.

The prepacemaker nucleus (PPN) in the midbrain of the gymnotiform electric fish Eigenmannia provides the only known neuronal input to the medullary pacemaker nucleus, which triggers each electric organ discharge (EOD) cycle by a single command pulse. Electrical stimulation of the PPN elicited two distinct forms of modulations in the pacemaker frequency, abrupt and brief accelerations, hence referred to as 'chirps', and gradual frequency shifts with a time constant of approximately one second. The associated EOD modulations were indistinguishable from natural communication signals. Depending upon the site of stimulation, the two forms of modulation could be elicited alone or superimposed. Stimulation sites eliciting only chirps could be separated from sites eliciting only gradual shifts by as little as 60 µm. The magnitude of the elicited chirps depended upon the timing of the pulse stimulus with reference to the phase of the pacemaker cycle.

Extracellular and intracellular recordings of single PPN neurons revealed that an action potential from a single neuron generates a chirp, and that the magnitude of the chirp depends upon the timing of the action potential with reference to the phase of the pacemaker cycle. Depolarization of such neurons by current injection produced bursts of chirps, and intracellular injection of Lucifer Yellow labeled a large type of PPN neuron which could also be retrogradely labeled from the pacemaker with horseradish peroxidase (HRP). Although the jamming avoidance response, which consists of gradual frequency shifts in the EOD, was elicited during the unit recording, the spike activity of these neurons had no relation to the JAR. The abrupt and brief acceleration in a chirp triggered by an action potential of a PPN neuron is too large and too sudden compared to the gradual shifts in the JAR. These observation thus preclude a possible participation of chirp-related PPN neurons in the JAR.

177.1 LEARNING RECEPTIVE FIELDS FOR COLOR CONSTANCY. T. Poggio and A. Hurlbert*. Artificial Intelligence Laboratory, Mass. Institute of Technology, Cambridge, MA 02139.

Recent physiological, psychophysical and computational results suggest that color constancy is achieved by filtering the retinal signal in separate chromatic channels through a center-surround receptive field with a narrowly peaked center and a very large, shallow, inhibitory surround. Computational analyses of how to recover surface reflectance from the image intensity signal, in which reflectance is mixed with illumination, yield exactly that filter. In Land's most recent demonstration, a filter of this type acts on Mondrians under varying illumination to yield constant color designators that match human color perception. Physiological data suggest that color-coded cells in primate cortical area V4, thought to be involved in mechanisms underlying color constancy, have very large ("non-classical") receptive fields. We show that a simple algorithm can learn color constancy from a set of examples, and that what the algorithm learns is a filter of this type.

The examples are pairs of images: an input image of a Mondrian under smoothly varying (across space and spectrum) illumination, and an output image of the Mondrian as if under uniform, white illumination (the reflectance image). Given enough examples (the number depends on the Mondrian size), the algorithm synthesizes the optimal linear filter that, acting on each input image, would yield its correct output image. Computer simulations show that the learned filter has a sharp positive peak and a broad negative surround. Given new input images with either linear or logarithmic illumination gradients, the filter acts on them to yield the correct output images.

This simple algorithm learns how to achieve color constancy in a restricted Mondrian world. We have implemented it on a network of interconnected simple processors that may be interpreted as a biological network of neurons and synapses.

177.2 HUE, LUMINANCE, AND HUE/LUMINANCE CELLS IN FOVEAL V1, V2 AND V4 OF THE BEHAVING MACAQUE MONKEY. Takashi Yoshioka*, Bruce M. Dow and Robert G. Vautin*. (SPON: Roberta Pentney). Department of Physiology, School of Medicine, SUNY, Buffalo, NY 14226.

Color includes "hue" and "luminance" (or brightness) as attributes. Colors such as orange and brown have the same hue but different luminance (brightness, lightness) levels with respect to some common background. Previous neurophysiological studies of color vision have tended to ignore the luminance component in color perception. In the present study, through the use of a color video display (Chromatics) we have examined the responses of single cells and cell clusters in macaque visual cortex (the foveal portions of areas V1, V2 and V4) to a color set which includes such "dark" colors as black, brown, coffee, olive, maroon, indigo and gray, as well as more traditional "bright" colors like white, orange, yellow, green and red. Recordings are conducted in alert monkeys trained to hold their eyes on a fixation point for several seconds at a time. After careful receptive field mapping and selection of the preferred spatial stimulus (a bar or spot), each cell is tested with a set of 19 colors (8 bright, 8 dark, 3 achromatic) presented as a 500 msec pulse of light (or dark and/or color) on a gray background of intermediate luminance. Our present data sample includes 700 cells with mapped receptive fields, and 500 cells with complete color tuning curves, roughly 50% from V1, 25% from V2 and 25% from V4. The cells fall into 3 major groups which we refer to as hue (H), luminance (L) and hue/luminance (HL). H cells are sensitive to hue but insensitive to luminance (e.g., responsive to both orange and brown). L cells are sensitive to luminance but insensitive to hue. HL cells are sensitive to both hue and luminance, requiring either positive or negative luminance contrast for activation of their color sensitivity (e.g., responsive to either orange or brown but not both). L cells constitute 49% of the total, with about the same proportion in each of the 3 visual areas. H cells constitute 7% and HL cells 44% overall, with H cells more common in V4 than in V1, and HL cells more common in V1 than in V4.

The data suggest that hue and luminance are processed both separately, by H cells and L cells, and together, by HL cells. Three-dimensional reconstruction of the cytochrome oxidase stained tissue, including a number of microelectrode marking lesions, is currently in progress, with a view toward establishing correlations between physiology and anatomy. Supported by NIH grant EY02349.

177.3 **NEURONAL ACTIVITY IN V1, V2 AND V4 IN MACAQUE MONKEY PERFORMING A VISUAL MATCHING TASK.** N.K. Logothetis, R. Vogels and P.H. Schiller. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA

Visual cortex in mammals consists of several distinct visual areas which are assumed to subserve different functions. In an effort to further clarify the differences among these areas, this study examined the extent to which single cells in V1, V2 and V4 exhibit modulated activity during the performance of a visual matching task.

A male rhesus monkey performed a matching task. The start of each trial was indicated by the appearance of a white fixation spot. After the monkey fixated it, four stimuli of different colors or orientations were presented peripherally, with one of them centered within the receptive field (RF) of the cell under study (which stimulus appeared at this location was randomized by trial). After 500 ms the fixation spot was replaced by a cue stimulus identical to one of the four peripheral stimuli. The monkey's task was to saccade to the matching (target) stimulus. In V1 81 cells, in V2 87 cells, and in V4 188 cells were examined during the performance of this task.

While in V1 and V2 cells could be activated by the appropriate visual stimuli used in the task, their activity was not modulated by the appearance of the cue stimulus. In contrast, in V4 such modulation was evident: in 5% of the cells activity was significantly elevated when the stimulus in the RF matched the cue; in 10% of the cells activity was elevated independent of which stimulus was present in the RF; in 3% of cells activity was related to the execution of saccades.

In addition a strong oblique effect was found with gratings as indicated by reduced accuracy and increased latency (Correct %: 55 for oblique, 95 for horizontal vertical; latency: 689 ms and 295 ms respectively). A uniform distribution of preferred orientations was found, however, in all three visual areas.

These results suggest that in the visual system V4 is one of the first stations at which extraretinal modulatory activity is evident using a matching paradigm.

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177.4 SELECTIVE SUPPRESSION OF RESPONSES OF FOVEAL V1, V2 AND V4 CELLS IN THE MACAQUE DURING A COLOR MATCHING TASK. Bruce M. Dow, Takashi Yoshioka* and Robert G. Vautin*, Department of Physiology, School of Medicine, SUNY, Buffalo, NY 14226.

We have trained macaque monkeys to do 2 visual tasks, a dim task and a color task, on a color video (Chromatics) display screen. The dim task was the standard one introduced by Wurtz (1969). The color task was a new one which we designed. In this task the fixation point (FP) assumes one of 4 colors: red, yellow, green or blue. The "stimulus", which comes on 1 sec later and remains on for 500 msec, can likewise be red, yellow, green or blue. The color task requires the monkey to release his response lever within about 500 msec if the FP and stimulus colors are the same, but to hold and wait for a later dimming of the FP if the 2 colors are different. Following completion of training, single cell recordings are obtained using chronic techniques as previously reported (Vautin and Dow, J. Neurophysiol. 54:273, 1985). An implanted eye coil (CNC Inc., Seattle, WA) is used to monitor eye position. Each cell is tested with its preferred spatial stimulus (a bar or spot) established on the basis of careful receptive field mapping. The dim task is conducted with a set of 8 bright colors, 8 dark colors, and 3 achromatic colors. The color task consists of 16 separate conditions, 4 types of "same" trials (red-red, blue-blue, etc.) and 12 types of "different" trials (red-green, red-yellow, etc.). All testing is done with 8 interleaved repetitions of each stimulus condition.

We have obtained complete data, including color tuning curves under both control (dim task) and test (color task) conditions, from a total of 250 cells, approximately equally divided among the 3 visual cortical areas V1, V2 and V4 (foveal regions). The main finding, present in 61% of all tested cells, is selective suppression of responses. Enhancement is present in only 4% of all cells, with mixed suppression/enhancement in 16%, and no effect in 19%. Suppression tends to be selective for FP color, most commonly blue, least commonly yellow. Suppression typically lasts for the first 250 msec of the 500 msec stimulus ON period, approximately coinciding with the monkey's "same" trial reaction time. Suppression is stronger in V1 than in V4, stronger with decreasing receptive field eccentricity, stronger in cells with a distinct luminance threshold, and stronger in cells with orientation selectivity. A possible explanation for the results is that blue FP color causes a threshold elevation in a luminance-dependent, border-detecting system (which is known to lack blue cone input). Experiments are currently in progress to test this hypothesis, and to look for possible histochemical correlations. Supported by NIH grant EY02349.

177.5 CORTICAL AFFERENTS TO AREA V1 IN THE CEBUS MONKEY. A.P. B. Sousa*, R. Gattass*, M.C. Piñon* and M. G. P. Rosa* (SPON: C.G. Gross) Dept. Neurobiology, Inst. Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

The topographic organization of areas V1, V2 and MT, in the Cebus, have been previously described (Gattass et al., Soc. Neurosci. Abstr., 10: 474, 1984, and Soc. Neurosci., 12: 1366, 1986). Here we describe the cortical afferents to area V1 as revealed by injections of retrograde fluorescent tracers. In 5 Cebus we made combined injections of two fluorescent tracers placed at different eccentricities. Injections were placed in V1 in the regions of representation of both the superior and inferior visual quadrants, at eccentricities ranging from .6 to 54 deg. The portion of the visual field represented at the injection site in V1, was determined by concurrent electrophysiological recording.

The results showed that V1 receives homotopic projections from V2 and MT, as defined electrophysiologically. Following injections in the inferior quadrant we found labelled cells in the banks of the lunate sulcus, in the pre-lunate gyrus, in the posterior bank of the superior temporal sulcus, regions which may correspond to areas V3, V4 and V4t described in the macaque. After injections in the upper quadrant we found labelled cells in the ventral surface, in a strip of cortex anterior to V2, a region which may correspond to areas V3 and V4 as defined electrophysiologically (umpublished observations). In addition, following centrally located injections, both in the upper and lower quadrants, we found labelled cells in the banks of the occipito-temporal sulcus; while after peripherally located injections we found labelled cells in the calcarine sulcus, in a region corresponding to area prostriata, and in the parieto-occipital cleft, in the superior bank of the medial parieto-occipital sulcus and on the medial surface, an area which may correspond to area PO described in the macaque (Covey et al., Neurosci. Abstr., 8: 681, 1982). In all areas the labelled cells were located exclusively in the infragranular layers, while in V2 the cells were found both in the infragranular ans supragranular layers. (Research supported by CNPq, FINEP and CEPG/UFRJ)

177.6 **MULTIPLE VISUAL AREAS BETWEEN V2 AND MT IN THE OWL MONKEY.** M.I. Sereno, C.T. McDonald, and J.M. Allman. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Previous mapping experiments in owl monkey extrastriate cortex suggested that the region between the representation of central vision in V2 and area MT was occupied by a single visual area, DL (dorsolateral crescent). Degeneration and HRP studies of the interhemispheric connections of owl monkey visual areas (Newsome and Allman, J. Comp. Neurol., 194:209, 1980; Cusick, Gould, and Kaas, J. Comp. Neurol., 230:311, 1984), however, revealed a prominent labeled band located about halfway between V2 and MT. Since callosal connections in the initial stages of the hierarchy of visual areas are roughly correlated with vertical meridian representations, which usually define borders between areas, we decided to reexamine this region in detail.

The visuotopic organization of this region varies more from animal to animal than does the organization of V2, MT, and DM. An additional vertical meridian representation was usually found in the dorsal half of the region between V2 and MT, approximately at the location of the callosal band described above. It separates two strip-like, mirror-image representations of most of the lower field. The posterior area congruently adjoins the horizontal meridian of V2 and the anterior area congruently adjoins the horizontal meridian of DL proper. For a given dorsoventral level, receptive fields in the anterior area are more eccentric than receptive fields in the posterior area, which in turn are more eccentric than receptive fields in V2. Medially, both areas adjoin DI (dorsointermediate area). Thus, there are three representations of the lower visual field between V2 and MT. The most anterior area, DL proper, closely resembles V4t in macaque monkeys. The homologies of the other two areas are presently less clear.

The visuotopic organization of the ventral half of the region between central V2 and MT is more complex. There are multiple, small representations of parts of both upper and lower fields. The lower field representations are not continuous with the ones described above. The topography here does not seem consistent with the idea of there being thin, parallel, dorsoventrally continuous strip-like areas between V2 and MT, containing only dorsal lower fields and ventral upper fields.

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177.7 ELECTRICAL STIMULATION OF AREA MT AND MST IN THE MONKEY CEREBRAL CORTEX: EFFECTS ON PURSUIT EYE MOVEMENTS. H. Komatsu and R. H. Wurtz Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20892 USA.

Chemical lesion of the foveal representation within the superior temporal sulcus produces a directional pursuit deficit in monkeys (Dursteler, Newsome, and Wurtz 1987): a reduction in speed of pursuit for a target moving toward the side of the lesion. The area damaged includes the foveal representation of MT (MTf) and the adjacent lateral part of MST (MST1). To clarify the nature of the directional bias, the present experiments determined the effects of electrical stimulation applied to these areas during pursuit eye movements. We applied electrical stimulation (biphasic pulses, 0.2ms in width, 500Hz, 0.4s in duration with a current of 50-150 μA) through microelectrodes after pursuit had been initiated. At many sites in MTf and MST1, we observed a directional effect: clear deceleration with stimulation during pursuit away from the stimulated hemisphere and weak acceleration with pursuit towards it. The current threshold was about 50 μA, and the stronger the current, the greater the effects. The velocity of pursuit also influenced the effects of stimulation: the higher the velocity, the greater the effects. We compared the effects of electrical stimulation in conditions where retinal slip information was available (closed-loop) and removed (open-loop). There was little difference in the effects under the two conditions, suggesting a loss of retinal-slip information during stimulation. Electrical stimulation during fixation evoked slow eye movements toward the side of the stimulation, but the effects were much weaker than during pursuit.

Directional effects were observed only when the stimulation was applied in MTf and MST1. Stimulation applied to extrafoveal MT produced no effects, however, when the stimulation was applied before the monkey made a saccade to the target moving in the contralateral visual field, a marked reduction in the initial speed of pursuit occurred. This is reminiscent of the retinotopic deficit following chemical lesions in the extrafoveal MT. Weak transient decreases in eye velocity in either direction occurred with stimulation of the intermediate area of MST representing the peripheral visual field. No consistent effect was observed with stimulation of dorsal-medial MST (MSTd).

Our experiments indicate that the areas of MT and MST involved in the control of pursuit eye movements in a directionally selective manner are limited to subregions MTf and MST1. The dependency of the stimulation effects on eye velocity suggests that eye velocity information is modulating the visual motion information used for the maintenance of pursuit and is enhancing a directional bias toward the stimulated hemisphere.

177.8 RECOVERY OF FUNCTION FOLLOWING CHEMICAL LESIONS OF CORTICAL AREA MT. Dwayne S. G. Yamasaki and Robert H. Wurtz. Lab. Sensorimotor Res., National Eye Inst., Bethesda, MD 20892, USA.

Chemical lesions of area MT in the extrastriate cortex of the monkey produce deficits in the initiation of pursuit eye movements (Newsome et al., J. Neurosci., 1985), but this pursuit recovers within days to weeks. In the present experiments we investigated two aspects of this recovery process in the weeks following such a lesion: changes in the receptive fields of cells in the adjoining areas of MT, and the effects of visual experience.

Monkeys were trained in a step-ramp paradigm to pursue spots of light moving at 16°/sec. We then injected one to three microliters of a neurotoxin, AMPA, into an identified region of MT. We observed a deficit for initiation of pursuit to targets moving in any direction in a retinotopically localized region of the contralateral visual field.

In the single cell studies, we wanted to know if the receptive fields of MT cells might expand toward the area of the visual field damaged by the neurotoxin; such enlargement of receptive fields might compensate for loss of visual information in that part of the visual field. In two monkeys, we recorded cells through several guide tubes directed toward regions of MT adjacent to the future site of the chemical lesion, but far enough away to be spared any direct effect of the neurotoxin. In the days following the injection, we again sampled cells from these same guide tubes. We did not see selective expansion of the receptive fields toward the injection site. We continued to find cells with receptive fields comparable in size to those seen before the lesion, but in addition, we saw a scattering of cells with receptive fields larger than those seen before the injection. Receptive fields of these cells expanded in all directions. We do not know whether the field expansion is related to recovery from the lesion, or is a non-specific effect in cortex surrounding a damaged area.

In one monkey, we determined the effect of visual pursuit experience on the recovery process. The monkey was fitted with a mask attached to its implanted head holder that denied it visual motion experience. Control experiments had demonstrated no effect of wearing the mask for a week on the pursuit performance in two normal monkeys. Immediately following the chemical injection, the mask was placed on the monkey. The next day we verified that the monkey showed a deficit in a few tests of pursuit. Previous monkeys tested with comparably small lesions showed substantial recovery within a week of the lesion. In contrast, this monkey, denied visual motion and pursuit experience, showed little recovery within the same time period. We do not yet know the extent of the change in time course of this recovery.

177.9

WITHDRAWN

177.10 CORTICAL CONNECTIONS OF A DORSAL VISUAL AREA IN SQUIRREL MONKEYS. R. E. Weller, G. Steele and C. G. Cusick. Dept. of Psychology, Univ. of Alabama at Birmingham, AL 35294 and Dept. of Anatomy, Tulane Univ., New Orleans, LA 70112.

Recent anatomical studies in squirrel monkeys identified a dorsal area of cortex, rostrally bordering Area 18, possessing strong connections with the rostral Dorsolateral Area, DL (Cusick, C. G., Anat. Rec., 218:30A, 1987). This dorsal region appeared to be a different area from DL, an area bordering lateral Area 18. We have investigated the cortical connections of this region of cortex, named the Dorsal Area (D). Injections of 0.15-0.2 µl of 1% wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), 3% Fast Blue, or 2% Fluoro-Gold were made in D in adult squirrel monkeys. After perfusion, a large part of surface cortex including D was removed, flattened, and sectioned parallel to the surface of cortex. The remainder of the brain was sectioned coronally. Sections were processed for HRP using tetramethylbenzidine or O-dianisidine as chromagens. Injection sites and patterns of connections were related to architectonic borders of areas visible in sections stained for cytochrome oxidase activity or myelin.

Most regions of cortex that had connections with D contained both retrogradely labeled cells and anterogradely labeled projections. The strongest connections of the Dorsal Area were with rostral DL. Connections were also present with cortex in both banks of the caudal Sylvian fissure and the caudal superior temporal sulcus, in the location of the Middle Temporal Area (MT). Two locations on the ventral surface of the hemisphere were labeled, both in the inferior temporal sulcus. The more caudal focus was approximately 5-6 mm rostral to the 17/18 border. The rostral focus was in ventral inferior temporal cortex. Injections in D also resulted in sparse connections with cortex in the region of the Frontal Eye Field, and weak and variable connections with caudal DL and lateral Area 18.

The connections of the Dorsal Area are distinct from those of the adjoining area, DL. These distinctions support designating D and DL as separate areas. Thus, as found in other primates, squirrel monkeys have multiple visual areas rostral to Area 18 or V2. The strong connections of D with cortex in the region of MT and in the posterior parietal lobe suggest that D is involved in the relay of visual information to posterior parietal cortex. Finally, similarities between the connections of D of squirrel monkeys and the Dorsointermediate and Dorsomedial Visual Areas of owl monkeys raise the possibility that D and one of these areas are homologous in these two New World primates.

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177.11 **VISUAL AREA PIP: AN EXTRASTRIATE CORTICAL AREA IN THE POSTERIOR INTRA-PARIETAL SULCUS OF MACAQUE MONKEYS.** D. J. Felleman, A. Burkhalter and D. C. Van Essen. Biology Div., 216-76, California Institute of Technology, Pasadena, CA 91125.

We have identified PIP, the posterior intra-parietal area, as a visual cortical area that is located at the posterior end of the intra-parietal sulcus (IPS), extending from the floor of the sulcus near its junction with the parieto-occipital sulcus (POS), to dorso-medial portions of the prelunate gyrus. PIP is located within V3A as originally identified by Van Essen and Zeki (J. Physiol., 227:193,'78), but in a region they reported as having a complex topographic organization. PIP can be distinguished from V3A and other nearby areas on the basis of its topographic organization and laminar patterns of connections with several well defined visual cortical areas.

We studied some of the cortical connections of PIP by making injections of anatomical tracers (HRP, 3H-proline, and fluorescent dyes) into physiologically and anatomically identified sites in V3 (n=3), VP (n=3), and V4 (n=13). The locations of injection sites, transported label, the myeloarchitectonic borders of V3 and the pattern of interhemispheric connections were displayed on unfolded, two-dimensional maps of cortex.

Injections into V3, VP, and V4 each led to two distinct foci of labeling within a restricted region of the lunate sulcus and intra-parietal sulcus. Although this region is delineated by a single callosal-free zone surrounded by a callosal-recipient ring, the separation between foci is sufficiently large (2 to 10 mm) and consistent (16 of 19 cases) that there is a sound basis for considering these as distinct visual areas. The lateral focus, on the anterior bank of the lunate sulcus, corresponds to V3A, while the medial focus corresponds to PIP. Lower-field PIP is located medially, in the fundus of the IPS, while upper-field PIP is located dorsal and slightly lateral on the pre-lunate gyrus. PIP receives feedforward connections from areas V3 and VP that are reciprocated by cells primarily in infragranular layers of PIP. Upper and lower field V4 provide feedback projections to PIP that are reciprocated by cells in both the supragranular and infragranular layers of PIP.

Feedforward projections were found from all three areas to the ventral intra-parietal area (VIP), in and near the floor of the IPS, and from V4 to the lateral intra-parietal area (LIP) on the lateral bank of the IPS. We are unsure whether V3 and VP also project to LIP, owing to uncertainties as to how the border between VIP and LIP should be identified. No projections were seen medial to PIP to area PO, (Covey et al., SNS Abstr., 8:681, 82), possibly because our injection sites were in regions representing relatively central portions of the visual field.

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177.12 RETROGRADE CONNECTIONS OF THE SULCAL REGION OF THE SUPERIOR PARIETAL LOBULE (AREA 5) IN THE MACAQUE. G.R. Stoner*, G.J. Blatt and R.A. Andersen (Sponsor: P. S. Churchland) The Salk Institute for Biological Studies, La Jolla, California 92] 38-92] 6.

Area 5 of the superior parietal lobule occupies four continuous regions in posterior parietal cortex: (]) a posterior region (PEc), (2) a medial region (PGm), (3) a rostral area which lies on the convexity of the superior parietal lobule (PE) and (4) a lateral region along the medial bank of the intraparietal sulcus (PEa). At the fundus of the intraparietal sulcus, area 5 adjoins the ventral intraparietal area (VIP) whereas posterior and medial to area 5 is the extrastriate visual area PO.

In the present study, retrograde fluorescent dyes, diamidino yellow (DY) and/or fast blue (FB) were placed in the sulcal region of area 5 (i.e., area PEa). Prior to delivery of the tracers, single unit recordings were made throughout various regions of area 5. The majority of cells tested were responsive to contralateral joint position and/or tactile stimulation. Many cells were responsive to multiple joint manipulation and also responded to touching the skin overlying the joint area. Some cells were responsive to bilateral or ipsilateral somatosensory stimulation and only a few cells responded to a visual stimulus.

Retrogradely labeled cells from injections in the sulcal region of area 5 (i.e., PEa) were mainly from other somatosensory cortical areas. Areas that had moderate-heavy label include area 7b, somatic sensory cortex area 2, second somatosensory cortex area SII and local connections with other regions of area 5: PE, PEc and PGm. Labeled cells were also seen in the medial bank of the Sylvian fissure and in various thalamic nuclei including the central-lateral nucleus (CL), lateral posterior nucleus (LP) and light label in some divisions of the pulvinar. In one case, heavily labeled cells were found in the anterior cingulate gyrus (area 24).

Two or three clusters of densely labeled cells were found in visual area PO. These labeled cells were seen in both the medial and lateral regions of PO, posterior to area 5. An additional cluster of labeled PO cells was observed from a dye injection made on the opposite bank of the intraparietal sulcus, (i.e., in area LIP or POa). Thus, area PO sends afferents to both the medial and lateral banks of the intraparietal sulcus (i.e., to area PEa and LIP respectively). From the area 5 injections, there was no obvious labeling of cells in any other extrastriate visual areas. In one case, however, there were a few labeled cells in LIP and area 7a, but this was probably due to leakage across the intraparietal sulcus. In other cases where the injection site was entirely restricted to area 5, no labeling in area LIP or 7a was observed.

These findings show that area PEa has extensive somatic input. In addition, though, area PEa also receives some visual information from extrastriate area PO. The significance of this visual input to the sulcal region of area 5 is not clear. A possibility is that input from the visual world may combine with other incoming sensory information (e.g., joint position) to enable area 5 cells to send appropriate signals to cells in motor and/or premotor cortex.

177.13 THE LATERAL INTRAPARIETAL AREA (LIP) IN THE MACAQUE: ASSOCIATIONAL CONNECTIONS AND VISUAL RECEPTIVE FIELD ORGANIZATION. G.J. Blatt, G.R. Stoner* and R.A. Andersen. The Salk Institute for Biological Studies, La Jolla, CA 92138-9216.

Area LIP, the lateral intraparietal area, is a cortical field in the caudal portion of the lateral bank of the intraparietal sulcus in the macaque. The present study consisted of two parts: 1) an anatomical tracing study using the methods of tritiated amino acid autoradiography and fluorescent dyes to map the anterograde and retrograde connections of area LIP and 2) an extracellular recording study to determine the receptive field organization of visually responsive LIP neurons.

The LIP injections were made on a 15 degree anterior-posterior angle through a recording chamber traversing area 5 in the superior parietal lobule which is primarily a somatosensory area (i.e., joint position, touch, etc.) This approach prevents the spread of tracers into visual area 7a.

A comparison of associational connections of area LIP with adjoining area 7a show some common connections but also important differences. Both LIP and 7a have connections with each other and to some extrastriate visual areas such as PO, dorsal prelunate (DP) and the medial superior temporal area (MST). However, area LIP and not 7a has a reciprocal projection with the medial temporal area (MT) and, has connections with areas V3A, V4 and the ventral intraparietal area (VIP). A previous study from our lab has shown that area 7a has strong connections with temporal lobe areas TF, TH, the caudal superior temporal sulcus (STS) and the cingulate gyrus. Area LIP, however, is connected with TF and TEO and lacks connections with the caudal STS and cingulate gyrus. Finally, only LIP and not 7a projects to the superior colliculus and anterior pretectal nuclei and LIP has stronger connections with the frontal eye fields than does area 7a.

Functionally, area LIP contains both visually responsive cells and an abundance of neurons responsive to saccadic eye movements. In the present studies, single unit recordings were made in anesthetized macaca fascicularis monkeys using an eye ring preparation to stabilze the eye. The receptive fields of visually responsive neurons in LIP revealed a local organization with neighboring cells tending to have partially overlapping receptive fields. An orderly progression of receptive field location was observed as the recording electrode moved tangentially relative to the cortical surface. However, discontinuities and/or duplications in the visual representation of the contralateral hemifield were observed. This suggests that within area LIP, the overall topographic representation was disorderly. Receptive fields of area LIP neurons were found to be smaller in size than those found in adjoining area 7a and unlike area 7a, were almost exclusively confined to the contralateral visual field.

These findings suggest that area LIP is an anatomically and functionally distinct visual area from area 7a. Area LIP has extensive connections with areas known to play a role in 1) saccadic eye movements and 2) processing of motion information suggesting that it is an important cortical area for visuomotor integration.

177.14 CORTICAL CONNECTIONS OF INTRAPARIETAL AND INFEROTEMPORAL VISUAL AREAS IN THE MACAQUE MONKEY: A DOUBLE-LABELLING STUDY. A. Morel* and J. Bullier. INSERM-Unité 94, 69500 Bron, France.

It is generally concluded from anatomical and behavioral studies that parietal and inferotemporal cortex process different aspects of visual information coming from the occipital cortex. The degree of separation between these two visual pathways was examined using multiple injections of retrograde and anterograde tracers (fluorescent dyes, WGA-HRP and tritiated amino acids) in cynomolgus monkeys. Following paired injections in the lateral bank of the intraparietal sulcus (area POa) and caudal inferotemporal cortex (area TEO) a large number of cortical areas were labelled and we focused on the connections with the prestriate, superior temporal sulcus(STS) and frontal cortex.

The results confirm that areas POa and TEO receive projections from largely separated areas of the occipital lobe, area POa from dorsal prestriate cortex (including dorsal V4) and TEO from ventral prestriate cortex (primarily ventral V4). However, some regions of the prestriate cortex, like area V3, project to both POa and TEO. Similarly, in the cortex of the STS rostral to the middle temporal area (MT), projections to POa and TEO are largely separated although some areas, particularly in rostral half of the STS, provide common projections to POa and TEO. In the prestriate as in the STS cortex, a small proportion of neurons (3-10%) send collateral projections to both parietal and inferotemporal areas.

Concerning the afferents from the frontal cortex, area POa receives extensive projections from cortex within both branches of the arcuate sulcus (AS) and the caudal half of the principal sulcus (PS) where several cyto- and myeloarchitectonic divisions could be recognized. The prefrontal projection to TEO arises from a small region located in the rostral bank and adjacent gyral surface of the descending branch of the AS. This region which falls within the zone of projection to POa contains only few neurons (less than 1%) with collaterals to POa and TEO.

In addition to their common connections with several regions of the prestriate, STS and frontal cortex, areas POa and TEO are also reciprocally connected. This suggests that these two parietal and inferotemporal areas are not part of completely separated cortical pathways, and that there are multiple cortical sites in which the two may interact.

177.15 CORTICAL AREAS PROJECTING TO ANTERIOR INFERIOR TEMPORAL GYRUS AS DEMONSTRATED BY RETROGRADELY TRANSPORTED WHEAT GERM AGGLUTININ CONJUGATED TO HORSERADISH PEROXIDASE IN THE MACAQUE. C.L. Martin-Elkins and J.A. Horel. (SPON: R.W. Stach) Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

The inferotemporal cortex (IT) consists of the relatively ill-defined middle temporal (mtg) and the inferior temporal (itg) gyri which are partially separated by the anterior middle temporal sulcus in the macaque. IT plays an important role in the acquisition and retention of visual information. This cortex is exclusively visual in function, but does not receive projections directly from striate cortex, but rather indirectly through prestriate cortex. Although considered to be a single, homogenous visual area in the majority of studies on this pathway, functional subdivisions within IT are beginning to emerge. Recently, cooling studies in this laboratory have demonstrated impairments in performance of a delayed matching task (DMS) when anterior itg is cooled. Conversely, cooling mtg, posterior itg, or foveal prestriate cortex was without effect on DMS with delays of 45s.

Visual information is thought to chain down from striate cortex through the prestriate and then posterior IT to ultimately reach anterior IT. The fact that interrupting this putative pathway providing visual information to anterior itg does not result in deficits similar to those produced by direct suppression of itg, raises the question of how anterior itg receives the visual information necessary for performance of DMS.

As a preliminary step in determining the pattern of cortical connections between anterior itg and other visual cortical areas, wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was injected into anterior itg. Retrogradely labeled cortical cells were mapped both contralaterally and ipsilaterally in the temporal pole, posterior itg and the parahippocampal gyrus. Labeled cells were particularly numerous in the ipsilateral parahippocampal gyrus in both infra- and supragranular layers. Ipsilaterally, cell bodies were mapped in orbitofrontal cortex, the lower bank of sts, the insula, and the subiculum. Few, if any, cells appear to be labeled in mtg consistent with evidence that itg may function independently of mtg in the performance of DMS. (Supported by NINCDS Grant NS 1829-05.)

177.16 THE INFERIOR TEMPORAL CORTEX OF THE MACAQUE MONKEY: I. REGIONAL DIFFERENCE IN RESPONSE PROPERTIES OF CELLS. K. Tanaka, Y. Fukada*#, M. Fukumoto*$ and H. Saito. NHK Sci. & Tech. Res. Labs., #Teikyo Univ. Facul. Arts, $Tokyo Univ. Facul. Med., Tokyo, Japan.

To learn the process of integration of pattern information, we have studied response properties of cells in the inferior temporal cortex (IT), which is assumed to be the center of the pattern vision. Since the previous lesion studies suggested regional difference of function within IT, a wide antero-posterior extent of the lateral surface of IT was explored, using semichronically prepared monkeys (M. fuscata). The monkeys were anesthetized with N_2O/O_2 and immobilized with gallamine triethiodide during the single-cell-recording. Responses of individual cells were initially tested with the whole range of the stimuli which included white, black, and colored rectangles and stripes of various sizes, photographs of various regular patterns as well as several tens of animal and plant 3-D models. For cells which were activated most effectively by the presentation of complex 3-D objects, we further tried to make clear which component or combination of components of the stimuli were crucial for the activation, by using 2-D models made of white, black and colored papers.

Of 503 IT cells so far studied, 333 showed clear responses (mainly excitatory for 319 and inhibitory for 14). There was a wide variety both in their stimulus selectivity and the size of the receptive fields. However, a large part of the variety is due to regional difference between the posterior and anterior parts of IT. Cells in the posterior part (P2-A5) had small (the root of area < 5 deg) receptive fields near the fovea, and most of them were activated by such simple patterns as a bar and a spot. For some cells, either orientation or color was not crucial. The other cells were selective only for the orientation or the color. Many of the nonoriented cells prefer small objects to long ones, and many of the noncolor cells prefer either light or dark contrast to the other. Cells with similar properties clustered in single penetrations.

Cells in the anterior part of IT (A5-A23) had large (7-30 deg) receptive fields which included the fovea or adjoined it at their medial margin. A majority of the responsive cells showed more integrated selectivity than the cells in the posterior IT. The essential feature for the activation was the shape more complex than oriented contours, a combination of shape and color, or that of shape and texture (see the accompanying paper for examples). There were also cells for which we failed to extract the trigger feature from the set of effective 3-D objects. The variety within single penetrations was generally large, although the clustering of cells with similar properties was seen in some penetrations.

From these results, we suggest that the coding of visual patterns of objects takes place in the anterior IT while more basic analyses of the central visual field are carried out in the posterior IT.

177.17 THE INFERIOR TEMPORAL CORTEX OF THE MACAQUE MONKEY: II. THE LEVEL OF COMPLEXITY IN THE INTEGRATION OF PATTERN INFORMATION. H. Saito, K. Tanaka, M. Fukumoto*# and Y. Fukada*$. NHK Sci. & Tech. Res. Labs., #Tokyo Univ. Facul. Med., $Teikyo Univ. Facul. Arts, Tokyo.

Recent studies have established the presence of cells responding specifically to faces in a small cortical region of the superior temporal sulcus. This may encourage the idea that the concepts of various objects are represented by the activity of respective gnostic cells. In this study, we tested this possibility by examining the response selectivities of cells in the inferior temporal cortex (IT) which is thought to be the center of the object recognition system.

Experiments were performed on anesthetized macaques prepared as described in the companion paper. Of cells recorded in the anterior part (A5-A23) of IT, 18% were well activated by simple patterns such as bars and spots. The vast majority (299, 82%) were unresponsive to such patterns, and therefore the responsiveness to various real objects was tested for them. The standard set of objects included realistic plastic models of vegetables and fluits (30 kinds), colored feather brushes (5 kinds), colorful miniature models of various animals made of wools or vinyl sheats (10 kinds), and realistic models of the human head and hand.

One hundred and forty seven cells (50%) showed reliable and selective responses to some particular real objects. To know the simplest essential features for the activation, we examined responses to 2-D patterns of various complexities which contained some of the components of the object's pattern. For 54 cells (37% of the 147 cells), the essential requirement for their activation was found to be a combination of spatial parameters (shape, texture), or of shape and color. For example, the trigger feature of a cell which responded selectively to a model of a zebra was found to be a pair of orthogonally oriented stripes placed within an elliptic frame, and that for a cell which specifically responded to a banana was found to be the combination of an elongated and tip-sharpened profile, yellowish color and short parallel lines on it. The trigger feature of a cell which responded selectively to an apple was found to be the combination of a circular disk and a short bar, and the stimulus requirements of cells which selectively responded to hands were reduced to the combinations of a circular disk and several bars extruding from it radially with special angles. For the rest (63%), we could not reduce the stimulus requirements to 2-D patterns. However, the vast majority responded to several different objects comparably, suggesting that these cells were activated by some common features shared by the objects.

Though there was a population of cells (20) which responded only to faces, we suggest that IT, as a whole, codes the visual patterns of objects not by gnostic units but by combinations of multiple cells each of which represents a feature of a moderate complexity.

177.18 **POTENTIALS EVOKED BY FACE STIMULI AND NON-FACE STIMULI IN THE HUMAN ELECTROENCEPHALOGRAM.** **O.-J. Grüsser, K. Bötzel* and B. Häussler*.** Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, Germany-West.

Electroencephalographic responses evoked by changes in pictorial patterns were recorded in 43 adult human subjects (26 females, 17 males). The responses from electrodes T5, T6, Cz, Pz, Oz and the lower occipital region were quantitatively analyzed (international 10/20 recording scheme). The stimuli were projected to a 4 x 6 degree binocularly viewed field. The patterns changed within 6 ms every 2.5-4.5 sec according to a random program; average luminance of successive slides was the same. Average evoked potentials were obtained by means of a digital computer.

Paradigm(1): Schematic faces in normal and upside-down position and scrambled patterns changed in random order. The most prominent response differences between normal and upside-down faces and between faces and non-face stimuli were obtained at the electrodes Cz, T5 and T6 (with no significant left/right difference) with a positive peak at Cz at about 160-180 ms latency, followed by a small negative peak at about 200-250 ms and a slow positive deflection between 300 and 700 ms. Sex differences were most prominent at electrode Cz.

Paradigm(2): A very schematic round face, a deformed schematic face with the same outline and a circle with the same mouth, nose and eye signals, but randomly distributed (non-face), followed each other in random order as in paradigm (1). Category-dependent differences (face/non-face) were again obtained at electrode Cz, T5 and T6 with a significant side difference between T5 and T6 for a peak between 150 and 180 ms.

Paradigm(3): Identical black-on-white or white-on-black line drawings of a face, a tree or a chair were used, altogether 160 slides in semi-random order. At Cz and Pz a response characteristic for faces was found with three prominent peaks. Especially a P 150-maximum was very pronounced in the EEG responses to face stimuli but absent with chair or tree stimuli. Difference curves (face-chair, face-tree, chair-tree), auto- and cross-correlation functions between these difference curves supported the hypothesis of "face-specific" responses.

Paradigm(4): 4 x 6 degree slides (black and white photos) of 54 different human faces, 53 vases and 53 pairs of shoes were projected as in paradigm(3). Face-specific responses (N 140-160, P 210-240, N 300) were more marked in female than in male subjects and again most pronounced at electrode Cz.

Paradigm(5): A recognition task was included in paradigm(4); 9 out of 160 items were memorized 20 minutes before the recording session. Essentially the same evoked potentials were obtained with the unknown stimuli as in (4), but a late positive wave appeared in addition (450-600 ms) in the responses to all stimuli.

We assume that the "face-specific" responses do not originate in the temporo-occipital cortical face region but in deep limbic structures or in the basal parts of the temporal lobe.

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177PO AREAS 7 AND 5 IN CAT: VISUAL AND SOMATOSENSORY RESPONSES OF SINGLE NEURONS AND AFFERENT SENSORY PATHWAYS. C.R. Olson, R.J. Krauzlis* and S.M. Lipkin*: Dept. of Psychology, Princeton University, Princeton, N.J. 08544.

The aim of these experiments was to map out visual and somatosensory divisions of parietal association cortex in the cat. Two approaches were employed. First, we characterized regional variations of sensory responsiveness by single-neuron recording in alert cats. Second, we analyzed regional variations of afferent connectivity by use of retrograde tracers. Convergent results obtained from the recording and connectional experiments suggest that in cat, as in primate, parietal association cortex consists of sharply distinct visual (area 7) and somatosensory (area 5) divisions.

Single-neuron recording in alert cats. We have analyzed neuronal sensory responsiveness by chronic recording in alert, head-restrained cats. Recording sites spanned a broad cortical strip encompassing the anterior lateral and middle suprasylvian gyri. Area 7. Throughout the middle suprasylvian gyrus, with the exception of a narrow anterior strip, neurons were responsive to visual stimulation and firing appeared frequently to be correlated with eye movements, gaze angle and fixation distance. Some neurons were responsive to somesthetic stimulation. These had very large bilateral receptive fields and their responses were markedly reduced during active fixation of a visual target. No obvious auditory responses occurred. Area 5. Within a cortical strip including the anterior part of the middle suprasylvian gyrus and the anterior lateral gyrus, neurons were responsive exclusively to somesthetic stimuli. They possessed comparatively small receptive fields and were arranged somatotopically. In a large central sector, neurons with receptive fields on the forelimb were encountered. Small medial and lateral aggregates of neurons were responsive, respectively, to hindlimb and head stimulation.

Connectional analysis. We have compared patterns of retrograde transport obtained following nine tracer injections in area 5 to patterns observed in a previous study from our laboratory following 14 injections in area 7. Sensory projections to area 5 derive from somesthetic cortical areas (SI, SII, SIV and two other zones bounding area 5 medially and laterally); sensory projections to area 7 derive from extrastriate visual cortex. Motor projections to area 5 derive from area MI and are somatotopically organized; motor projections to area 7 originate in the frontal eye fields. Areas 5 and 7 both receive projections from cingulate, insular and parahippocampal cortex, but the projections arise in each case from nonoverlapping groups of neurons.

178.1 A SYSTEM FOR MULTIPLE MICROELECTRODE RECORDING FROM THE NEOCORTEX OF WAKING MONKEYS. V.B. Mountcastle, G.F. Poggio, H.J. Reitbock*, A.P. Georgopoulos, K.O. Johnson, M.B. Longerbeam*, M.A. Steinmetz, J.R. Phillips, D.K. Brandt*, and C. G. Habbel*.Bard Laboratories of Neurophysiology, Dept. Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have modified and adapted for recording from the neocortex of waking Rhesus monkeys a method for multiple microelectrode recording originally devised by Reitbock, et al. (1,2). A common sliding drive allows micropositioning of 7 independent electrodes in steps of 2 um. Microelectrodes are quartz glass fibers (OD 80 um) with central metal cores (30 um) of Pt-Rh alloy drawn to the desired tip size and ground to the desired form. Electrodes of 2-5 um tips and 3-4 megohms impedance (at 1 Khz) penetrate the dura of 3-4 Kg monkeys, and record satisfactorily from neocortical neurons. The drive is held securely over the region to be explored; the electrodes pass through 300 um guide tubes grouped along their distal portion in a flexible bundle and held in any chosen spatial arrangement within a 5 mm stainless steel implant inserted with hydraulic seal in a small craniotomy opening.

Microprocessor controlled multiple channel processing systems have been designed and constructed for display and discrimination of single neuron impulse activity. Gain, noise filtering, and automatic impedance testing is provided for each electrode. Any electrode may be switched to any of eight processing channels. Each channel consists of a waveshaping filter and a differential amplitude discriminator. Bidirectional electrode movement, as well as signal switching, is obtained via the microprocessor which displays and updates on the console terminal the electrode depth, impedance, and selected channel assignment. A second, independent, microprocessor based system is used to collect, buffer, and encode in real time all event data which are transferred to an experiment-controlling minicomputer whenever convenient.

Exploratory recordings were made in the visual, parietal, and somatic sensory areas; they established the reliability of the method and the stability of recording. The first experiment in a trained macaque was made in the motor cortex of an animal working in a 3D reaching task. In 10 daily sessions, 59 of 66 attempted penetrations were successful; 355 cortical neurons were studied during execution of the reaching paradigm. Seven neurons were recorded simultaneously 15 times; 6 - 14x; 5 -16x; 4 - 11x; 3 - 10x; and 2 - 2x. Analyses of these and other results will be presented.

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1. Reitbock, H.J., et al. (1981) Neurosci. Lett. Suppl. 7:148.
2. Reitbock, H.J. (1983) J. Neurosci. Meth. 8:248-262.

178.2 MASS CORRELOGRAMS OF VISUALLY RESPONSIVE NEURONS IN CORTICAL AREA 18 OF THE CAT. S. Reinis and D.S. Weiss*, Department of Psychology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The function of the central nervous system depends on multiple parallel interactions of large numbers of neurons which together form extremely complex networks where each unit is connected with hundreds of other cells. A meaningful study of brain function depends, therefore, on the analysis of the neuronal circuitry using both morphological and physiological methods. However, when complex interactions are investigated, existing methods for analysing neuronal relationships of neurons are hardly adequate. For this reason, we introduced the method of calculating "mass correlograms", where the frequency distribution of the intervals between the peaks of the mass firings of many neurons around the recording microelectrode are calculated and summarized.

These peaks usually do not represent a single neuronal spike, but are rather formed by several neurons firing simultaneously. In this study, we present mass correlograms recorded in the vicinity of functionally defined, complex cells in the area 18 of the cortex. When the cells respond to an adequate visual stimulus (a light bar moving at the optimal angle, speed and direction), the mass correlogram shows an early regular rhythmic activity with a repeated interval of about 3 ms. The rhythmic interactions may be traced to approximately 100 ms from the beginning of the correlogram. Then, between 500 and 2000 ms, the numbers of interspike intervals gradually increase, and far exceed the frequency of the early rhythmic interactions.

These findings suggest that the analysis of the moving visual stimuli in area 18 of the cortex depends on an early rhythmic interaction of large numbers of neurons, and on an even larger number of long-term interactions which last up to two seconds and possibly more. If we assume that the synaptic delay is about 1 ms, then the neuronal network for the detection of the moving visual stimulus is composed of thousands of successively activated neurons.

All experiments conformed to the guidelines approved by the Canadian Council of Animal Care.

178.3 QUANTITATIVE METHODS FOR THE STUDY OF DEPTH PERCEPTION. H. A. Mallot* and H. H. Bülthoff, Inst. f. Zool. III, Joh. Gutenberg-Univ., Saarstr. 21, D-6500 Mainz, FRG and Center for Biological Information Processing, MIT, E25-201, 45 Carlton St., Cambridge, MA 02139, USA.

Most studies on depth perception in neurophysiology have focused on binocular disparity and used artificial stimuli (bars or random dot stereograms). On the other hand, the importance of pictorial cues (e.g. shading and texture gradients), especially for monocular viewing and for distant scenes is well known in the psychophysics of depth perception. Interaction of different cues is used to disambiguate equivocal information. We have designed experiments to study different cues in human psychophysics which can be used in electrophysiological and behavioral experiments as well.

Computer graphics provide a powerful tool to generate natural images of simple objects (ellipsoids) that provide depth cues in a completely controllable way. Different shading models and ray-tracing were used to compute dull and shiny surfaces. Textured surfaces and texture gradients were generated by means of a solid modelling technique. In addition, stereo image pairs could be interlaced on the color CRT-monitor. The interlace signal of the monitor triggered shutter glasses such that the views of the stereo pair were displayed alternatingly to the corresponding eye.

Quantitative measurement of perceived depth was performed by a cross comparison between different depth cues. In a first series of experiments, a small local stereo probe had to be adjusted interactively to the perceived surface. By this procedure, a local (pointwise) depth map is measured. In a second experiment, we displayed two entire images on the screen one of which could be changed interactively in elongation. This experiment allows a cross comparison of any two cue-combinations at a higher level surface representation.

The following results on the psychophysics of depth perception have been obtained: (1) If no edges (discontinuities in image intensity) are present, stereo information can still be used ("intensity-based stereo"). (2) Perceived depth is greater when more information is available (accumulation). (3) Edge-based stereo vetoes intensity-based stereo in contradictory cases. (4) Shape from shading is a rather weak cue; it is vetoed by edge-based but not by intensity-based stereo. (5) Texture gradients by themselves are also weak but seem to improve the performance when combined with other cues such as shading. (6) Surface interpolation between sparse edges is significantly improved by shading and intensity-based stereo. In certain contradictory cases, the interpolated surface is perceived as "subjective surface".

Very little is known on the neurophysiological correlates of these results, since most of the discussed depth cues are missing in stimuli composed of light bars, edges, and gratings. In future work, we plan to adapt the experiments described here for studies with alert monkeys.

178.4 CAN GERBILS WITH LESIONS OF PRIMARY VISUAL CORTEX USE RETINAL IMAGE SIZE TO ESTIMATE DISTANCE IN A JUMPING TASK? D. P. Carey, L. A. Booth* and M. A. Goodale, Dept. of Psychology, The University of Western Ontario, London, Ont., N6A 5C2.

The Mongolian gerbil (Meriones unguiculatus) can be easily trained to jump accurately from one surface to another over a wide range of distances. Previous experiments in our laboratory have shown that gerbils use a number of different dynamic and static depth cues to calibrate the amplitude of their jump, and that the final estimate of the distance to be jumped is a weighted average of these different cues (Ellard, Goodale, & Timney, Beh. Brain Res., 14, 1985). Gerbils with lesions of area 17 and 18, in contrast to animals with lesions of the superior colliculus or pretectal nuclei, were found to be impaired on this task (Ellard, Goodale, Maclaren Scorfield, & Lawrence, Exp. Brain Res., 64, 1986). Nevertheless, they still showed some residual capacity to calibrate their jumps on the basis of visual cues. Unlike normal animals, gerbils with lesions of area 17 and 18 did not appear to use motion parallax cues to gauge the distance to be jumped. Since retinal image size has also been shown to be a powerful cue for normal gerbils in this situation (Goodale, Ellard, & Booth, unpublished observations), there was a possibility that this same learned cue to depth could be utilized by animals with lesions of area 17 and 18. To test this hypothesis, we first trained gerbils to jump over a gap between two platforms which varied randomly from 10 to 36 cm. Changing the size of the landing platforms on 'probe' trials resulted in systematic errors in the calibration of the jump, suggesting that retinal image size was being used to estimate the distance of the gap. In other words, the gerbils overjumped their usual landing position if the landing platform on probe trials was smaller than the one they were familiar with, and underjumped if it was larger. After large lesions of the posterior neocortex which included all of area 17 and much of the surrounding extrastriate cortex, the animals showed some difficulty with longer jumps but in general were surprisingly unaffected by the lesion. Moreover, the amplitude of their jumps on probe trials revealed that they were still sensitive to the retinal image size of the landing platform. These results suggest that an important learned cue to depth, retinal image size, may not depend entirely on the geniculostriate pathway and its cortical elaboration, but instead may involve other retinofugal systems which may be largely subcortical. (This work was supported by Grant #A6313 from the Natural Sciences and Engineering Research Council of Canada to M. A. Goodale).

178.5 CAN MARKOV ANALYSIS PROVIDE AN INDEX OF HUMAN INFORMATION PROCESSING DURING VISUAL SCANNING? M. Rizzo, R. Hurtig, Division of Behavioral Neurology and Dept. of Speech Pathology and Audiology, Univ. of Iowa, Iowa City, Iowa 52242

The parameters of eye movements typically associated with the scanning of complex images are the number, location, duration and sequence of fixations. Previous studies of scanpaths have emphasized the feature dependence of fixations and their spatial distribution and have neglected how the pictures were scanned. It is reasonable to speculate that the sequential dependence of fixations in a scanpath should vary depending on (a) the external stimulus features and their spatial arrangement, (b) the biological or personal significance of stimuli, and (c) the result of previous exposure.

We applied a standard mathematical technique to analyze the overall predictability of successive fixations in scanpaths in an attempt to develop a new index of human visual information processing. We obtained a quantitative measure of the sequential dependence of fixations in scanpaths by calculating the transitional probability of moving from one region of a stimulus to others. We applied this analysis to fixations extracted from the digitized scanpath data of 29 normal subjects recorded using standard electro-oculography with a minimum resolution of 0.5 degrees. They viewed complex images (including upright and inverted faces and picture scenes) under differing sets of instructions. The analysis was based on a division of the picture stimuli into cells defined by specific image features or by spatial locations. From the first order Markov matrix of the fixations defined by the cells it was possible to calculate a statistic known as the asymmetric lambda. The asymmetric lambda can be taken as an index of how constrained a scanpath is by external stimulus features or by internal cognitive processing factors.

Results showed a characteristic range of overall predictability for sequential fixations that varied for different classes of stimuli. Asymmetric lambdas for scanpaths were lower for processes requiring abstract judgments rather than for those requiring mere descriptions of physical stimulus characteristics. These data show that sequential properties of scanning like the spatial distribution of fixations may depend on stimulus type and goals of search. The sequential properties of scanning can be used as an index of higher neural processes associated with cognitive tasks such as learning, recognition and goal directed search of complex visual displays.

178.6 REDUCTION OF VISUAL CORTICAL PROCESSES TO A COMMON ALGORITHM. G. J. Carman. Division of Biology, Caltech, Pasadena, CA 91125.

Early visual processes, such as those for motion and stereo, make use of different retinal cues in order to derive information about the visual world, such as the presence of edges and the relative depths of surfaces. It may be useful to examine the similarities between such seemingly different visual processes in hopes of discovering whether they can be reduced to a common algorithm, with the goal of obtaining an understanding of how cortex functions as a network. Here I propose a general associative algorithm which makes use of a representation obtained from graph theory to solve the early visual problems of motion correspondence and binocular correspondence. In so doing, the algorithm derives representations of the boundaries of objects from monocular and binocular retinal information over time.

This algorithm accepts as input two sets of tokens, T1 and T2, with each token giving the attribute(s) for a position. For example, in motion or stereo correspondence the tokens could give the sign of the local luminance contrast as a function of position along local, 1-dimensional samples from the retinal image. The algorithm determines which token(s) i of T1 are associated with which token(s) j of T2 by an optimization of an objective function subject to applicable constraints. For example, the optimization could seek to minimize retinal displacement for motion, or retinal disparity for stereo. If there are N tokens in each set, then there are $2^{N \times N}$ possible distinct associations between two arbitrary sets of tokens, requiring on the order of N^2 neurons. In many cases, including motion and stereo, the constraints for a given process manifest themselves as restrictions on this large solution space, requiring only on the order of N neurons to obtain the optimal solution. This solution gives a new local set of tokens T3 which, together with an another set T3' produced in parallel, provide the input to the next level in this process of "folding together of dimensions" to produce progressively more abstract representations.

Simulations of this algorithm on random dot images demonstrate that it can solve the correspondence problem for motion and stereo, combining two samples over time or space, respectively, into a single representation of edges and surfaces with relative depth. A hypothetical implementation of this algorithm given the input to striate cortex leads to the formation of orientation selectivity and its loss at the center of hypercolumns. Iterative application of this algorithm can in principle generate a series of representations which lead to hierarchical, object-centered representations which are thought to be implemented within extrastriate cortex.

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178.7 RECOVERING 3-D STRUCTURE FROM MOTION: SOME NEW CONSTRAINTS. V. S. Ramachandran*, S. Cobb*, D. Rogers* (SPON: R. M. Boynton). Psychology Department, C-009, University of California, San Diego, La Jolla, CA 92093

If a 2-D parallel projection of a rotating object is cast on the screen, viewers can recover its 3-D structure ("structure from motion" or SFM). How is this achieved? We find that the visual system derives SFM by using a wide repertoire of short-cuts and "rules-of-thumb." These rules include: (1) Velocity: We superimposed two coaxial opaque random dot cylinders of identical diameter spinning at two different speeds. What we saw was a single object even though no rigid interpretation was theoretically possible. The dots were seen to occupy two different depth planes rotating with identical angular velocities with the faster dots always being seen nearer (as though there was a small cylinder inside a larger one). Thus the brain derives SFM by responding directly to velocity cues rather than by using an algorithm that seeks rigid interpretations. In fact, the system is quite willing to discard rigidity in order to use velocity. Also, when the two cylinders were presented side by side, the faster one appeared much more convex. (2) Occlusion: We generated a single opaque rotating 3-D cylinder. Although this display could depict either the convex front surface or the concave back surface of a cylinder, it was always perceived as convex. When we did reverse it perceptually it looked non-rigid. The brain prefers the convex percept because otherwise it cannot "explain" the appearance and disappearance of dots at the borders. (3) Visual Inertia. A transparent 3-D cylinder was made to "oscillate" on its axis, i.e. it rotated by 30° and then reversed direction. The cycle was repeated continuously. Surprisingly, the cylinder appeared to rotate continuously instead of appearing to rock, an example of visual "inertia." 4) Reversal of Direction. Two superimposed transparent planes of dots moved in opposite directions at a constant linear velocity. As each dot reached the vertical border it reversed direction and retraced its path. Instead of two co-planar sheets of dots we perceived a "cylinder." The mere reversal of direction at the border generated a depth separation.

We conclude that velocity cues can profoundly influence the derivation of 3-D struture although we do not rule out the possibility of an additional rigidity-based algorithm. By the collective use of several crude short-cuts the system achieves the same biological goal as it could with a single sophisticated algorithm.

178.8 A PARALLEL DISTRIBUTED PROCESSING MODEL FOR THE ABILITY TO OBTAIN THREE-DIMENSIONAL STRUCTURE FROM VISUAL MOTION IN MONKEY AND MAN. R. M. Siegel. The Salk Institute for Biological Studies, La Jolla, CA 92037.

Rhesus monkeys and human subjects can both detect the change in three-dimensional structure of a cylinder using only motion cues. Two motion areas, the middle temporal area (MT) and the medial superior temporal area (MST) appear to be involved in this analysis based on single unit recordings and the effects of chemical lesions in the monkey. In order to understand how neurons in area MST might form receptive fields sensitive for structured motion from area MT input, a model has been constructed based on the known physiological and anatomical evidence.

The parallel distributed processing model consisted of three layers. The properties of the one hundred processing units in the input layer were chosen to be homologous to those of a set of area MT neurons. Each was broadly tuned for a given velocity at a retinotopic location and projected to all ten units of the middle layer. The middle layer units projected to an output layer consisting of a single unit. This last unit signaled whether the input display was structured or not and was modeled after a MST neuron. The output of a unit in the middle or output layer was given as a sigmoidal function of a weighted linear sum of the outputs from the units in the input or middle layer respectively. All the weights were initially chosen at random; the weights were then iteratively adjusted to minimize the difference between the model's output unit and the known structure of the input configuration. The input stimuli were a collection of velocity profiles of 10,000 structured and unstructured cylinders of 6^0 diameter. Each cylinder had 1-64 points on its surface and rotated at 1-10^0/sec.

The model rapidly "learned" to correctly identify whether or not the input velocity flow field was structured. Psychophysical analysis of the model using the same stimuli as in previous work (Andersen and Siegel, Soc. Neurosci. Abstr., 1986) demonstrated that it satisfied the biological constraints of fraction of structure, number of points, and global spatial analysis. The "receptive fields" of this representation in the middle layer were complex, yet symmetrical, functions in both the spatial and velocity domain. Given the designed similarities of this network to the motion analysis system in primates, it is predicted that similar fields will be found in areas MT and/or MST.

This model provides a solution to the problem of extracting structure from motion that is distributed among a number of units. It differs from previous models in that it uses the motion flow field, does not rely on algebraic formulations and can easily be mapped onto known cortical structures. Lastly, this model demonstrates that the use of parallel architectures that are closely modeled on the cortical representation is a computationally efficient means to solve problems in vision. (Funded by The Salk Institute, San Diego Supercomputing Center and PHS NS07457-02.)

178.9 **LOCALIZED RESPONSES TO LOW CONTRAST MOVING RANDOM DOT PATTERNS IN HUMAN VISUAL CORTEX MONITORED WITH POSITRON EMISSION TOMOGRAPHY.**
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The magnocellular system of the visual pathway is characterized by its sensitivity to low contrast moving patterns. The ascending stages in the magnocellular system are the P-alpha retinal ganglion cells, the magnocellular laminae of the LGN, layer 4C-alpha and 4B of striate cortex, and the middle temporal area (MT). Neurons in the magnocellular laminae of the LGN are sensitive to stimulus contrast of less than 10% while the neurons in the parvocellular laminae are much less sensitive (Kaplan & Shapley J. Physiol. 330:125-143, 1982). In deoxyglucose experiments Tootell (J. Neurosci., 1987) found that low contrast stimuli selectively activated layers 4C-alpha and 4B of striate cortex and area MT with relatively little activation of adjacent cortical structures. We have used stimulus evoked changes in cerebral blood flow monitored with positron emission tomography (PET) to map the portions of the human visual cortex responsive to low contrast stimuli. Data were analyzed using the image subtraction technique (Fox et al. Nature 323:806-809, 1986). Subjects were instructed to fixate on a small dark fixation point in the center of a random pattern of dots with a local contrast of 8% and a 10% dot density. Data were generated by subtracting the response to a control stimulus condition from a test stimulus condition (see Fox et al. J. Neurosci. 7:913-922, 1987). In the control condition the dots were stationary; in the test condition the array of dots moved at 16 degrees per second with the direction of movement for the array changing every 2 seconds. Eight movement directions presented in pseudorandom order were used. In both the control and test conditions, data were collected over a 40 second period. There were a total of 17 test-control comparisons in 6 subjects. The data were combined using a stereotactic system (Fox et al. J. Comp. Asst. Tomog. 9:141-153, 1985) and averaged together to reduce the background noise level. The largest response was centered in striate cortex. The second largest response was located in the fundus of the temporal-occipital-parietal pit (TOPP) (Polyak The Vertebrate Visual System,1957; Talairach & Szikla Atlas D'Anatomie Stereotaxique du Telencephale, 1967). This region is included in a bilateral lesion found in a patient with a selective impairment of perception of motion (Zihl et al. Brain 106:313-340, 1983) and is a possible candidate for the location of area MT in human visual cortex. (Supported by NIH grants EY03851, RR 07003, NS-06833, HL-13851, NS-07025 and NS-0094 and grants from the Gordon Trust and the Sloan Foundation.)

178.10 PSYCHOPHYSICAL MEASURES FOR CHANGES IN DIRECTION OF MOTION.
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Dynamic random dot motion displays were used to measure sensitivity to changes in direction of motion. Motion sensitive units in striate cortex are thought to process motion for separate retinal directions of flow, while units found in higher centers, such as mediotemporal cortex, combine more than one simultaneous direction of flow. Thus far, there have been no reports of cells tuned to sequential changes in the direction of motion.

We obtained thresholds for changes in motion direction by adjusting the percentage of dots moving coherently on a random noise field. There is no translating pattern present. This presents the visual system with signal-to-noise task for motion. This ratio can be varied independently from dot speed, direction, and display brightness.

Sensitivity to changes in direction increases monotonically with the angle between the two directions. Over a wide range of velocities, detection thresholds for linear motion, expressed in terms of the signal-to-noise ratio, are lower than or nearly equal to changes in speed and/or direction. In no condition is threshold for change lower than that for simple direction.

Using above-threshold motions, a repeated sequence of alternating directions (a zigzag trajectory) is seen either as such ("component motion") or as an oscillation superimposed on a continuous mean direction of flow ("composite motion"). The latter is seen at faster rates of alternation (above 4 Hz), and with narrower angles separating the two component directions. At intermediate values of alternation (3-4 Hz), the percepts may change spontaneously. This is a temporal analog to earlier experiments with pairs of superimposed drifting gratings, in which percepts were either "composite" plaids or "component" gratings moving in separate directions.

The underlying mechanism for these percepts was explored with motion after effects (MAEs). After adapting to a zigzag stimulus sequence for more than 1 min, MAEs are seen with test fields of stationary dot patterns. There is no MAE specific to adaptation for changing directions as distinct from simple motion. Even for the slowest zigzag adapting fields, seen as "component motion," MAE is a steady drift in a direction opposite to the mean of the component directions. This holds true even for separations as wide as 130 deg, which argues for the existence of populations of cells broadly tuned for direction. A more central mechanism must be responsible for the sequential combination of these components into various perceived trajectories.

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178.11 EVIDENCE OF AN INTRINSIC TEMPORAL CODE FOR PICTURES IN STRIATE CORTEX NEURONS.
Barry J. Richmond, Lance M. Optican*, and Timothy J. Gawne*, (SPON. M. Segraves). Laboratory of Neuropsychology, National Institute of Mental Health, and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

In the preceding abstract we reported that information transmitted about pattern, duration, and luminance was greater when all three features were considered simultaneously rather than separately. This synergy requires a consistent, unconfounded, mapping from stimulus features to neuronal responses. However, information theory does not require that the neural code be interpretable in terms of these features. Nonetheless, we have identified an intrinsic coding scheme that would allow pattern, duration, and luminance to be extracted.

The responses of the 15 striate cortical neurons reported in the preceding abstract were analyzed further. The stimuli were 16 one-dimensional Walsh patterns, presented at each of 7 luminance combinations (112 stimulus conditions) for seven neurons, and for 4 luminances and 5 durations (320 stimulus conditions) for 8 others. For each neuron, the responses were decomposed into their principal components (PCs). These components are orthogonal patterns of temporal activity that define the axes of a multidimensional space. Responses with different temporal waveforms have different PC coefficients and map to different points in this PC space.

In a planar space defined by two PCs, no relation between stimulus features and neuronal responses was apparent. However, in a space defined by the first three PCs, the responses elicited by an individual Walsh pattern, irrespective of duration or luminance, appeared to lie near a plane. Thus, we approximated the responses to each of the 16 patterns by their best-fit planes. The average residual variance per plane was 4.6% $\pm$ 4.1 (SD, n = 112) for the 7 neurons tested with 7 luminances, and 12.0% $\pm$ 5.2 (n = 128) for the 8 neurons tested with 4 luminances and 5 durations. Since the coefficients of the PCs are uncorrelated, if the responses to a given pattern had not been well approximated by a plane, the residual variance would have been at most 33%.

In any one neuron's PC space many of the planes representing the 16 stimulus patterns appeared easily differentiable. This geometrical structure demonstrates that the generation of neuronal responses obeys certain rules, which form an intrinsic temporal neural code for visual features. A response is potentially decodable, to determine the stimulus pattern irrespective of duration or luminance, if the plane into which the response falls can be ascertained. Information about duration and luminance is then encoded relative to that plane. Since three points determine a plane, such a decoding scheme may require as few as three complementary neurons sharing related codes.

178.12 STRIATE CORTEX COMPLEX NEURONS DO NOT CONFOUND PATTERN, DURATION, AND LUMINANCE.
Timothy J. Gawne*, Barry J. Richmond, and Lance M. Optican*. Laboratory of Neuropsychology, National Institute of Mental Health, and the Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

We have used Shannon's information theory to show that temporal modulation of a neuron's response contains information about stimulus pattern. Other stimulus features, such as duration and luminance, can also modulate the response. Does the neuron transmit information about these features as well?

A rhesus monkey fixated while stimuli, 2.5 degrees wide, were flashed on the receptive fields of neurons located 2° eccentrically. The stimuli were 16 one-dimensional, optimally-oriented Walsh patterns. They were presented at one of four luminance combinations (luminance range: 0.004 - 5.0 ft-lamberts), and flashed on for one of five durations (32 - 256 ms). All 320 stimulus combinations were presented to eight cells. A continuous spike density function was obtained by convolving the spike train with a Gaussian pulse (σ = 3 ms). Then, principal components (PCs) were extracted from 320 ms segments of the densities (Richmond et al., J. Neurophysiol., 1987). The coefficients of the PCs form a multivariate measure of the temporal modulation of the responses.

Transmitted information measures the discriminability among inputs given an output for a pair of input and output codes. Information is low when inputs are confounded among outputs. We related four stimulus input codes to each of two response output codes. The input codes were: 1) the four luminances alone, 2) the five durations alone, 3) the 16 patterns alone, and 4) the 320 combinations of these features. The two output codes were: 1) the coefficients of the first three PCs (T3) and 2) the number of spikes in the response (Ts).

	all 320	pattern	duration	luminance
T3(bits)	1.07$\pm$.08(SE)	0.32$\pm$.04	0.20$\pm$.05	0.19$\pm$.02
Ts(bits)	0.46$\pm$.04	0.15$\pm$.02	0.06$\pm$.02	0.05$\pm$.01

T3 was two to four times greater than Ts for all input codes. Ts for pattern was much greater than that for duration or luminance, whereas T3 had approximately the same magnitude for all three features. Strikingly, the transmitted information based on the input code of all 320 stimulus combinations (1.07 bits) was substantially greater than that predicted by the sum of the information from the individual codes (0.71 bits).

The codes for the three stimulus features were synergistic: more was known about the stimulus when a code that simultaneously accounted for all three features was used. Similar synergy was also observed in another seven cells tested with 16 patterns and 7 luminances at one duration. This synergism implies that the different stimulus features were not confounded by the neuron.

178.13 CATS SEE SUBJECTIVE CONTOURS. M. Bravo* and R. Blake* (SPON: C. Enroth-Cugell). Cresap Neuroscience Lab., Northwestern University, Evasnton, IL 60201

The formation of subjective contours allows a parsimonious and usually accurate representation of object boundaries from complex and incomplete visual information. The human visual system presumably constructs these illusory contours at an early level of processing and uses them in subsequent stages. We now have evidence that cats see subjective contours.

Subjective contours were formed by a stimulus configuration in which four disks, each with a cut-out quadrant, are positioned to create a Kaniza illusory square. To prevent the cats from learning the particular configuration of disks that creates the square, we presented the cats with a movie of a subjective square undergoing apparent motion. The stimulus was devised such that if subjective contours are not seen, the disks forming the illusion appear to spin about their centers. If subjective contours are seen, then a square moving across the display is seen in addition to the local spinning motion.

Before presenting these displays to cats, we evaluated the saliency of the subjective square on naive humans. These observers could readily discriminate a movie with an embedded subjective square that moved over successive frames from one of randomly rotating disks. Performance remained accurate over a range of frame rates. Observers did not see global motion with disk configurations that did not produce subjective contours. At slow frame rates it was possible to find these other configurations as they moved across the screen, but as frame rate was increased these configurations eluded detection.

Using a forced choice procedure and food reinforcement for correct responses, we trained two cats on a number of displays that successively approximated the final display described above. The cats were then tested for twenty days on different versions of the final display and their performance was consistently above chance over this period. Performance was not impaired by increasing the frame rate, indicating that the cats were not relying on recognition of the disk configuration to discriminate between movies with and without the subjective square. Rather, these results strongly suggest that the cats were seeing the subjective contours. Evidence that non-primate mammals perceive subjective contours argues against the idea that subjective contours are cognitive in origin.

178.14 OSCILLATORY MOTION THRESHOLDS MEASURED BY THE VISUAL EVOKED POTENTIAL. A.M. Norcia* and W.W. Wesemann* (SPON: E. Keller). Smith-Kettlewell Eye Research Foundation, 2232 Webster St., San Francisco, CA 94115.

Visual evoked potentials were recorded in response to small periodic phase shifts of sinusoidal luminance gratings. The grating position was shifted back and forth in square wave steps at rates between 12 and 40 displacements per sec. Displacement amplitude was increased in a series of 19 linearly spaced steps over a period of 10 sec. The amplitude and phase of the evoked response ocurring at the direction reversal rate were determined by a Discrete Fourier Transform. The lower threshold for motion was determined by extrapolation of the function relating VEP amplitude to displacement amplitude. These thresholds were compared to psychophysically determined thresholds as a function of the spatial frequency and contrast of the grating as well as the temporal frequency of the motion.

The VEP amplitude versus displacement function consisted of an initial rising portion usually followed by a saturation of amplitude at displacements well short of 180 deg. The phase of the evoked response was nearly constant, although phase leads with increasing displacement were sometimes noted.

VEP and psychophysical thresholds were nearly constant over the range of spatial frequencies between about 1 and 16 c/deg and over the range of temporal frequencies between 15 and 40 direction reversals per sec. Best thresholds were in the range of 15 to 20 arc sec. The present results with the VEP replicate previously reported psychophysical results (Wright and Johnston, Vision Res., 25:187, 1985). The oscillatory motion threshold lies between the range of the visual hyperacuities such as vernier offset acuity and stereoacuity which have thresholds of under 10 arc sec and grating resolution which ranges between 40 and 60 arc sec. Oscillatory motion thresholds were found to be elevated in observers with strabismus/amblyopia.

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178.15 VISUAL EVOKED POTENTIALS AND EEG ACTIVITY UNDER DIFFERENT LEVELS OF ANESTHESIA IN RAT. D.F. Sisson* and J. Siegel, Institute for Neuroscience and School of Life and Health Sciences, University of Delaware, Newark, DE 19716 and J.H. Lukas*, U.S. Army Human Engineering Lab., Aberdeen, MD 21005.

The use of EEG as a predictor of anesthesia induced changes in visual evoked potentials (VEP's) was examined in acute rat preparations. VEP's were generated in male, Wistar rats (500 - 750 g) by binocular, strobe-light flashes and recorded from Area 17 simultaneously by cortical screw (left) and micropipette (right). EEG activity was measured at the cortical screw, and its spectral composition was analyzed by Fourier transform. Anesthesia level was varied by chloral hydrate intravenously in 10 mg doses. Heart rate was used as a gross measure of anesthetic level, and throughout the experiment sufficient doses were administered to maintain heart rate between 250 and 350 bpm.

Within this gross range of anesthetic levels, two distinct forms of VEP's were observed. Some VEP's were similar to those reported from awake, chronic preparations. The major differences were increases in latency to peak of all components and attenuation of the late components P3 and N3 relative to those observed in chronic animals. In other VEP's N1 was either greatly attenuated or entirely absent, and the amplitude of the P1 component was concomitantly increased. During intervals when normal VEP's were observed, a major EEG frequency band between 4 and 5 hz was always present; a minor frequency band between 15 and 25 hz was often observed. During intervals when N1 was attenuated, most EEG frequencies were in a band from less than 1 hz to 3 hz with little contribution from frequencies higher than 10 hz. Heart rate was only weakly correlated to VEP shape and only at the extremes of the range allowed. Normal VEP's were obtained at 350 bpm while VEP's with attenuated N1 components were obtained at 250 bpm. Between these extremes either VEP type could be observed at any heart rate.

Laminar analysis of normal VEP's recorded from a micropipette show polarity reversal of P1 near cortical layer IV and of N1 and P2 in deeper layers. VEP's with attenuated N1 components show polarity reversal of most of the potential near layer IV with only minor alterations in deeper layers suggesting that N1 attenuation is a sign of cortical depression and that in this state, the reflection of direct geniculate input to cortex can be observed unobscured in the VEP.

The categorization of VEP's by EEG activity helps to control one source of variability in evoked potential data, and increases the reliability of comparisons of VEP's measured over long time periods.

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178.16 VALIDATING CLUSTER ANALYSIS OF VISUAL EVOKED POTENTIALS BY Q-FACTOR ANALYSIS. D.S. Weiss* and S. Reinis (SPON: J. Landolt). Dept. of Psychology, University of Waterloo, Waterloo, Ont., Canada, N2L 3G1.

Given the inherently complex and variable nature of the EP waveform, there is little consensus among researchers as to the best approach for analysing EP datasets. Consequently, EP datasets are subjected to a wide variety of statistical procedures, usually with some loss of relevant information. One method of analysing EP waveforms is Euclidean distance cluster analysis (complete linkage). This technique is independent of score distribution, and incorporates latency, amplitude and symmetry information into the clustering solution. However, the cluster solution (dendrogram) does not actually describe the waveform features which separate the clusters, and statistical inferences drawn from the solution are difficult and problematic. Therefore, a method of analysing EPs is required which will independently verify the cluster solution without loss of information and which will allow statistical inferences to be drawn from the data.

Q-factor analysis with varimax rotation is a method for clustering objects according to attributes. When applied to EP data, the data matrix is transposed so that individual EPs are variables (columns) and voltages at successive time points are cases (rows). Before factoring, a correlation matrix is computed between EPs across time points, thus preserving information about EP amplitude and wave shape characteristics. Principal factor axes are then calculated and rotated, thereby organizing the original EP dataset into clusters according to the rotated factor loading matrix. The Q-factor solution can then be used to cross-validate the Euclidean distance dendrogram. Q-factor scores, calculated from the rotated factor loadings, represent "ideal" waveforms which model the characteristics of the original EPs within each cluster, adding the dimensions of wave shape and features analysis to the cluster solution.

Clustering solutions for 72 sets of eight visual EPs, recorded from four cats, were cross-validated by Q-factor analysis. The eight EPs in each dataset (responses to the onset and movement of a light bar stimulus presented at eight different angles) were represented by three principal factors, which typically accounted for approximately 94 percent of the variance within the dataset. Of the 273 clusters obtained from the Euclidean dendrograms, 244 were successfully replicated by the Q-factor loading matrix (89.4 percent).

All experiments conformed to guidelines approved by the Canadian Council of Animal Care.

178.17 VISUAL CALLOSAL PROJECTIONS IN THE FERRET. Rosemarie B.Rayos del Sol-Padua,* Joyce Brown* & E.Hazel Murphy. (SPON: A.J. Haroian). Department of Anatomy, Medical College of PA., Phila., PA., 19129.

The callosal projections between visual cortical areas in the adult pigmented ferret have been studied, using combined physiological and retrograde tracing techniques, in order to characterise the tangential and laminar organization of these projections. Three groups of animals were used. In one group, the corpus callosum (CC) was sectioned and gelfoam, soaked in 20% HRP, was inserted between the cut ends of the callosal axons. In a second group, multiple injections of 20% HRP were made unilaterally throughout the dorsal posterior surface of the cortex, encompassing much of areas 17 and 18. In the third group, the 17/18 border was identified by physiological mapping of the visual field and injections of 20% HRP were made in this location. Following a survival of 1-2 days, animals were perfused, the brain cut and alternate sections were processed for HRP reaction (TMB chromagen) to visualise callosal cells,for cytochrome oxidase (CO) to aid in identification of the 17/18 border, and for Nissl to aid in identification of laminar boundaries.

The HRP material was drawn and analysed, using light and darkfield optics and a camera lucida, to determine the tangential extent and laminar organization of the callosal cell zone at the 17/18 border. The callosal cell zone in area 17 had a mean width of 2.3mm. The width of the callosal cell zone in area 18 was more variable in width and in the density of callosal cells, but had a mean width of 5.3 mm. The 17/18 border was clearly distinguishable in CO and Nissl material, and analysis of HRP filled cells at this border indicated a clear distinction in the laminar organization of callosal cells in these 2 cytoarchitectonic areas. In area 17, virtually 100% of the callosal cells are in laminae II/III. In contrast, in area 18, approximately 60% of callosal cells are in laminae II/III, and most of the remainder are equally divided between laminae V and VI. Fewer than 5% are located in lamina IV. These differences in tangential extent and laminar organization of the callosal projections between areas 17 and 18 must reflect differences in their funtional contributions to these 2 cytoarchitectonic regions of cortex.

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178.18 PROPERTIES OF AREA 17/18 BORDER NEURONS CONTRIBUTING TO THE VISUAL TRANSCALLOSAL PATHWAY IN THE CAT. M.E. McCourt*, J. Boyapati* and G.H. Henry* (SPON: D.G. Albrecht). Dept. of Physiology, JCSMR, The Australian National University, Canberra, A.C.T. 2601 Australia

The response properties and laminar distribution of area 17/18 border neurons contributing to the visual callosal pathway in the cat were studied using single-unit recording, electrical stimulation and neuroanatomical labelling techniques. A total of 203 units driven either ortho- (O: N=165) or anti-dromically (A: N=38) from stimulating electrodes in either the corpus callosum (CC) or the area 17/18 border of the contralateral visual cortex (CH) were obtained from 7 anesthetized, paralyzed cats. Forty-three percent (87/203) of the callosally-recipient (O) or projecting (A) neurons were also activated from stimulation at the ipsilateral lateral geniculate nucleus. The laminar distribution of 144 callosal units (O=110; A=34) was determined from electrode track reconstruction. Seventy-five percent (109/144) resided in layers 2/3 and 4ab. The reconstructed distribution matched closely that derived from the injection of horseradish peroxidase at the site of the CH stimulating electrodes. Neurons in the complex family (C and B cells: Henry, G.H., Brain Res., 133:1, 1977) comprised 68% (81/119) of the cells which could be classified according to receptive field (RF) type. S-cells accounted for 30% (36/119); 2% were non-oriented. The ratio of O to A units was not significantly different across RF type. The ocular dominance (OD) distribution for 83 callosal units (O=67; A=16) was trimodal: 36% were monocular (OD classes 1 and 7) and 32% were binocular (OD=4). OD distribution was similar across RF types and across O and A units. Estimates of transcallosal conduction time (CT) and synaptic delay (SD) were derived for 132 units which could be driven from both the callosum and contralateral visual cortex. CT was obtained by subtracting spike latency from stimulation at CC (Lcc) from that resulting from stimulation at CH (Lch). Mean CT for all cells was 2.52 ms. Mean CT for 58 C-family units was 2.94 ms (≈11 m/s velocity), and was significantly longer than CT in 26 S-cells, which averaged 1.80 ms (≈18 m/s). Four percent of callosal units possessed CT's suggesting unmyelinated axonal conduction (≈2 m/s). CT's measured from O and A units were not significantly different. SD was computed as 2Lcc-Lch. For O cells, SD indexes synaptic delay plus spike initiation latency; for A cells the SD value indexes only spike initiation latency. For 22 A cells, mean SD was 0.26 ms, consistent with previous measures of spike initiation latency. In 101 O cells, the modal SD was 1.20 ms. Subtracting spike initiation latency and post-synaptic depolarization delay in the generation of the action potential yields an SD estimate for O cells of ≈0.8 ms. Most O cells are thus monosynaptically activated by callosal afferents. Mean SD values for both O and A units did not differ significantly as a function of RF type.

178.19 THE ORGANIZATION OF CALLOSAL CONNECTIONS IN THE OCCIPITAL VISUAL CORTEX OF THE PIGMENTED RABBIT. A.M. Grigonis. Department of Anatomy, Hahnemann University, Broad and Vine, Philadelphia, PA 19102-1192.

In the adult pigmented rabbit, the projection of the corpus callosum connecting the striate visual cortex of both hemispheres has been well documented. The cells which make up the callosal projection between the two visual hemispheres are localized around the border between striate and occipital cortices. The tangential extent of the distribution of callosal cells is limited to the lateral third of the mediolateral extent of the striate cortex. The radial distribution of callosal cells in the striate cortex consists of a majority of cells present in laminae II and III, with few cells in laminae IV and V. In the present study the tangential and radial organization of callosal cells were examined in the occipital visual cortex of the pigmented, Dutch-Belted rabbit. Normal adult rabbits received multiple injections of 20% HRP throughout both striate and occipital visual areas in one hemisphere (total injected amount, approx. 15μl). Retrogradely labelled cells were visualized by reacting coronal sections throughout the visual cortices of the opposite hemisphere with tetramethylbenzidine. One out of every four sections were stained with thionin in order to identify cytoarchitectonic patterns which were used to define the cortical regions. The organization of callosal cells in the occipital cortex of the rabbit forms a more complex pattern than in the striate cortex. The callosal cells found in the occipital cortex are distributed similarly to the striate callosal cells in the rostral half of the occipital cortex. More caudally, however, there is a wider distribution of callosal cells in the occipital cortex, and there are bands of cells which are present throughout the mediolateral extent of the occipital cortex, which are not found in the striate cortex. Also, there is a different radial distribution of callosal cells between the rostral and caudal portions of the occipital cortex. In rostral regions of the occipital cortex, the proportion of cells found in each of the laminae is similar to the proportion found in the striate cortex; however, in more caudal regions, there is an increased proportion of cells located in laminae II and III than in laminae IV and V. The results suggest that in the occipital cortex of the rabbit, the callosal projection consists of an uneven distribution of cells compared to the projection in the striate cortex, which may reflect functional differences between striate and extrastriate cortical regions.

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179.1 GABA AND ACETYLCHOLINE SYNTHESIS IN THE OFFSPRING'S VESTIBULE OF PROPYL-THIO-URACYL TREATED RATS. L. Cerón* and G. Meza. Dept. Neurociencias, I.F.C., UNAM. Apdo. Postal 70-600, 04510 México, D.F., México.

It is currently accepted that vestibular sensory cells have chemical synapses in which GABA and acetylcholine (Ach) function most probably as afferent and efferent neurotransmitters, respectively. To provide evidences in that respect, the enzymes of synthesis of both putative neuromediators (glutamate decarboxylase, GAD, for GABA and choline acetyltransferase, ChAT, for Ach) were measured in homogenates of vestibules of Long-Evans rats. To investigate GAD and ChAT localization, propyl-thio-uracyl (PTU) was administered to pregnant rats and GAD and ChAT were measured in the progeny's vestibule. PTU causes congenital hypothyroidism to offsprings of PTU-treated animals, preventing among other effects, the arrival of efferent connections to the vestibule, thus providing a model in which sensory cells are naturally isolated from their efferent synapses.

We found that GAD and ChAT activities were present in vestibular homogenates of normal Long-Evans rats, findings that provide evidence of GABA and Ach as neuromediators in the rat vestibule. When these enzymes were measured in the vestibule of 38-day-old congenitally hypothyroid rats (CHR) a 70% decreased ChAT with respect to normal rats was found; in contrast, GAD was not modified. When new-born CHR were treated with thyroxine hormone this PTU effect on ChAT was prevented.

These results provide evidence of the efferent bouton's localization of ChAT and that the sensory cell contains GAD being Ach and GABA their respective neurotransmitters.

179.2 MORPHOLOGY OF VESTIBULAR PRIMARY AFFERENTS IN JUVENILE TURTLES, *PSEUDEMYS SCRIPTA*. A.M. Brichta and E.H. Peterson. Dept. Zoological & Biomedical Sciences and College of Osteopathic Medicine, Ohio University, Athens, Ohio, 45701.

As part of a study aimed at understanding the sensory control of cervical musculature in *P. scripta* we are using the neuronal marker horseradish peroxidase (HRP) to characterize the functional architecture of primary vestibular afferents. Experiments were conducted *in vitro* and *in vivo* using juvenile turtles (2-3" carapace length) to minimize transport distances and the number of sections over which neurons must be reconstructed. We made injections into anterior or posterior branches of C.N.VIII or into individual ampullary nerves. In some cases we back-filled secondary vestibular cells by iontophoretically injecting HRP into the medial longitudinal fasciculus.

Peripheral nerve endings within the cristae, are of three morphological types; 1) calyceal - chalice-like endings with limited branching; 2) bouton - finely varicose endings with extensive branching; 3) dimorphic - having characteristics of both. Within the cristae, calyceal and dimorphic endings are located centrally while bouton endings are more peripheral. This sequestered arrangement corresponds to the known distribution of vestibular receptors in *Pseudemys* (Jorgensen, '74). Parent axons of each ending type differ significantly in diameter (calyx= 1.64±.04, dimorphic= 1.41±.03 and bouton= 0.75±.05 um). Diameters of these peripheral processes are correlated with the size of their somata and central processes. Thus vestibular primaries in *Pseudemys* form three size classes which also differ in their peripheral morphology and distribution.

Central processes of vestibular primaries enter the brainstem and bifurcate. The two daughter branches run longitudinally, lateral to the vestibular nuclei, and drop off multiple, finely beaded collaterals in the transverse plane. Collectively these collaterals form a three dimensional orthogonal lattice traversing the vestibular nuclei. Although each centrally projecting vestibular primary gives off collaterals throughout much of the vestibular complex, each appears to have a preferential site of termination. The presumed targets of these afferents, secondary vestibular neurons, are bipolar and are aligned transversely, parallel to incoming primaries. Thus individual collaterals can be expected to contact only a small number of secondary vestibular cells. The architecture of vestibular primaries, taken together with the morphology of secondary vestibular cells suggests that there may be a relatively precise mapping of the vestibular end organ onto second order vestibular neurons.

179.3 SYNAPTIC COVERING OF CELLS IN THE MEDIAL VESTIBULAR NUCLEUS IN THE GERBIL. G.A. Kevetter. Departments of Otolaryngology and Anatomy & Neuroscience, University of Texas Medical Branch, Galveston, Texas 77550

The Medial Vestibular Nucleus (MVN), one of the four primary vestibular nuclei, receives an input from the vestibular nerve. Cells in the MVN, in turn, send projections in three major pathways: the vestibulo-ocular pathways, the vestibulospinal system, and the commissural pathways. We are investigating the morphology and ultrastructure of the medial vestibular nucleus in order to provide a morphological substrate for sensory-motor integration that occurs in this nucleus. In this study we calculated the percent of the cell surface covered by synaptic boutons (Synaptic Covering Ratio or SCR). Control animals were perfused and processed for routine electron microscopy. Experimental animals received large injections of HRP into the vestibular labyrinth. After 48 hour survival period, the animals were perfused, processed with a routine HRP reaction, and processed for EM as the control animals. Thin sections were cut through the MVN and observed. The perimeter of 2 cells cut approximately through the center of the cell was photographed for each block. The cell was considered to be cut through the center if the nucleolus was prominant in the nucleus. The presence of a nucleolar satellite in females was also used as indicative of the approximate largest surface area of the cell. The percent of the cell perimeter that was covered by synaptic boutons was calculated. In 13 cells used as controls, an average of 31.5% of the cell surface was covered by boutons. Most of these boutons contained round clear vesicles. The trend is for the larger cells to have a greater SCR than the smaller neurons. The range of surface area of the neurons investigated was large (Range = 74.3-1389.2 μm^2; $\bar{X}$ ± SEM = 342.31 ±114.68). In material in which the majority of vestibular afferents had been labeled with HRP, a proportional covering ratio was determined. Seven neurons have been studied to date. The neurons sampled were the first two neurons which were sectioned through the center of the cell regardless of whether or not HRP-labeled boutons were present. Thus far, cells in the experimental group have had a lower SCR (17.0%) than somas in the control group and the cells sampled thus far have been smaller (Range = 74.3-262.3 μm^2; $\bar{X}$ ± SEM = 177.43 ± 26.504). Three of the neurons investigated in the experimental material did not have any labeled boutons apposed to the perimeter, indicating that they were not second-order vestibular neurons. The total SCR of second order neurons (16.1%) was the same as that of neurons without labeled boutons (16.4%). The proportional covering of vestibular afferent input to the second order neurons was 16.5%. These morphological findings suggest that a significant proportion of the primary vestibular input synapses directly on the soma of second order neurons. (Supported by NSF grant BNS-84-18559)

179.4 VESTIBULAR COMMISSURES IN THE GERBIL. S.D.Newlands* and A.A. Perachio+. Departments of Otolaryngology and Physiology & Biophysics+, University of Texas Medical Branch, Galveston, TX 77550

The direct connections across the midline between the bilateral vestibular nuclei were examined in the Mongolian gerbil (*Meriones unguiculatus*). Horseradish peroxidase was ionophoretically injected unilaterally into one of the major vestibular nuclei in each experimental animal. The animals were sacrificed two days later and processed with DAB as the chromagen. Each serial transverse section from a series spanning the rostral-caudal extent of the vestibular nuclear (VNC) was evaluated for crossing fibers, contralaterally labelled neurons, and contralateral terminal fields. Contralateral to the injection, the majority of labelled neurons were in the VNC and nucleus prepositus hypoglossi (PrH); additional cells could be found scattered in the reticular formation. Contralateral terminal fields were primarily seen in the VNC, PrH, abducens nucleus, and the parvocellular reticular area just ventral to the lateral vestibular nucleus (LVN). The most extensive commissural system involved reciprocal and non-reciprocal vestibulo-vestibular connections of the medial vestibular nuclei (MVN). The number of retrogradely labelled cells and crossing fibers after MVN injection were an order of magnitude greater than seen for comparable injections in the other nuclei. An average of 94% of contralateral filled neurons were in the MVN following an MVN injection. Furthermore, terminal fields were seen throughout the VNC contralaterally, most densely in the MVN but also prominent in the LVN. In all animals injected in the lateral, superior (SVN), and descending vestibular nuclei (DVN) an average of 69% of the contralaterally labelled neurons were observed in the MVN with the rest distributed among the other nuclei, dependent upon the injection site. Few contralateral terminal fields were observed following injections into the LVN, DVN, and SVN. Most of the crossing fibers between the bilateral VNC coursed through the medial longitudinal fasciculus (MLF) in the dorsal half of the brainstem. The majority of labelled fibers crossed the midline directly at the level of injection. At the rostral pole of the VNC, the crossing fibers were largely seen at the floor of the fourth ventricle while more caudally fibers were more ventrally located but seldom were observed ventral to the MLF. Midline lesions were made in the the commissural system to investigate the possibility that only a discrete bundle of the crossing fibers were actually commissural (vestibulo-vestibular) as opposed to vestibular nuclear efferent projections (vestibulo-ocular, vestibulo-spinal, etc.). We found that partial transection of the midline brainstem decreases the presence of retrogradely filled neurons contralaterally, dependent upon the level of the lesion and the injection site. The vestibular commissural system in the gerbil therefore appears diffuse and limited primarily to reciprocal connections of the bilateral medial vestibular nuclei. These findings differ from findings in the cat where connections between the other vestibular nuclei appear more prominent. (Supported by NASA grant NAG 2-26 and NIH grant NS24391)

179.5 THE EFFERENT AND AFFERENT CONNECTIONS OF THE TANGENTIAL VESTIBULAR NUCLEUS AND THE CENTRAL PROJECTIONS OF THE AMPULLARY NERVES OF THE CHICKEN. R.G. Cox* and K.D. Peusner. (SPON: R. Walsh). George Washington University, Washington, D.C. 20037.

Neurons and fibers were labeled with horseradish peroxidase (HRP:Sigma type VI) using 6-8 week old chickens, unless otherwise stated. Deposition of HRP into the tangential nucleus (TN) and into the second cervical level (C2) of the spinal cord shows that the TN axon output is restricted to a tract which courses anteriorly through the ventrolateral vestibular nucleus without branching before entering the contralateral medial longitudinal fasciculus (MLF). Within the MLF, the TN axons course posteriorly, form collaterals that innervate the abducens nucleus, and then proceed to C2 levels of the spinal cord. This pathway, taken by axons of the 2 main neurons in TN, principal and elongate cells, is found in 1 day, 1 week, and 7 week old animals. No labeled cells were found in either TN after HRP deposition into the uvula, flocculus, pontine reticular formation, nucleus piriformis, nucleus jumeaux, vestibulo-cerebellar nucleus, retrotangential nucleus, or the dorsomedial part of the medial vestibular nucleus (MVNdm).

The TN receives afferents from the colossal vestibular fibers (spoon endings), small collaterals of fine ampullary fibers, flocculus, and C2 level of the spinal cord. The latter input prevails in 1 day, 1 week, and 7 week old chickens.

The ganglion cells of each ampullary nerve occupy discrete portions of Scarpa's ganglion (VG). Ganglion cells of the posterior ampullary nerve (PAN) occupy the posterior VG, while cells of the anterior ampullary nerve (AAN) and the lateral ampullary nerve (LAN) occupy the anterior VG, dorsal and central parts, respectively. The central fibers of these ganglion cells occupy discrete portions of the TN before bifurcating into ascending and descending tracts. The PAN occupies the posterior half of TN, restricted to the dorsal part, while the AAN and LAN share the anterior half of TN, with the AAN dorsal and the LAN ventral. The ascending PAN is associated with the nucleus piriformis, while the ascending AAN and LAN each occupy discrete cell groups of the vestibulo-cerebellar nucleus. In the descending vestibular nucleus (DVN), the descending PAN runs dorsocentral, the AAN ventromedial, and the LAN ventrolateral until they converge at the posterior tip of DVN. The initial part of the ascending tract and the anterior 2/3 of the descending tract of each ampullary nerve form collaterals to the MVN, ventrolateral and dorsomedial parts. These collaterals form a distinctive tract that courses posteriorly within medial regions of the MVNdm. The colossal vestibular fibers, distributed in the 3 ampullary nerves, conform to the ampullary pathways as described. Supported by NIH grant R01 NS 18108.

179.6 INTRACELLULAR INJECTION OF HRP IN VESTIBULAR TYPE I NEURONS IN CAT. T. Ohgaki*, I.S. Curthoys and C.H. Markham. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Horizontal canal secondary Type I neurons in ketamine anesthetized cats were identified by their characteristic firing pattern in response to horizontal rotation and monosynaptic activation by stimulation of the vestibular nerve. Cell bodies or axons were penetrated with microelectrodes containing 6% HRP, and HRP was injected intracellularly for 100-200 nA minutes. After 10-24 hours, animals were perfused. The brain was cut in 100 μm horizontal or coronal sections. The sections were treated for HRP using the diaminobenzidine method, placed on slides and counterstained.

Fifty-three neurons were stained and each neuron was reconstructed in the horizontal plane and converted to the coronal plane or vice versa. Type I neurons were located in the rostral portion of the medial vestibular nucleus (MVN). The cell bodies were large and had rather elongated shapes and rich dendritic arborization. Some of these neurons had axon collaterals which projected to ipsilateral MVN near their own cell bodies.

The neurons were divided into two groups, those which projected to the contralateral abducens nucleus (excitatory Type I) and those which projected to the ipsilateral abducens nucleus (inhibitory Type I). Stem axons of excitatory Type I neurons crossed the midline and bifurcated into rostral and caudal branches in the contralateral MLF. Two or three tertiary collaterals arising close to the bifurcations distributed terminals in a relatively wide area in the contralateral abducens nucleus. Some of these collaterals projected to contralateral MVN. Some of the rostral and caudal stem axons had collaterals which projected to the contralateral nucleus prepositus hypoglossi (PH) or nucleus raphe pontis.

There were at least 4 classes of inhibitory Type I neurons, all of which distributed to a relatively limited region in the ipsilateral abducens nucleus. These had either (1) no further collaterals, (2) a branch entering and descending in the MLF, (3) a branch descending and terminating in ipsilateral PH, (4) a branch which ran rostrally, terminating ipsilaterally in the nucleus raphe and nucleus reticularis pontis caudalis areas containing pause neurons and premotor burst neurons.

The results show that the secondary Type I neurons, especially inhibitory Type I's, project not only to abducens nuclei but also to areas associated with the genesis of quick vestibular eye movements and saccades, thus confirming previous physiological studies.

179.7 MEMBRANE PROPERTIES AND POTASSIUM CONDUCTANCES IN ADULT PIGEON VESTIBULAR HAIR CELLS. M.J. Correia[#+], B.N. Christensen[+], and L.E. Moore[+]. Departments of [#]Otolaryngology and [+]Physiology & Biophysics, Univ. Texas Med. Br., Galveston, TX 77550

Membrane properties and K^+ conductances have been studied in 42 enzymatically dissociated vestibular hair cells from the adult pigeon. Twenty-four hair cells were from the anterior crista, 8 were from the horizontal crista, 7 were from the utricle, and 3 were from the lagena. All hair cells were studied using whole-cell patch clamp electrodes filled with either KCL-EGTA or K-Gluconate-EGTA solutions. The electrode impedance varied from 3-5 MΩ. The extracellular bath contained pigeon Ringers in the following concentrations (mM): Na - 152.0; Cl - 162.0; K - 3.5; Ca - 2.5; Mg - 1.0; Glucose - 17.0; and HEPES - 10.0. The mean steady state potential at which zero current was measured was -47 mV (SD = 14, n = 42). Fifty-seven percent of the cells had zero-current membrane potentials between -50 mV and -70 mV. Voltage clamp-I/V curves and voltage clamp admittance functions were calculated. The admittance functions were produced by measuring membrane current, during voltage clamp while applying small level (<5 mV, rms) sum of sines voltages (bandwidth - 1-500 Hz) superimposed on but following the transient phase of step clamp depolarizations and hyperpolarizations of various magnitudes. The admittance functions were fit with an equivalent circuit model for a range of membrane potentials between -56 mV and -86 mV for 16 cells. The mean (±SEM) membrane potential was -69 (±2) mV. The model assumed an isopotential soma described by the equation:

$$Y = j2\pi f C_m + G_m + G_1/(1 + j2\pi f r_1),$$

where C_m is the membrane capacitance; G_m is the frequency independent steady state conductance; G_1 is the frequency dependent conductance; r_1 is the relaxation time constant; f is frequency and $j = \sqrt{-1}$. Mean (±SEM, n = 25) best fit parameter values were: C_m = 1.4 (±0.2) pF/cm^2; G_m = 3.0 (±1.1) mS/cm^2; G_1 = -3.5 (±1.2) mS/cm^2; R_m = 3.0 (±1.5) kΩ-cm^2 and r_1 = 0.6 (±0.3) s. Based on the asymptotic estimate of the corner frequency of the admittance function, the mean (±SEM) membrane time constant was 4.6 ms (±0.7 ms, n = 25). The average (±SEM) input capacitance was 9.7 μF (±1.5 μF, n = 25). Hair cell surface area was calculated to be 438 μ^2 using measurements of dissociated cells' mean (±SEM) lengths (16.2 ±0.8 μ, n = 38) and widths (7.1 ±0.3 μ, n = 38) and the equation for the surface area of a cylinder. Assuming the cilia increase the surface area by 252 μ^2 (Ohmori, H., *J. Physiol.*, 359:189,1985), the mean specific capacitance would be 1.4 μF/cm^2 and the mean specific membrane resistance would be 3.3 kΩ-cm^2. The input impedance was measured from the I/V relation at potentials between -51 and -69 mV and the mean (±SEM) value was 2.9 GΩ (±0.4), n = 5. This work was supported in part by grants from ONR and NASA.

179.8 OTOLITH AFFERENT POLARIZATION VECTORS AND SENSITIVITIES IN THE ANESTHETIZED GERBIL. J.D. Dickman[#] and M.J. Correia[#+]. Departments of [#]Otolaryngology and [+]Physiology & Biophysics, Univ. Texas Med. Br., Galveston, TX 77550

Previously, the preferred axis of maximum sensitivity (polarization vector) for otolith afferents has been determined in primates (Fernandez & Goldberg, *J. Neurophysiol.*, 1976) and cats (Loe, et al., *J. Physiol.*, 1973). The present study addresses the sensitivity and polarization vector orientation for rodent otolith afferents in gerbils (*Meriones unguiculatus*). The animals were initially anesthetized with Nembutal (40 mg/kg), with supplementary injections of Ketamine (25 mg/kg) given as needed. The animal's head was stereotaxically positioned such that at zero degrees pitch and zero degrees roll, the horizontal semicircular canals were coplanar with the earth horizontal plane. Post-ganglionic single fiber afferent responses to small angle pitch and roll rotations (±15 and ±25 deg) about an earth horizontal axis were recorded using solution filled (3M NaCl) micropipettes. To date, responses from 21 isolated utricular and saccular afferents (recordings from both the left and right superior and inferior branches of the VIIIth nerve) have been examined. Sensitivity and polarization vector values were determined using the methods derived by Peterka (*IEEE Biol. Eng.*, 1981). The sensitivity of gerbil otolith afferents ranged widely, with a mean (±SEM) sensitivity of 42.13 (±6.03) spikes/sec/g. This sensitivity value is quite similar to that reported for primates (Fernandez & Goldberg, 1976) and cats (Loe, et al., 1973). The calculated mean (±SEM) resting discharge rate (response rate when the polarization vector is aligned perpendicular to gravity) was 51.73 (±7.36) spikes/sec, which is slightly lower than the discharge rate reported for the monkey. Like Peterka (1981), we adopted the following coordinate reference system: +z is directed out the top of the head; +y is directed out the back of the head; and +x is directed out the left ear. For the 21 units examined, the calculated polarization vectors generally aligned into two groups when projected on to the coronal head plane. Thirty three percent of the units had polarization vectors located between 0-30 deg above the ipsilateral ear. Another thirty eight percent of the units had polarization vectors located between 0 - ±30 deg off the z-axis. When projected on to the horizontal plane, the polarization vectors were also generally aligned into two groups. Thirty percent of the units had polarization vectors located between 0-60 deg away from the animal's -y-axis (nose) toward the ipsilateral ear. Another thirty percent of the units had polarization vectors located between 0-30 deg behind the ipsilateral ear toward the back of the animal's head (+y-axis). Although the current sample of examined units is small, it appears that rodent otolith afferent sensitivities and polarization vectors may be similar to those reported for primates and cats.

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179.9 DOES HISTAMINE HAVE A ROLE IN THE SEMICIRCULAR CANAL OF THE FROG? P.S. Guth,[1] C.H. Norris,[2] D.B. Quine*,[3] and G. Housley [1]. Depts. of [1]Pharmacology and [2]Otolaryngology, Tulane Univ. Med. Sch., New Orleans, LA 70112; [3]Wildlife Research, INHS, Champaign, IL 61820.

We have found that histamine has both a suppressive effect and a facilitatory effect on spontaneous and evoked afferent nerve activity in the isolated semicircular canal of the frog.

Beginning with concentrations as low as 20 micromolar, histamine has a facilitatory effect that increases up to concentrations of 100 to 200 micromolar. At higher concentrations the facilitatory effect wanes and a suppressive effect appears. At concentrations of 1 millimolar and greater, histamine is markedly suppressive. Pyrilamine and tripelennamine are H_1 blocking agents. Both suppress normal canal spontaneous and evoked activity at concentrations as low as 60 micromolar. Cimetidine, an H_2 blocking agent, is also suppressive. Its actions can be detected at concentrations as low as 20 micromolar.

The histidine decarboxylase inhibitor, α-fluoromethyl histidine, induces a dose-dependent reduction in spontaneous firing rate.

The cholinomimetic agent, carbachol, which in μM concentrations causes an increase in firing rate of the afferent nerve is felt to act presynaptically on the hair cell to cause it to release the endogenous afferent transmitter (Bernard et al., Brain Res. 338, 225-236, 1985; Guth et al., Acta otolaryngol. 102, 186-194, 1986). This facilitatory effect of carbachol is antagonized by μM concentrations of pyrilamine, implying that histamine is involved "downstream" of the carbachol receptor.

This evidence, taken together, seriously suggests a role for histamine in semicircular canal function and a new site of action for the antihistamines which are effective in vertigo and motion sickness.

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179.10 **KYNURENIC ACID AND 2-AMINO 5-PHOSPHONOVALERIC ACID BLOCK SPONTANEOUS ACTIVITY OF THE VESTIBULAR SYSTEM PRIMARY AFFERENTS** **Rosario Vega and Enrique Soto. Departamento de Ciencias Fisiológicas, ICUAP, Universidad Autónoma de Puebla, P.O.Box 406, Puebla, México.**

Excitatory amino acid agonists have been shown to produce a dose dependent increase in the activity of primary afferents of the vestibular system. This effect seems consistent with the idea of a *glutamatergic* transmission at the recepto-neural junction between hair cells and primary afferent fibers. In this report we present the results obtained with excitatory amino acidic antagonists (Kynurenic acid KY and 2-amino-phosphono-valeric acid APV). These drugs seem useful to distinguish between different kinds of amino acidic receptors (Watkins, Trends in Pharmacol. Sci. 5 (1984) 373; Coleman et al, Brain Res. 381 (1986) 172).

We studied the effects of KY and APV on the basal activity of the primary afferents from the sacculi and lagena of the axolotl *(Ambystoma mexicanum)* inner ear. Drugs were applied manually by micro perfusion in the vicinity of the synapses (20 μl in a 5 ml bath). This method of drug application produces a brief increase of drug concentration followed by quick dilution. APV and KY inhibit the basal activity of primary afferents in a dose dependent manner. KY exerts a more intense and long lasting inhibitory effect; it completely inhibits the basal activity when applied at 10 mM; APV inhibition is less intense and at the same concentration reaches a 60% inhibition of basal activity.

These results are consistent with the hypothesis of an amino acidic mediated transmission at the afferent synapse in the vestibular system. They also support the idea of a Kainic-Quisqualic type receptor sustaining the basal activity of the vestibular system primary afferents in the axolotl.

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179.11 RESPONSE DYNAMICS OF CANALICULAR AFFERENT NEURONS IN THE BULLFROG.

L. Hoffman* and V. Honrubia, Div. of Head and Neck Surgery UCLA School of Medicine; Los Angeles, California 90024.

Activity from individual neurons innervating the horizontal and anterior cristae was recorded from bullfrogs to describe their response dynamics to a series of sinusoidal stimuli. Following surgical exposure of the vestibular nerve, glass microelectrodes filled with 0.2 M KCl were used to penetrate the anterior branch in an area between Scarpa's ganglion and the anastomosis with the posterior branch. Neural spike trains representing the cell's spontaneous activity and responses to sinusoidal stimuli were analyzed on-line to determine the spontaneous firing rate coefficient of variation (CV), and gain and phase to the stimulus sinusoids. Units were identified as innervating the horizontal or anterior cristae based upon the direction of yaw rotation which produced excitatory and inhibitory modulation of spontaneous activity. Transfer functions quantifying each unit's response gain and phase across the stimulus bandwidth (usually 0.00625 to 3.2 Hz) were determined with methods that have been used to describe the response dynamics of canal afferents of the cat (Tomko et. al., J. Neurophys. 45:376, 1981). The elements used to derive the best-fit transfer functions included zeroes and poles, the adaptation operator, and s^K (fractional exponent of s).

We found that the transfer functions describing the response dynamics of the bullfrog's canalicular afferents assumed one of three basic forms. A second order equation, which included a gain term and two poles, provided the best fit for cells which had values of CV less than 0.5. These have been found to correspond to the smallest and most numerous afferents innervating the semicircular canals in the bullfrog (Honrubia et. al., Laryngoscope 95:1526, 1985). Most of the remaining units exhibited response properties which required additional terms to fit the data. These properties included phase leads (re: acceleration) at the low frequency stimuli. The responses of some cells to high frequency stimuli had phases that asymptotically approached -90° (re: acceleration), and were best fit by transfer functions that included an adaptation operator. However, the high frequency response phase from the majority of cells exhibiting low frequency phase leads approached asymptotes less than -90°. These data required the implementation of s^K which provided the phase shift necessary to fit this response characteristic.

This work was supported by grants from the NIH (NS08335 and NS09823), the Pauley Foundation, and the Hope for Hearing Research Foundation.

179PO CAT VESTIBULAR UNITS RESPONDING TO VIBRATION. James H. Fuller Dept. Oral Anatomy, Univ. Illinois, Chicago, IL 60612

Single units were recorded in the vestibular nuclei in cats trained to make accurate eye and head movements to light targets. Seven categories of units were designated based on firing rates associated with head movements (Categories 1-4) or eye movements (5-7). Initial tests were passive whole body rotation (vestibular input), head fixed in space rotation (neck proprioceptor input), and ocular saccades between targets. Vestibular stimulation resulted in related modulation in Category (Ctg) 1,2,3, and 7 units. Neck sensory modulation was weak in Ctg 1, strong in 2 and 3, absent in Ctg 7 (units in Ctg 4-6 will not be considered). Only Ctg 7 units showed activity related to eye movements (burst and/or tonic).

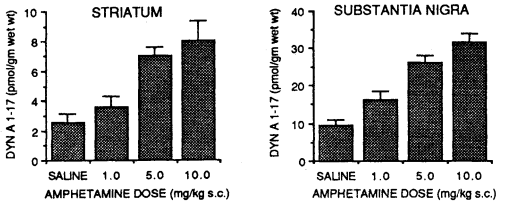
In another procedure the head was braked for 50-200 msec during identical repeated voluntary head movements between targets. The temporal events of head braking were: 1) 0.0 msec, solenoid coil activation; 2) 3.0-4.0 msec, brake plate contact; 3) 6.0-7.0 msec, brake engagement; 4) 10-15 msec, clear head deceleration. Ctg 1 and 2 units had a discernible leading and trailing edge with the onset and offset of the brake. The response was less stereotypical in Ctg 3 units with a series of two or more aggregates of spikes during the engagement of the brake. Characteristic of Ctg 1, 3, and 7 units was a single early spike, latency of ca. 1.5 msec, jitter of ca. 60 microsec. The early spike typically had the shortest latency, least jitter, and most reliable response when the brake occurred in "matched" directions. For Ctg 1 and 3 units, the matched direction was the same as vestibular sensitivity, whereas for Ctg 7, it was the same as the eye movement sensitivity (which was opposite vestibular sensitivity).

A likely stimulus to account for the early spike was the surge of vibration along the head holder shaft prior to actual braking. This stimulus was most effective during active head movements (ca. 80% in matched, and 25% in unmatched); brake activation with a stationary head was less effective. Other forms of vibration were examined in Ctg 3 units; e.g., mechanical tapping of the shaft produced similar responses, although of longer latency (3-4 msec). Other evidence suggests these responses originate from paciniform receptors located in the cervical perivertebral area. The early spike may reflect the direct pathway shown by Hikosaka and Maeda (Exp. Brain Res., 1973) between cervical receptors and the vestibular nuclei, and may be the origin the neck-eye or cervico-ocular response (COR). It is most prevalent in units having no apparent association with eye movement; the COR is likely not essentially related to the eye.

180.1 INCREASES IN STRIATONIGRAL DYNORPHINS FOLLOWING REPEATED AMPHETAMINE INJECTIONS. K.A. Trujillo, R. Day and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109.

Prodynorphin neurons in the striatum project heavily to the substantia nigra pars reticulata. Anatomical, biochemical and behavioral evidence suggests that this descending pathway may modulate, and be modulated by, ascending nigrostriatal dopamine neurons. In the present study we administered repeated amphetamine injections to rats in order to study the influence of nigrostriatal dopamine on striatonigral prodynorphin neurons.

Adult male Sprague-Dawley rats were injected with amphetamine sulfate (1.0, 5.0 or 10.0 mg/kg s.c.) once daily for seven days, and sacrificed 1 hour or 24 hours following the final injection. The striatum and substantia nigra were removed and assayed for four prodynorphin peptide products: dynorphin A 1-8, dynorphin A 1-17, dynorphin B 1-13 and alpha neo-endorphin. In animals sacrificed 24 hours following the final injection there was a dramatic, dose-dependent increase in both the striatum and the substantia nigra for all four prodynorphin products, suggesting that repeated amphetamine administration causes an increase in synthesis and/or decrease in release of these peptides from striatonigral prodynorphin neurons. The increase in peptide s levels was much less pronounced in animals sacrificed 1 hour following the final injection, suggesting that acutely, amphetamine causes release of stored peptides. These results lead us to suggest that amphetamine-dependent release of s dynorphins results in an increased synthesis of prodynorphin peptide products in the striatonigral pathway. These results are consistent with the suggestion that striatonigral prodynorphin neurons are modulated by dopamine.



180.2 EFFECTS OF CHRONIC MORPHINE TREATMENT ON BETA-ENDORPHIN IMMUNOREACTIVE FORMS IN RAT BRAIN. D. M. Bronstein and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

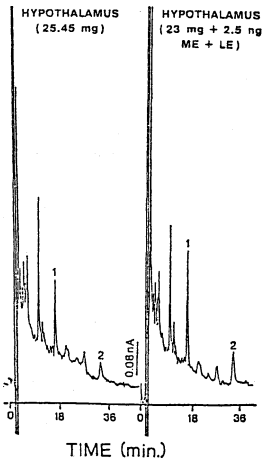
Even prior to the discovery of the enkephalins, it was postulated that morphine tolerance/dependence might be the result of a decrease in endogenous opiate activity due to negative feedback effects of exogenously administered opiates. It has since been shown by a number of investigators that 1-2 weeks of morphine administration causes no change in the concentration of Beta-endorphin immunorectivity (BE-ir) in the pituitary or in various brain regions. However, BE-ir measured in these studies did not distinguish between different immmunoreactive BE forms. In the brain, the majority of BE-ir consists of BE(1-31) and to a lesser extent, the carboxy terminal cleaved BE(1-27), which has been suggested to act as an endogenous antagonist to BE(1-31). Although no changes are observed in total BE-ir in morphine tolerant animals, it is hypothetically possible that there is increased processing of BE(1-31) to BE(1-27), (i.e. brains from morphine treated animals might contain a greater ratio of "antagonist" BE relative to opiate active BE). The purpose of the present study was to determine whether there were differences in the concentrations of BE(1-31) and BE(1-27) in the brains of control, morphine tolerant , or morphine withdrawn animals.

Male Sprague-Dawley rats were implanted with 2 morphine (75 mg/pellet) or placebo pellets on Day 1 and an additional 3 pellets on Day 4. In the morning of Day 7, animals were injected with 2 mg/kg of naloxone (to induce precipitated withdrawal in morphine tolerant animals) or saline and killed 30 min later. The results of radioimmunoassays showed, as expected, that there were no changes in the concentrations of total BE-ir in the hypothalamus, midbrain (containing the periaqueductal grey region), or caudal medulla (including the nucleus tractus solitarius) as a result of any of the experimental manipulations. Samples from the 4 groups were then applied to Sephadex G-50 chromatography columns and developed with a 1% formic acid solvent containing .01% bovine serum albumin. The collected fractions (1.3 ml) were assayed for BE-ir. There were no apparent differences between the 4 treatment groups in the amounts of BE(1-31) and BE(1-27) detected in any of the brain regions examined. The results of this study indicate that chronic morphine treatment does not appear to alter the processed forms or the content of BE stored in the brain. However, these results do not preclude the possibility that the rate of POMC processing or BE(1-31) turnover may be affected by morphine administration.

180.3 Assay of Enkephalins from Rat Brain By Liquid Chromatography - Electrochemical Detection, L. H. Fleming* and N. C. Reynolds, Jr., Dept. of Neurology, University of Wisconsin Medical School (Milwaukee Clinical Campus), Milwaukee, WI 53201

Several groups have used electrochemistry (ED) as the basis of detection for brain extracts of neuropeptides separated by liquid chromatography (LC). (Sauter, A. and W. Frick, J. Chromatogr. 297:215, 1984, Mousa, S. A. and G. R. Van Loon, Life Sci. 37:1795, 1985 and Dawson, R., Jr., J. P. Steves, J. F. Lorden, and S. Oparil, Peptides 6:1173, 1985). In these reports, alternative analytical methods were not used to verify peak identity or to evaluate quantitative data.

Our current work has focused on the development of an LC-ED assay for enkephalins from rat brain extracts. Most of the difficulties in devising a workable protocol were in three areas: 1) Elimination of early and late eluting electroactive contaminants from tissue, 2) discovering the origin of other electroactive interfering substances and eliminating them and 3) maintaining an adequate recovery of endorphins to allow detection with the present ED technology. The chromatogram of hypothalamus shown below was obtained using a "clean-up" protocol designed to maximize recovery (78%) while eliminating interfering substances. To evaluate peak identity and purity, several analytical techniques were used, including peak current ratios and radioimmunoassay. This LC-ED technique allows for the quantification of both methionine- and leucine-enkephalin in a single assay performed on extracts from rat brain regions.



1 = Methionine enkephalin
2 = Leucine enkephalin

180.4 ISOLATION OF HUMAN SYNENKEPHALIN FROM PHAEOCHROMOCYTOMA TISSUE. R. Corder*, R.C. Gaillard* and J. Rossier. (SPON: Philippe Magistretti). Dept. of Medicine, University Hospital, 1211 Geneva 4, Switzerland, and Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif-sur-Yvette, France.

The amino-terminal region of the enkephalin-peptide precursor, pro-enkephalin, is composed of a non-opioid polypeptide of unknown function referred to as synenkephalin (SYNENK). However, antisera raised in rabbits against SYNENK purified from bovine adrenal medullary chromaffin granules are highly species-specific and unsuitable for studies of the functional significance of either human or rat SYNENK. Using a radioimmunoassay which employs an antiserum raised against the synthetic tyrosinylated carboxy-terminal sequence of SYNENK (YEESHLLA) and the same peptide as standard and tracer, preliminary investigations have shown human adrenal medullary phaeochromocytoma tissue to be a rich source of SYNENK-like material. The identified immunoreactivity possessed gel filtration and ion exchange characteristics consistent with the weakly acidic 8KDa polypeptide predicted from cDNA studies. Thus we elected to purify human SYNENK using these tumours as a tissue source.

Sections (total wt. 70g) from three adrenal medullary tumours, identified histologically as phaeochromocytoma, were extracted in 15 volumes 0.1M HCl. The resultant extract was fractionated using $(NH_4)_2SO_4$ to produce a SYNENK enriched precipitate at 50% saturation. SYNENK was then purified by employing sequentially the following chromatographic procedures: gel filtration (Sephadex G50, 2.6 x 96cm), anion exchange HPLC (Protein Pak DEAE 5PW, 7.5 x 75mm), reverse phase HPLC (Nova-Pak Phenyl, 3.9 x 150mm) and cation exchange HPLC (UltroPac TSK CM-3SW, 7.5 x 150mm). The carboxy-terminal SYNENK RIA was used throughout to identify the SYNENK peptide. A single peak of immunoreactivity was obtained from each chromatographic step, with cation exchange HPLC yielding a homogeneous peak (25nmoles) suitable for chemical characterization. The NH_2-terminal sequence was determined using 0.2nmol of this peptide, using an Applied Biosystems 470-A sequencer. This identified the NH_2-terminal Glu residue and confirmed the sequence predicted by cDNA studies as far as the 27th residue.

Thus, using an antiserum specific for the 67-73 sequence of human SYNENK, we have isolated and chemically characterized the authentic human peptide. The raising of specific antisera against this peptide should allow the development of an immunoassay for pathophysiological studies of SYNENK in man.

180.5 CHARACTERIZATION OF METHODOLOGY FOR CONCURRENT MEASUREMENT OF SMALL PROENKEPHALIN FRAGMENTS IN CSF, TISSUE AND BLOOD. D.L. Lucas* and T.L. Yaksh* (SPON: J.R. Daube). GI Hormone Research Lab. and Lab. of Neurosurgical Research, Mayo Clinic, Rochester, MN 55905.

Processing of the proenkephalin molecule will result in several short forms of the form: ( )-YGGFM-( ). We have characterized a separative-radioimmunoassay (RIA) methodology which permits concurrent measurement of several enkephalin derived peptides in a single sample. Samples (tissue extracts, ventricular or spinal superfusates, EDTA plasma) are passed over a C_{18} disposable column (Sep-pak). Hydrophilic components are eluted with distilled H_2O (5 ml) and discarded. Hydrophobic elements are eluted with 50% acetonitrile. The sample is lyophilized, reconstituted in .5 ml of borate buffer and passed over a YM-2 filter (Amicon, 14 mm, nominal cutoff of 2000 DA) using a centrifugation holder. A second .5 ml of buffer is passed through the filter and combined. The filter is then inverted and washed by centrifugation with .5 ml buffer to measure the levels of encrypted enkephalin in >2000 DA fragments in the retentate. The filtrate is lyophilized, reconstituted in buffer (.5 ml) and an aliquot placed on an LKB HPLC (Rainin short one C_{18} column). The column is eluted at a rate of .5 ml/min with a gradient of 10-60% acetonitrile in .1 M acetate buffer (pH = 4). Each 1-min HPLC sample is incubated with trypsin (Try: Sigma 25 µg for 1 hr at 37°C) followed by carboxypeptidase B (CPB: Sigma .05 mg for 1 hr at 37°C). The reaction is stopped by boiling for 10 min. Met-enkephalin (ME) RIAs are carried out (antibody N-382: carboxy terminus directed; sensitivity 9 fmole/tube: interassay reliability: 8%). Table 1 indicates the recovery of the molecular forms of standard containing encrypted enkephalin. The HPLC elution studies revealed reliable separation of the several forms. Examination of spinal perfusate samples has indicated that during the addition of substance P to the spinal perfusate, the ratio of ME to total encrypted ME activity rises from 1.7±0.4 to 7.6±0.9. (NIH grant NS-16541 to TLY.)

Table 1

Molecule	MW	Concentration (pmol/ml)	1	2	4	5
YGGFM	574	5	100	101	71	-
YGGFM	574	35	100	107	84	-
YGGFMR	730	5	41	108	132	-
YGGFMRF	877	5	35	96	73	-
YGGFMRGL	900	5	9	88	79	-
Peptide B	3657	5	2	86	6	56

1: % cross reactivity with ME RIA; 2: % conversion of molecule to moles of ME by trypsin + CPB; 3: % recovery through Sep-pak; 4: recovery of ME equivalents in YM-2 filtrate; 5: recovery of ME equivalents in YM-2 retentate.

180.6 CHARACTERIZATION OF OPIOID RECEPTOR SUBTYPES IN THE BRAINSTEM WITH RESPECT TO ANALGESIA. N. Al-Rodhan* and T.L. Yaksh* (SPON: B.F. Westmoreland). Department of Neurologic Surgery, Mayo Clinic, Rochester, MN 55905.

There is increasing pharmacological and biochemical evidence to suggest that opiates exert their effects through heterogenous receptor populations (µ, δ, κ, σ, ε). Subtypes of these receptors have been postulated to exert specific effects at different sites in the central nervous system. This study is part of an ongoing series of investigations to characterize the distribution of opioid receptor subtypes in the brainstem of the rat and their role in analgesia. In the present studies, focussing on the periaqueductal gray (PAG) region, we have systematically examined the dose dependency and the relative activity of several µ and δ receptor preferring ligands following their intracerebral administration through chronically implanted guides placed stereotaxically into the PAG. All injections were given in a volume of 0.5 µl saline. Antinociception was measured using: 1) the hot plate test (HP; 52.5°C; baseline latency = 10.8±2.6 sec; 60 sec cutoff), and 2) the tail flick test (TF; baseline latency = 2.9±0.6 sec; 6 sec cutoff). Each rat received no more than 3 injections, at least one of which was with a "probe dose" of drug known to be effective. In this series of experiments, the ability of naloxone (1 mg/kg, i.p.) to reverse the effects of the PAG microinjection was assessed using the lowest dose of opiate producing a just maximal effect. As indicated in the table, the ordering of agonist potency on the HP and TF was: SUF > PL017 = MOC = DAGO = DSTLE >> DPDPE. Slopes for the dose response curve of SUF, PL017, MOC, DAGO and DSTLE did not differ. The degree of reversal produced by the high dose of naloxone was similar for all agonists (e.g. 65 to 85% reversal).

Conclusions: (1) These studies in the rat suggest that the opioid receptor in the PAG which modulates thermal antinociception displays a structure-activity relationship suggesting the primary characteristics of a "µ" receptor. (2) Failure of DPDPE to act over a broad range of concentrations suggests the absence of δ receptors in the PAG modulation of thermal nociception and suggests that DSTLE may mediate antinociception by an effect on a µ-receptor. (Supported by NIH grant NS-16541, TLY.)

Specific Ligands		HP (nmol)		TF (nmol)	
Receptor	Drug	ED_{50} (±95% CI)	Slope (±95% CI)	ED_{50} (±95% CI)	Slope (±95% CI)
µ	SUF	0.04 (0.007 - 0.25)	26 (-18 - 70)	0.06 (0.03 - 0.11)	37 (14 - 59)
	PL017	1.2 (0.8 - 1.6)	39 (16 - 62)	2.1 (1.3 - 3.3)	41 (19 - 63)
	MOC	1.2 (0.6 - 2.1)	28 (15 - 41)	2.2 (1.4 - 3.5)	32 (17 - 46)
	DAGO	3.3 (0.09 - 120)	13 (-15 - 42)	0.9 (0.3 - 2.7)	19 (-0.02 - 38)
δ	DSTLE	1.0 (0.9 - 1.2)	39 (33 - 46)	1.2 (0.8 - 2.0)	36 (15 - 57)
	DPDPE	> 7.7	—	> 7.7	—

180.7 HETEROGENEITY OF KAPPA OPIOID RECEPTORS ACROSS SPECIES. A. Mansour, H. Akil, W. Essman*, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

There is a wealth of evidence to suggest the existence of species-differences in opioid receptors. Among the more striking differences have been those described for kappa receptors. While pursuing these findings we have been able to show not only a differential localization of kappa receptors across species, but also a heterogeneity of kappa sites. Several lines of evidence support this conclusion.

I. Selective kappa agonist, such as U50,488H, have differential affinities for kappa sites labelled with [^{3}H]bremazocine across species, while non-selective compounds such as bremazocine and EKC do not. Brains of monkey, rat, pigeon and guinea pig were frozen in isopentane (-30°C) and forebrain sections were thaw-mounted on microscope slides. The brain sections were incubated with [^{3}H]bremazocine (0.5-0.6 nM) in a 50 mM Tris HCl (pH = 7.4 at 25°C) buffer (in the presence of a 300 fold excess of DAGO and DPDPE) and varying concentrations of either U50,488H, Cambridge 20, U69,593, PCP, ethylketazocine (EKC), cyclazocine, tifuladom, nalorphine, or bremazocine. Following a 60 min incubation, the sections were washed in four 4 min Tris (pH = 7.5, 4°C) washes, dried and quantified with a beta counter. Representative Ki values for U50 and EKC (nM) are presented below.

	Monkey	Rat	Pigeon	Guinea Pig
U50, 488H	3.5	140.9	165.1	7.1
Ethylkectazocine	1.3	1.1	5.9	1.8

II. U50,488H competition studies with varying concentrations of [^{3}H]bremazocine (0.06-6.0 nM) in the presence of mu and delta blockers, resulted in parallel curves in some species (e.g. guinea pigs) and non-parallel, multiple site curves in others (e.g. rat).

III. Direct binding experiments with the selective kappa agonist U69,593 suggest that it binds to two sites. Brain sections were incubated for 2 hrs with varying concentrations (0.15 - 20.0 nM) [^{3}H]U69,593 in a 50 mM Tris buffer (pH = 7.4, 25°C), followed by four 1 min Tris (pH = 7.5, 4°C) washes. Non-specific binding was defined by 1 uM bremazocine and quantification of binding was as described above. In monkey cortical sections, for example, scatchard analysis suggests that U69 binds to two sites with Kds of 0.4 and 14.5 nM as determined by the LIGAND program. Similarly, in the guinea pig forebrain two sites can be resolved with Kds of 0.6 and 44 nM, respectively. While further work is needed to pharmacologically characterize each of these binding sites, these results and those presented above support the notion of a heterogeneity of kappa opioid receptors with possibly distinct functions.

180.8 EVIDENCE FOR KAPPA OPIATE RECEPTOR HETEROGENEITY BETWEEN RAT AND GUINEA-PIG BRAIN. K. Reinertsen*, W. Bowen, and J.M. Walker. Sect. of Biochem., Div. of Biology and Medicine and Dept. of Psychology, Brown Univ., Providence, RI 02912.

We have investigated binding sites labeled by [^{3}H]-(-)-bremazocine ([^{3}H]-(-)-BREM) and [^{3}H]-(-)-ethylketocyclazocine ([^{3}H]-(-)-EKC) in rat and guinea-pig brain membranes. We present evidence for two subclasses of kappa receptor based on the following criteria: a) affinity for the kappa-selective ligand U50-488H and b) sensitivity to a low temperature/high salt incubation condition.

Assays were carried out with 2 nM ^{3}H-ligand for 60 or 90 min at 25°C in 10 or 50 mM Tris-HCl (pH 7.4) containing 100 nM DAGO and 100 nM DSTLE to mask mu and delta sites, respectively. Nonspecific binding was determined with 10 uM levallorphan. The ability of U50-488H and dynorphin analogs to displace 2 nM [^{3}H]-(-)-BREM binding to membranes from rat brain (RB) and guinea-pig brain (GPB) was assessed. U50-488H was 8-fold more potent in GPB than in RB. By contrast, the dynorphin analogs D-Ala^2-dynorphin-1-13-NH$_2$ and D-Ala^2,F$_5$Phe^1-dynorphin-1-13-NH$_2$ were nearly equally potent in the two tissues. Thus, there appear to be two types of [^{3}H]-(-)-BREM binding sites which differ in their affinity for U50-488H. Guinea-pig brain contains a preponderance of [^{3}H]-(-)-BREM binding sites with high affinity for U50-488H, while rat brain contains a preponderance of sites with lower affinity for U50-488H. Dynorphins bind with nearly equal affinity to both sites, indicating that they are kappa receptors.

Binding of 2 nM [^{3}H]-(-)-BREM and [^{3}H]-(-)-EKC to membranes from rat brain, guinea pig brain, and guinea pig cerebellum (GPC) under the above incubation condition was compared to binding in incubations carried out in presence of 50 mM K-phosphate (pH 7.4) with 400 mM NaCl at 4°C for 4 hrs. For both ligands, the low temp/high salt condition produced only a moderate (30 - 40%) enhancement of binding in the guinea-pig tissues. However, the low temp/high salt condition produced a marked (140 - 155%) enhancement of binding in the rat tissue. Thus, rat brain contains binding sites for [^{3}H]-(-)-BREM and [^{3}H]-(-)-EKC whose ability to bind ligand is markedly enhanced by incubation at low temperature in presence of high salt. This site is largely absent in guinea-pig brain. Ligand selectivity analysis of this site is in progress to determine its relationship to kappa receptors.

These results support the notion of heterogeneity within the kappa opiate receptor class. Rat brain is reported to contain fewer kappa receptors than guinea-pig brain. This difference may in fact reflect species variation in kappa receptor subtypes and differences in optimal assay conditions for these receptors.

180.9 DEVELOPMENT OF COMPOUNDS SELECTIVE FOR KAPPA-OPIATE RECEPTORS. L. Toll, J.A. Lawson* and G.H. Loew*. Division of Life Sciences, SRI International, Menlo Park, CA 94025.

Structure activity studies, receptor characterization and receptor purification have proceeded at a much slower rate for kappa-opioid receptors than for mu and delta receptors, partly for the lack of suitable ligands selective for this site. This problem has been partially alleviated by the discovery of the benzodiazepine opiate tifluadom and, more importantly, the benzamides U-50,488 and U-69,593, developed by VonVoigtlander and colleagues. The lengthy synthesis and the chiral nature of the Upjohn compounds make extensive SAR studies time-consuming and expensive. The chirality of the compounds necessitates difficult resolutions to determine absolute structural requirements for binding to the kappa receptor. We have recently developed a new class of compounds which are structurally related to U-50,488, but which are readily synthesized and which are achiral. In this class of compounds, small structural changes modulate affinities and selectivities so that high affinity compounds with significant selectivities for the kappa or mu receptor are obtainable. In addition to the benefits for structure activity studies, several positions are available for the insertion of a moiety which could bind irreversibly to the receptor and may be suitable for affinity labeling of the kappa or mu receptor. Data will be presented on binding affinity and selectivity of these achiral aminoamide compounds, and on the usefulness of these compounds as potential affinity probes for kappa and mu receptors.

180.10 LOSS OF MU OPIOID RECEPTORS IN THE NUCLEUS ACCUMBENS FOLLOWING MESOLIMBIC DOPAMINERGIC LESIONS. E.M. Unterwald, G.F. Koob*, and R.S. Zukin. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461 and *Res. Inst. Scripps Clinic, La Jolla, CA 92037.

The modulation of dopaminergic function by opioids in the mesolimbic system is well documented. Opioid receptors and peptides have been found in relatively high concentrations in areas of the limbic system that are extensively innervated with dopaminergic neurons. The present study investigates the nature of the opioidergic-dopaminergic interaction in the nucleus accumbens. The organization of the different opioid receptor types with respect to their pre- and postsynaptic localization and their relationship to dopaminergic neurons is determined.

Dopaminergic terminals in the nucleus accumbens of rats were destroyed by unilateral injection of 6-hydroxydopamine (2 µl; 4 µg/µl). Separate groups of animals received unilateral injections of ibotenic acid (0.5 µl; 10 µg/µl) in order to lesion postsynaptic elements and cell bodies. Fourteen days after the lesions, the fraction of each opioid receptor type lost was determined by binding studies on nucleus accumbens membranes. The highly specific ligands used were [^{3}H]-D-Ala^2, N-Phe^4, Gly-ol^5-enkephalin (DAGO) for mu receptor assays, [^{3}H]-D-Penicillamine^2, D-Penicillamine^5-enkephalin (DPDPE) for delta receptor assays, and [^{3}H]-bremazocine in the presence of DAGO (100 nM) and D-Ala^2,D-Leu^5-enkephalin (DADLE) (100 nM) for kappa receptor assays. Quantitative in vitro autoradiography at the level of the light microscope was used to visualize the neuroanatomical pattern of the receptors on the lesioned and non-lesioned sides of the brain. The same specific ligands used in the receptor binding assays were used in the autoradiographic studies to identify each receptor type.

Results indicate that 6-hydroxydopamine lesions produced a 30% loss of mu opioid receptors in the nucleus accumbens. No change in mu receptor affinity was observed. Delta and kappa receptors remained unchanged. The time course of the loss of mu receptors paralleled the time course of the depletion of dopamine. These results suggest the absence of delta and kappa opioid receptors on presynaptic dopaminergic terminals in the nucleus accumbens. Approximately one-third of mu opioid receptors in the accumbens appear to be located on either the presynaptic dopaminergic terminals or on the neurons upon which they impinge. Preliminary results with ibotenic acid are consistent with the former hypothesis.

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180.11 DIRECT AUTORADIOGRAPHIC LOCALIZATION OF MU$_1$ BINDING SITES IN RAT BRAIN WITH A NOVEL OPIATE LIGAND. R.R. Goodman, M. Price* and G.W. Pasternak, The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College, New York, NY 10021.

A new naloxone derivative, NalBzoH (6-desoxy-6-benzoyl-hydrazido-N-allyl-14-hydroxydihydronormorphinone), has been characterized in our laboratory. Extensive binding studies demonstrate that the ligand labels mu$_1$ sites pseudoirreversibly. Binding to other classes of opioid sites is freely reversible. Using conditions to selectively label mu$_1$ sites, we directly examined the autoradiographic labeling of mu$_1$ receptors for the first time. Non-fixed thin rat brain sections (8 µm) were incubated with ^{3}H-NalBzoH (57.4 Ci/mmole) at 25°C for 60 min after which levallorphan (1µM) was added to some sections and the incubation continued for an additional 120 min to dissociate reversibly bound ligand, leaving mu$_1$ binding. Total binding was determined in sections incubated at 25°C for 60 min without the addition of levallorphan and nonspecific binding with sections preincubated with levallorphan (1µM) prior to the addition of the radioligand. The regional distribution of binding of the pseudoirreversible binding was very similar to mu$_1$ binding determined in rat brain using digital subtraction autoradiographic techniques or in the mouse using subtraction of grain counts. Initial studies demonstrate high concentrations of mu$_1$ binding in layers I and IV of the cerebral cortex, clusters in the striatum, the nucleus accumbens, medial and intralaminar thalamic nuclei, pyramidal cell layer of the hippocampus, superficial layer of the superior colliculus, medial geniculate body, mamillary body, and medial habenula. Moderate concentrations were noted in the periaqueductal gray, consistent with the suggestion of mu$_1$ mechanisms of morphine analgesia in this region. Interestingly, the highest concentration of mu$_1$ binding sites seen to date appear to correspond to the accessory nucleus of the optic tract. More detailed studies are currently in progress to determine the pre- or postsynaptic localization of mu$_1$ sites.

180.12 A NOVEL OPIATE ANALOG WITH VERY HIGH AFFINITY FOR MU$_1$ BINDING SITES. M. Price*, M. Luke*, E.F. Hahn* and G.W. Pasternak, [SPON: K.M. Foley]. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College and Rockefeller University, New York, NY 10021.

Over the past few years, our laboratory has synthesized and characterized a series of azines, hydrazones and acylhydrazones, such as naloxonazine. One goal of these studies was to develop a compound which could be used to selectively label mu$_1$ receptors. Attempts to use radiolabeled naloxonazine were only partially successful. Although binding could be demonstrated, the very high nonspecific binding prevented detailed studies. Similar difficulties were encountered with a series of other compounds. Recently, we synthesized a novel naloxone derivative, ^{3}H-NalBzoH (6-desoxy-6-benzoylhydrazido-N-allyl-14-hydroxydihydronor-morphinone), with high specific activity. In binding assays using bovine striatum, the ratio of total to nonspecific binding is approximately 10:1. The ligand labels two types of binding sites. Approximately 60% of binding is freely reversible and dissociates in the presence of a high concentration of levallorphan (1µM) at 25°C in under 2 hours. The remainder of the binding is resistant to dissociation and persists for up to 6 hours with no noticeable loss of specific binding. By 24 hours approximately 30% of this dissociation-resistant binding still remains. Competition studies indicate that the freely reversible binding is selective for morphine as opposed to enkephalins and therefore probably corresponds to mu$_2$ sites while the dissociation-resistant binding is sensitive to both morphine and the enkephalins and probably represents mu$_1$ binding. Although the mu$_1$ binding of this ligand is highly resistant to dissociation, it is not covalent. Thus, ^{3}H-NalBzoH is a novel opiate derivative which binds mu$_1$ sites pseudoirreversibly. In certain respects, it is similar to α-bungarotoxin which also labels its receptor tightly, but not covalently. Although covalent bonding would permit studies of the binding site under denaturating conditions, ^{3}H-NalBzoH still offers a number of advantages in the study and biochemical characterization of mu$_1$ opiate receptors.

180.13 A CHEMICAL MECHANISM FOR WASH-RESISTANT BINDING OF OPIATE AZINES TO OPIOID RECEPTORS. A. Garzon-Aburbeh*, A.W. Lipkowski*, and P.S. Portoghese. Dept. of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455.

Opiate azines such as naltrexonazine (NTX=N-N=NTX) have been reported to possess a wash-resistant binding component at opioid receptors. However, the chemical nature of such binding has not been reported. In view of the presence of thiols at opioid receptors, and our observation that such groups catalyze the hydrolysis of the C=N bond, we have investigated the possibility that NTX=N-N=NTX is converted to NTX=N-NH_2 which would then react with a membrane component. We have synthesized an unsymmetrical analogue of NTX=N-N=NTX where one of the hydrazone double bonds is reduced (NTX-NH-N=NTX). In this regard, two tritiated analogs (*NTX-NH-N= NTX and NTX-NH-N=NTX*) were prepared with the label at either part of the unsymmetrical molecule for binding experiments to mouse membranes. Only with *NTX-NH-N=NTX (5 nM) was the radioactivity retained (42% specific binding) in the membrane after three washes. This suggested that only the portion of the molecule carrying the hydrazine moiety accounts for the wash-resistant binding. After five washes of the membranes incubated with *NTX-NH-NT=NTX a new tritiated compound with high R_f (0.8) was detected in the washes whose R_f corresponds to a synthetic linolenoyl hydrazide of naltrexone. Moreover, after 5 washes, no wash resistance could be demonstrated with NTX=N-N=NTX. Reconstitution of the previously washed membranes with the wash components restored the wash-resistant binding, suggesting that some essential component was washed out during the washing procedure. This component is thought to be a phosphatide based on the fact that treatment of the washes with phospholipase A_2, followed by denaturation of the enzyme and reconstitution with the washed membranes, failed to show any wash resistance with NTX=N-N=NTX. Furthermore, adding phosphatidic acid, phosphatidyl serine, or phosphatidyl choline to the previously washed membranes restored the wash-resistant binding characteristic. On the basis of these experiments, the mechanism proposed involves the reaction of NTX-NH-NH_2 liberated in situ with the C-2 ester carbonyl group of a phosphatide to afford a fatty acid hydrazide of naltrexone. This mechanism is consistent with the fact that cerebroside sulfate, which does not contain an ester group, is unable to restore the wash-resistant binding of the washed membranes. The wash resistance of opiate azine and related ligands therefore can be viewed as the receptor-mediated formation of fatty acid hydrazide derivative of the opiate. Its lipophilic chain may give rise to increased affinity (wash resistance) due to hydrophobic interaction with a portion of the opioid receptor that normally accommodates the receptor-associated phosphatide.

180.14 **IN VIVO ACYLATION OF THE LOWER AFFINITY [^{3}H]DADL BINDING SITE BY THE ENANTIOMER OF SUPERFIT AND ITS PROTECTION BY THE DELTA ANTAGONIST ICI174864: EVIDENCE THAT ICI174864 INTERACTS IN VIVO WITH THE LOWER AFFINITY [^{3}H]DADL BINDING SITE.** K. C. RICE[1], A. E. JACOBSON[1]*, J. B. LONG[2], V. BYKOV[3]* AND R. B. ROTHMAN[3].
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[^{3}H]DADL labels two sites in vitro which are distinguished by interactions with mu ligands. The lower affinity mu-noncompetitive binding site, commonly identified as mu, is the delta binding site of an opiate receptor complex, and is termed the delta$_{cx}$ binding site. The higher affinity mu-competitive delta binding site, commonly identified as delta, is the delta binding site not associated with the receptor complex, and is termed the delta$_{ncx}$ binding site. Previous studies demonstrated that the i.c.v injection of ESF (enantiomer of superFIT,((3S,4R)-(-)-cis-N-[1-[2-(4-aminophenyl)ethyl]-3-methyl-4-piperidyl]-N-phenylpropaneamide hydrochloride) to rats 18-24 hr prior to sacrifice decreased the binding of [^{3}H]DADL to the delta$_{cx}$ and delta$_{ncx}$ binding sites, when the assay was conducted in the presence of 1 mM 2-mercaptoethanol (Rothman et al., Neuropeptides, in press). The goal of this study was to examine the interaction of the delta antagonist ICI174864 with the delta$_{cx}$ binding site in vitro, and in vivo utilizing ESF.

The in vitro affinities of ICI174864 for the delta$_{cx}$ and delta$_{ncx}$ binding sites were determined by displacement of 2 nM [^{3}H]DADL using membranes pretreated with the site-directed acylating agents BIT (2-(p-ethoxybensyl)-1-diethylaminoethyl-5-isothiocyanatobensimidisole)-HCl or FIT (N-phenyl-N-[1-(2-(p-isothiocyansto)phenylethyl)-4-piperidinyl]propanamide)-HCl, allowing the selective assay of the delta$_{ncx}$ and delta$_{cx}$ binding sites, respectively. [^{3}H]DADL binding assays were conducted without 2-mercaptoethanol using two different "salt solutions:" 1) 100 mM NaCl, 3 mM $MnCl_2$ and 2 uM GTP and 2) 100 mM choline chloride, 3 mM $MnCl_2$. The IC50 of ICI174864 for the delta$_{cx}$ and delta$_{ncx}$ binding sites were 2472 nM and 169 nM using condition 1, and 5538 nM and 733 nM using condition 2. These data demonstrate a Na-dependent high affinity interaction of ICI174864 with the delta$_{ncx}$ binding site, and a very low affinity interaction with the delta$_{cx}$ binding site, suggesting that ICI174864 should not interact in vivo with this binding site.

To test this hypothesis, 24 hr prior to sacrifice and preparation of membranes, rats received i.c.v. injections of vehicle (group 1), 25 ug ESF (group 2), 3 ug ICI174864 (group 3) or 3 ug ICI174864 15 min prior to 25 ug ESF (group 4). Binding parameters of the delta$_{cx}$ binding site were determined using the method of binding surface analysis. I.c.v. injection of ESF almost completely eliminated the delta$_{cx}$ binding site. Pretreatment of rats with ICI174864 prior to the injection of ESF (group 4), prevented the in vivo acylation of this binding site. In contrast, the density of mu binding sites, labeled with [^{3}H]FOXY (3,14-dihydroxy-4,5alpha-epoxy-6beta-fluro-17-methylmorphinan), were identical in all 4 groups.

These data demonstrate that the delta antagonist interacts with the delta$_{cx}$ binding site in vivo but not in vitro, clearly illustrating the failure of in vitro data to predict in vivo occurences. Furthermore the almost total loss of delta$_{cx}$ binding sites, commonly identified as mu, following i.c.v. injection with ESF without a concomitant loss of mu binding sites supports the hypothesis that the delta$_{cx}$ and mu binding sites are not the same entity.

180.15 ALTERATIONS IN KAPPA AND MU OPIOID RECEPTOR BINDING BY VOLATILE ANESTHETICS IN VITRO. C. Ori* and E.D. London (SPON: J.E. Johnson). Neuropharm. Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224.

The mechanism by which general anesthestics act is largely undefined. Although specific targets for the actions of some anesthetics, like ketamine or xylamine, have been hypothesized (Lawrence, D. and Livingstone, A., Br. J. Pharmacol., 73:435, 1981), a nonspecific mechanism, implying a general physical perturbation of macromolecules, might better explain the effects of a wide variety of structurally different substances which all produce general anesthesia. Many inhalational anesthestics can generally affect all macromolecules, including proteins and lipids (Mashimo, T. et al., Mol. Pharmacol., 29:149, 1986). Inhalational anesthestics also have effects on muscarinic (Aronstam, R. S. et al., Biochem. Pharmacol., 35:667, 1986) and nicotinic (Firestone, L. L. et al., Anesthesiology, 64:694, 1986) acetylcholine receptors, and opioid receptors (Daras, C. et al., Eur. J. Pharmacol., 89:177, 1983; Inoki, R. et al., Life Sci., 33 (Suppl. 1):223, 1983) which vary with the receptors and the anesthetics.

We investigated the in vitro effects of the inhalational anesthetics, nitrous oxide (N_2O) and halothane, on mu and kappa opioid receptor subtypes in membranes from the guinea pig brain. Membrane suspensions were bubbled with 100% N_2O and 2% halothane for 1 h to achieve saturation before they were used in binding assays. Untreated and oxygen-saturated membranes were used as controls. Mu receptor binding was assayed using [^{3}H]dihydromorphine as the ligand, and kappa binding was assayed with [^{3}H](-)ethylketocyclazocine in presence of 100 nM DADL and 30 nM morphine to block binding to delta and mu sites, respectively.

Both O_2 and N_2O produced slight increases in Kd's for mu binding (control, 0.87 nM; O_2, 1.40 nM; N_2O, 1.45 nM) with no changes in Bmax. Halothane greatly decreased the affinity (Kd = 2.70 nM) without affecting Bmax. The values of Kd for kappa binding sites were slightly but significantly increased both by O_2 and N_2O (control, 0.25 nM; O_2, 0.29 nM; N_2O, 0.31 nM), but only N_2O altered Bmax (control, 115 fmol/mg protein; O_2, 113 fmol/mg; N_2O, 84 fmol/mg). Halothane greatly decreased both affinity and density of kappa binding sites (Kd and Bmax, 0.20 nM and 38 fmol/mg protein, respectively).

The results demonstrate different effects of inhalational anesthetics on opioid receptor subtypes. Oxygen also showed some effects on these receptors, consistent with a general physical perturbation of the membranes. The observed differences might result from conformational changes in receptors due to effects on the lipoprotein structure of membranes, as influenced by the different physical properties of the gases (Lee, N. M. et al., Life Sci., 26:1659, 1980).

180.16 THC AND THC ANALOGS ALTER OPIATE RECEPTOR BINDING IN RAT BRAIN SECTIONS. J. Margulies* and R. P. Hammer, Jr. (SPON: J. Hardman). Dept. of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, HI 96822.

Delta-9-tetrahydrocannabinol (THC) is known to influence brain function, however, the mechanism and specific sites of drug action in brain are unknown. We have examined the effect of THC and water soluble THC analogs on binding of radioligands in various neurotransmitter receptor systems in vitro. These radioreceptor assays were performed on sections through the caudate nucleus of male Sprague-Dawley rats. Animals were decapitated, brains were removed and immediately frozen at -30°C. Sections through the caudate-putamen were taken; tissue was dried and stored at -50°C overnight. Radioligand receptor binding in the presence of graded concentrations of THC or THC analogs was measured by liquid scintillation counting. To study opiate receptor binding, sections were preincubated for 30 min at 4°C in 50 mM Tris-HCl, pH 7.4, 100 mM NaCl and 1 mg/ml BSA. Sections were then incubated for 1 hour at room temperature in 50 mM Tris-HCl, pH 7.4, 100 mM NaCl and 2.5 nM [^{3}H]naloxone, and rinsed for 15 min in ice cold 50 mM Tris-HCl, pH 7.4. Caudate sections were wiped off the slides with Whatman GF/B filters, and filters and tissue were dissolved in Cytoscint for scintillation counting. Nonspecific binding was determined in the presence of 2 uM etorphine. Opiate receptor binding was found to be affected by both THC and THC analogs in a dose dependent manner as follows.

THC Concentration	1 uM	100 nM	10 nM	1 nM	0.1 nM
% [^{3}H]naloxone Bound	9.3	43	73	89	99

THC Analog #1(SP-111A)	10 uM	1 uM	100 uM	10 nM	1 nM
% [^{3}H]naloxone Bound	51	65	72	76	97

While naloxone in a sodium-containing buffer is known to favor binding to the mu-subtype opiate receptor, we cannot rule out an interaction with the kappa-subtype opiate receptor based on these data alone. To further elucidate the interaction of THC with opiate binding sites, we examined the effects of THC and THC analogs on the binding of the kappa receptor radioligands, [^{3}H]bremazocine and [^{3}H]EKC. These preliminary studies suggest that the drug-induced alteration in opiate receptor binding is mediated by the mu-subtype opiate receptor. These data together with autoradiographic results illustrating the effects of THC on regional brain opiate receptor binding will be presented. (Supported by USPHS Awards DA04081 and HD19951 to R.P.H.)

180.17 EFFECTS OF FEEDING SCHEDULE: INVESTIGATION OF MORPHINE-INDUCED FEEDING. H.L. June,* J.R. Andrade, and M.J. Lewis. (SPON: L.H. Hicks). Dept. of Psychology, Howard University, Washington, D.C. 20059.

A growing body of evidence suggests that opioid agonists can significantly influence the feeding patterns of laboratory rodents. While there have been published reports of enhancement of feeding (Sanger and McCarthy, 1980; Tepperman, Hirst, and Gowdey, 1981), some researchers (Kumar and Stolerman, 1971) have reported an attenuation in feeding behavior. In an attempt to further specify the contingencies under which an opioid agonist influences feeding behavior, morphine was administered intraperitoneally to both 18hr food deprived and non-deprived rats. Then, in a subsequent study, using a separate group of animals, morphine was centrally administered to the lateral hypothalamus.

Male Sprague-Dawley rats were allocated to a deprived (D) (n=10) and a non-deprived (ND) (n=10) group. Food was withdrawn from the animals in the D group 18h prior to the experiment. Food intake measures were taken at 2, 4, and 6h intervals. Animals were injected with saline or morphine (.5, 1, or 1.5 mg/kg, i.p.). Fifteen non-deprived animals were implanted with cannulas in the lateral hypothalamus. Morphine (4.5 µg) was then injected with a 25 guage internal cannula.

The results indicate that non-deprived animals failed to show any significant changes in their consumption with any of the drug doses or time intervals. However, morphine significantly enhanced feeding during the first 2h in non-deprived animals, with the 1mg/kg dose producing the largest effect. At the 4 and 6 hr intervals, a nonsignificant increase was observed with the 1.0 and 1.5 mg/kg doses in these animals. Central administration of morphine resulted in a highly significant increase in feeding during the first 2h, then a moderate increase during the second 2h period. No effect was observed during the last 2h period.

These results suggest that while morphine may cause an increase in feeding, its effect appears to be due to feeding schedule and dosage. These results also confirmed that the effect of morphine on feeding appears to be centrally mediated, as have been reported by others (Tepperman, Hirst, and Gowdey, 1981).

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180.18 ALTERATIONS IN OPIOID SYSTEMS AND BEHAVIOR IN ADJUVANT-INDUCED INFLAMMATION OF THE RAT HIND LIMB. C. Stein**, M.J. Millan*, R.M. Arendt*, K. Peter** and A. Herz* (SPON: H.L. Altshuler). *Dept. of Neuropharmacology, Max-Planck-Institut für Psychiatrie, D-8033 Martinsried, F.R.G., +Dept. of Anesthesiology, Klinikum Grosshadern, D-8000 München 70, F.R.G.

We have previously characterized neurochemical and functional changes in adjuvant-induced polyarthritis in rats (Millan, M.J. et al., J. Neurosci., 7:77, 1987; Millan, M.J. et al., J. Neurosci., 6:899, 1986). This study evaluates behavioral characteristics and alterations in opioid systems in rats with adjuvant-induced inflammation of a single hind paw. Male Wistar rats received an intraplantar injection of Freund's adjuvant and developed inflammation within 24 hrs. Paw volume of the affected limb increased gradually up to day 4 and remained stable at about 3 times normal size up to day 35. Rats showed a persistent flexion of the inflamed limb and a reluctance to place weight on the paw. They showed a significant reduction in the rate of body weight gain and in food intake beginning on day 4. Acute nociceptive thresholds to pressure applied to the affected limb and motility (open-field test) were significantly reduced compared to controls. One week after inoculation immunoreactive dynorphin levels were significantly elevated only in the ipsilateral dorsal horn of lumbar spinal cord of affected animals. A supersensitivity to the analgesic action of morphine was seen primarily on the inflamed (as compared to uninflamed) paw. The behavioral and physiological changes suggest that adjuvant-induced inflammation of the hind limb in rats is associated with pain. The biochemical changes suggest that an increased noxious afferent input can modify the activity of DYN-containing neurones in anatomically correlated parts of the spinal cord.

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180.19 OPIATE RECEPTORS ARE INCREASED IN REGIONS OF DECREASED GLUCOSE UTILIZATION IN PATIENTS WITH TEMPORAL LOBE EPILEPSY MEASURED BY POSITRON EMISSION TOMOGRAPHY. J.J. Frost, H.S. Mayberg, J.S. Fisher, K.H. Douglass*, R.F. Dannals*, J.M. Links*, S.H. Snyder, H.N. Wagner, Jr.*, Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Animal models of epilepsy have indicated a role for opiate peptides and opiate receptors in seizure mechanisms. ^{11}C-carfentanil (CAR) was used to quantitate regional mu opiate receptor binding in patients with partial complex seizures and a unilateral temporal lobe seizure focus determined by electroencephalography. Regional glucose utilization was also measured using ^{18}F-fluorodeoxyglucose (FDG). All patients were studied in the interictal state. Quantification of CAR binding was achieved by compartmental modelling studies which indicated the close relation between the region/occipital cortex activity ratio and the value of K_D/B_{max}; the occipital cortex is used to determine nonspecific binding due to the low number of opiate receptors it contains. The region/occipital cortex ratio did not vary as a function of blood flow since near-equilibrium of CAR binding is attained during the measurement.

There was a significant ($p<.01$) elevation in CAR binding on the side of the electrical seizure focus in the termporal cortex but not in the amygdala or hippocampus in 13 patients. By contrast glucose utilization was decreased in the temporal cortex and amygdala. There was a significant relationship ($r=0.74$, $p=0.012$) between the increase in CAR binding in the temporal cortex on the side of the electrical focus and the decrease in glucose utilization.

The increase in opiate receptors may represent a component of an endogenous anticonvulsant system to limit the spread and severity of seizures as suggested by animal models. Measurement of neuroreceptors by PET in epilepsy patients circumvents many limitations of current animal models of epilepsy and can significantly improve our understanding of neurochemical mechanisms which initiate and modulate seizures.

181.1 LEARNING OF A CLASSICALLY CONDITIONED NICTITATING MEMBRANE/EYELID RESPONSE WITHOUT CEREBELLAR CORTEX. D.G. Lavond, J.E. Steinmetz, and R.F.Thompson. Department of Psychology, Building 420, Jordan Hall, Stanford University, Stanford, CA 94305.

Adult male New Zealand White rabbits had their left cerebellar cortex aspirated of crus I and II, paramedian lobe, the lateral margin of the vermis, and Larsell's HVI. After 1 week of recovery they were trained for classical conditioning of the nictitating membrane/eyelid response. The conditioned stimulus (CS) was a 350 msec, 85 dB SPL white noise. The unconditioned stimulus (UCS) was a 100 msec, 2.1 N/cm^2 corneal airpuff. The CS and UCS overlapped and coterminated on paired trials. Daily training consisted of 12 blocks of 9 trials per block, with the first trial of each block a CS alone test trial and the remaining 8 trials were paired. Group 1 was trained first on the lesioned side for 10 days, then contralaterally for 2 days, and again on the ipsilateral side for 2 more days. Group 2 was trained first on the unlesioned side, then on the ipsilateral side, and again on the contralateral side.

All rabbits learned the response without lateral cerebellar cortex as long as the underlying interpositus nucleus (IN) was undamaged. Learning took substantially longer when trained on the lesioned side (Group 1) than when trained on the unlesioned side first (Group 2). Group 2 took about as many trials for learning as reported previously for unoperated animals. In contrast, no learning occured on a side with damage to IN.

Learning occured very rapidly when Group 1 was switched to training on the unlesioned side regardless of whether IN damage prevented learning on the previous side. We have previously reported that although training is one-sided, learning occurs to some degree on both sides.

Two rabbits with extensive bilateral cortical aspirations, including the anterior cortex and most of the posterior vermis but sparing the flocculus and paraflocculus, did not learn on the side with damage to the IN but did learn to the side with an intact IN.

The present study demonstrates that the lateral cerebellar cortex removed is not essential for learning of the classically conditioned eyelid response. However, this cerebellar cortex normally plays a role in conditioning since it takes substantially longer to learn without it.

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181.2 DISRUPTED RETENTION OF THE CLASSICALLY CONDITIONED EYEBLINK RESPONSE IN THE ALUMINUM INTOXICATED RABBIT USING BRAIN STIMULATION AS A CONDITIONED STIMULUS. P. R. Solomon, D. Koota*, J. B. Kessler*, and W. W. Pendlebury. Dept. of Psychology, Williams College, and Dept. of Pathology, Univ. of Vermont College of Medicine.

We have previously reported that intraventricular injection of aluminum chloride in the rabbit disrupts both acquisition and retention of the classically conditioned eyeblink response and that this disrupted conditioning is correlated with the number of neurons that contain neurofibrillary tangles. We also reported that the disrupted conditioning was not related to illness, sensitivity to the air puff unconditioned stimulus (UCS) or motor deficits (Soc. Neuro. Abstr., 12: 380, 1986). We were not, however, able to rule out the possibility that altered sensitivity to the conditioned stimulus (CS) contributed to the conditioning deficits. To test this possibility, we used direct stimulation of the medial geniculate nucleus as a conditioned stimulus. We reasoned that if aluminum still disrupted conditioning, this would rule out the possibility that pathology of the primary sensory systems was responsible.

Rabbits were classically conditioned to emit an eyeblink conditioned response (CR) in response to electrical stimulation of the medial geniculate nucleus (CS) paired with a corneal air puff (UCS: 100 msec, 3 PSI) until they attained a criterion of two consecutive sessions of (100 CS-UCS pairings) of greater than 90% CRs. They then received intraventricular injections of 1% AlC_3 (100 µl), HCl (100 µl, to control for pH) or normal saline (100 µl). Ten days post-injection, each animal underwent a retention test consisting of 50 tone alone presentations. Whereas all saline and HCℓ controls gave at least 90% CRs during retention, no aluminum intoxicated rabbit emitted more than 30% CRs. Subsequent tests indicated that this disruption of conditioning could not be attributed to deficits in motor processes or illness.

Neuropathological examination of the tissue revealed widespread neurofibrillary tangle formation involving dorsal cortex, hippocampus, multiple brainstem nuclei, and cervical spinal cord anterior horn cells.

Considered with the results of previous work, these data suggest that aluminum-induced neurofibrillary degeneration disrupts retention of the conditioned response by affecting central associative processes.

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181.3 EFFECTS OF VARYING THE INTERSTIMULUS INTERVAL ON CLASSICAL EYELID CONDITIONING WITH PONTINE NUCLEUS STIMULATION AS A CONDITIONED STIMULUS. J.E. Steinmetz, D.G. Lavond and R.F. Thompson. Department of Psychology, Stanford University, Stanford, CA, 94305.

Stimulating electrodes were chronically implanted in the dorsolateral and lateral pontine nuclei of five groups of adult male New Zealand White rabbits. After a 1 wk recovery period, the rabbits received four daily sessions of classical conditioning training by forward pairing a pontine stimulation CS (a 120 uA, 200 Hz train of 0.1 msec pulses) with a 100 msec air puff US. Each daily training session consisted of 12 blocks of 9 trials per block with the first trial of each block a CS alone presentation and the remaining trials paired presentations of the CS and US. The five groups were given either a 150, 350, 600, 1100 or 2100 msec train of CS brain stimulation that coterminated with the 100 msec air puff US thus producing a 50, 250, 500, 1000 or 2000 msec interstimulus interval (ISI). After the initial four days of training all animals were given two days of training with the brain stimulation CS and air puff US and a 250 msec ISI.

Varying the ISI in the present study produced different levels of conditioning in the five groups of rabbits. Specifically, similar to previous ISI studies which have used peripheral CSs such as tones during training, manipulation of the ISI with the pontine stimulation CS produced a concave or "inverted-U" shaped function between conditioned response frequency and ISI. Relatively robust and rapid conditioning was observed with ISIs of 250 and 500 msec, moderate conditioning was obtained with ISIs of 1000 and 2000 msec and no conditioning was observed when a 50 msec ISI was used. All animals demonstrated robust conditioned responding after they were switched to the 250 msec ISI (i.e., 90% CRs by the second half of Day 2 of training). These data indicate that the characteristic ISI function observed for classically conditioned responses established with peripheral CSs can also be observed when classically conditioned responses are established with direct activation of cerebellar mossy fibers as a CS.

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181.4 LESIONS OF THE INTERPOSITUS NUCLEUS ABOLISH EYEBLINK CONDITIONING WHEN STIMULATION OF THE AUDITORY CORTEX IS USED AS A CS. B.J. Knowlton and R.F. Thompson. Dept. of Psychology, Stanford Univ., Stanford, CA. 94305.

Learning and retention of the classically conditioned eyeblink/nictitating mebrane (NM) response is dependent on the cerebellum and associated brainstem circuitry. Lesions of the cerebral auditory cortex do not affect formation of the simple learned response, but they have been shown to abolish heart rate discrimination conditioning when tones of different frequencies are used as CS+ and CS-. In the present study, 8 rabbits received stimulation of the auditory cortex as a CS in the standard NM conditioning procedure. The rabbits were implanted with stimulating electrodes in the auditory cortex, which was localized by evoking auditory field potentials. In 3 additional rabbits, stimulating electrodes were implanted in cerebral cortex in areas where no clear evoked potentials were recorded. These 3 rabbits failed to learn the conditioned response (CR). Of the 8 rabbits with stimulating electrodes in the auditory cortex, 5 were implanted with lesion electrodes in the dentate/interpositus region of the cerebellum ipsilateral to the eye trained. The other 3 were implanted with lesion electrodes in the contralateral dorso-lateral pontine nucleus (DLPN). After recovery from surgery, each rabbit was given paired training with CS stimulation currents beginning at 80 µA. An airpuff served as the US. Each rabbit was given daily sessions of 108 trials each. The CS current was increased by 20 µA each day, until learning occurred or until a maximum of 10 training sessions had been given. After overtraining, each rabbit received a lesion to the dentate/interpositus (DI) or the DLPN. After recovery, these rabbits were tested for CR retention. The 4 rabbits in which the anterior interpositus nucleus was destroyed did not relearn the CR in at least 5 days of training. These rabbits all learned the CR when trained on the contralateral eye. In one DI rabbit, much of the interpositus was spared and relearning took place. All of the rabbits with unilateral DLPN lesions retained the CR. These results suggest that stimulation of the auditory cortex can act as a CS in NM conditioning, and that it is similar to a peripheral CS in that conditioning is abolished by anterior interpositus lesions in both cases. By using direct brain stimulation, the connections between auditory cortex and the essential circuit for NM conditioning can be investigated.

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181.5 PROPOSED NEURAL PATHWAYS FOR CONDITIONED AND UNCONDITIONED STIMULI IN THE FEAR-POTENTIATED STARTLE PARADIGM. J.M. Hitchcock & M. Davis Depts. of Psychology & Psychiatry, Yale Univ., Ribicoff Res. Fac., Conn. Mental Health Ctr., 34 Park St., New Haven, CT 06508.

Classical conditioning is thought to involve the convergence of neural, pathways transmitting conditioned stimulus (CS) and unconditioned stimulus (US) information. We have previously found that the central nucleus of the amygdala and its efferent projection to the brainstem startle reflex pathway are necessary for fear-potentiated startle (Hitchcock & Davis, Beh. Neurosci., 100, 1986; Mondlock & Davis, Neurosci. Abs., 1985), a model of conditioned fear in which the acoustic startle reflex is enhanced in the presence of a light previously paired with shock. The present studies sought to delineate the neural pathways that transmit CS (light) and US (footshock) information to the amygdala.

To investigate the CS pathway, rats were trained with 20 light-shock pairings. One day later, separate groups of rats received sham operations or bilateral electrolytic lesions of various visual structures. Consistent with previous results (Tischler & Davis, Brain Res., 276, 1983), preliminary data indicate that lesions of the lateral geniculate nucleus (LGN) and aspiration of the visual cortex block performance of fear-potentiated startle tested after a longer recovery time (6 days) than previously used. Lesions of the insular and surrounding cortex, which receives afferents from the visual cortex and projects to the amygdala, also block performance. Further studies will delineate more exactly the critical cortical areas.

To investigate the US pathway, three tests were used: a) enhancement of startle that occurs during the 5-20 minutes after presentation of 10 brief footshocks in rapid succession (shock sensitization), b) acquisition of fear-potentiated startle (lesions made before training), and c) performance of fear-potentiated startle (lesions made after training, before testing). We have found that lesions of the central nucleus of the amygdala block shock sensitization, acquisition, and performance of fear-potentiated startle. However, lesions in a US pathway to the amygdala should block shock sensitization and acquisition, but not performance of fear-potentiated startle, because the US is not necessary for performance. Thus far, these criteria have been satisfied by combined lesions of the medial part of the medial geniculate nucleus (mMGN) and the posterior intralaminar nucleus (PIN) of the thalamus, which receive spinal afferents and project directly to the central nucleus of the amygdala.

Our current working hypothesis is that a CS pathway from the LGN to the visual cortex to the insular cortex and a US pathway from the spinal cord to the mMGN/PIN converge at the amygdala, which then projects to the startle reflex pathway and enhances the startle reflex during fear-potentiated startle.

181.6 ENHANCEMENT OF ACOUSTICALLY- AND ELECTRICALLY- ELICITED STARTLE BY ELECTRICAL STIMULATION OF THE AMYGDALA: LOCUS OF MODULATION AND TEMPORAL RELATIONSHIP. J.B. Rosen and M. Davis. Dept. of Psychiatry, Yale Univ., Conn. Mental Health Ctr., Ribicoff Res. Fac., 34 Park St., New Haven, CT 06508.

The acoustic startle response can be enhanced in the presence of a cue previously paired with a shock (fear-enhanced startle). Startle elicited by electrically stimulating various points along the startle pathway can also be enhanced by a fear-conditioned stimulus (Berg & Davis, Behav. Neurosci., 99:191-199, 1985). Previous work from our laboratory has shown that lesions of the amygdala block fear-enhanced startle (Hitchcock & Davis, Behav. Neurosci., 100:11-22, 1986) and that electrical stimulation of the amygdala enhances acoustic startle (Rosen & Davis, Neurosci. Abstr., 12:517, 1986). The present study demonstrates that activation of the amygdala can enhance both acoustically- and electrically-elicited startle in a very precise and predictable spatial and temporal manner.

Unipolar electrodes were chronically implanted in the amygdala and the acoustic startle circuit [ventral cochlear nucleus (VCN), paralemniscal region of the nucleus of the ventral lateral lemniscus (VLL), nucleus of the reticularis pontis caudalis (RPC) or the medial longitudinal fasciculus (MLF)] of Sprague-Dawley rats. Following recovery, rats were placed in a startle cage and presented with electrical stimulation of the amygdala (0.1 ms cathodal pulse) and either a 20 ms white noise burst or bilateral stimulation of the various nuclei along the startle pathway (1.0 ms cathodal pulse). Whole body startle amplitude was measured by an accelerometer attached to the cage, and in some cases, startle was measured by recording EMG responses through electrodes implanted into neck muscles.

Maximal enhancement of acoustic startle by amygdala stimulation occured when amygdala stimulation was given simultaneously with the onset of the acoustic startle stimulus. The time between amygdala stimulation and maximal enhancement of startle elicited electrically from the VCN or the VLL increased reliably to 1 and 3 ms, respectively. Amygdala stimulation did not enhance startle elicited electrically at the RPC or the MLF.

In agreement with previous results of fear-enhanced startle, modulation of startle by activation of the amygdala seems to enter the startle pathway at the RPC. The transit time from the amygdala to the startle circuit is approximately 3-4 ms. This suggests that only one or two synapses lie between the amygdala and startle circuit. Neuroanatomical tracing studies are in progess to delineate this pathway.

181.7 SENSITIZATION OF ACOUSTICALLY AND ELECTRICALLY ELICITED STARTLE RESPONSES BY FOOTSHOCK. N. Boulis*, M. Davis (SPON: M.Bowers), Departments of Biology and Psychiatry, Yale University, New Haven, CT 06508.

The acoustic startle reflex can be facilitated by the presentation of either a single footshock or a series of footshocks presented in rapid succession (footshock sensitization). This sensitizing effect develops gradually after cessation of footshocks, reaches a peak in approximately 10-20 min, and gradually dissipates over the next 20-40 min. The acoustic startle response is a short latency reflex mediated by brainstem and spinal cord structures. The present study evaluated the anatomical locus along this pathway where footshocks might ultimately alter neural transmission and, hence, the behavioral response.

A total of 32 rats were implanted with bilateral electrodes in either the ventral cochlear nucleus (VCN), an area just medial to the ventral nucleus of the lateral lemniscus (VLL), or the nucleus reticularis pontis caudalis (RPC). These sites contain the three brainstem synapses of the acoustic startle circuit (Davis et al., J. Neurosci., 1982, 2, 791-805). Following recovery, rats were given two matching sessions intended to establish levels of acoustic and electric stimulation that would elicit similar startle amplitudes. Two days later all animals were given a test session which consisted of an hour of alternating acoustic and electric stimulation presented at 30 sec intervals. After 20 min, half of the animals also received a train of ten footshocks (0.5 sec, 0.6 mA) presented at a rate of 1 shock per sec. Two days later the same test conditions were repeated, except that rats which previously did not receive footshocks were now given footshocks and vice-versa.

In all groups, acoustic startle elicited following shocks was significantly elevated over the response level present in the no-shock condition, indicating that prior footshock sensitizes the acoustic startle response. Startle elicited electrically from the VCN and the VLL were similarly facilitated by the presentation of prior footshock. In contrast, startle elicited from the RPC showed no sensitization, despite the fact that acoustic startle measured in these same animals during the same test session was facilitated. Based on these data, we conclude that footshock sensitization ultimately alters transmission at the RPC. A similar pattern of results was found previously when startle was facilitated in the presence of a light previously paired with footshocks (i.e., a conditioned fear stimulus, Berg & Davis, Behav. Neurosci., 1985, 99, 191-199). These and other data suggest that specific brainstem loci may mediate the behavioral changes that occur during footshock sensitization and conditioned fear.

181.8 **THE LATERAL AMYGDALOID NUCLEUS: SENSORY INTERFACE OF THE AMYGDALA IN FEAR CONDITIONING?** *Piera Cicchetti*, Joseph E. LeDoux, and Donald J. Reis* (SPON: M.S. Gazzaniga). Division of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

A specific subcortical pathway, the geniculo-amygdala projection, mediates the classical conditioning of emotional responses to acoustic stimuli in the rat (LeDoux et al., J. Neurosci., 4, 1984, 683-698; Neurosci., 17, 1986, 615-627). Within the amygdala, the central nucleus provides a link between the geniculo-amygdala pathway and efferent motor systems controlling both autonomic and behavioral emotional responses (Iwata et al., Neurosci. Abstr., 1986). The sensory receptive area of the amygdala during conditioning is unknown. However, anatomical tracing studies indicate that the lateral amygdaloid nucleus receives a major input from the acoustic thalamus (LeDoux et al., J. Comp. Neurol., 242, 1985, 192-213) and projects to the central nucleus (Krettek and Price, J. Comp. Neurol., 178, 1978, 225-254). The lateral amygdaloid nucleus may therefore serve as the sensory interface of the amygdala during fear conditioning. If so, lesions confined to this structure should disrupt fear conditioning.

Studies were performed on male Sprague-Dawley rats. Bilateral electrolytic lesions were placed in the lateral amygdala (n=6) or in the striatum immediately above the lateral amygdala (n=6). The lateral amygdala was also lesioned unilaterally (n=5). Controls were either unoperated (n=6) or in operated controls the electrode was lowered into the lateral amygdala bilaterally without passing current (n=8). After 14d, the animals were instrumented for computer-assisted recording of arterial pressure (AP) and subjected to classical conditioning trials involving the pairing of a pure tone with footshock. The next day, changes in AP and freezing behavior (cessation of somatomotor activity) elicited by the acoustic conditioned stimulus were measured during extinction trials (LeDoux et al, J. Neurosci., 4, 1984, 683-698). The rats were then sacrificed and the brains removed and processed histologically.

In unoperated controls the CS elicited increases in AP (18 ± 4 mmHg) and induced freezing behavior (114 ± 5 sec.). Comparable responses were seen in the operated control group (AP: 18 ± 3; freezing: 102 ± 14) and in the groups with striatal lesions (AP: 20 ± 3; freezing: 84 ± 17) or unilateral lesions of the lateral amygdala (AP: 15 ± 3; freezing: 103 ± 16). In contrast, bilateral lesions of the lateral amygdala greatly reduced the magnitude of both the AP (3 ± 1) and freezing (38 ± 18) responses ($p<.01$).

Lesions of lateral amygdala thus disrupt the classical conditioning of both autonomic and behavioral emotional responses. We propose that the lateral amygdala is a critical sensory link in the neural pathway through which acoustic stimuli are transformed into emotional signals.

181.9 AUDITORY CORTEX LESIONS DISRUPT THE ACQUISITION OF DIFFERENTIAL BRADYCARDIAC CONDITIONING TO TONAL STIMULI IN RABBITS.
A.H. Teich*, C.G. Gentile*, P.M. McCabe, T.W. Jarrell*, R.W. Winters, D.L. Liskowsky, and N. Schneiderman. Dept. of Psych., Univ. of Miami, Coral Gables, FL 33124.

Previous findings from our laboratory indicate that lesions of the auditory cortex disrupt the retention of differentially conditioned bradycardiac responses to tonal stimuli in rabbits (Jarrell et al., in press). In the present experiment, the effect of lesions of the auditory cortex on the acquisition of differential bradycardiac conditioning was examined. Lesions were made in either the auditory cortex or the visual cortex. After 7 days of recovery, animals received 7 days of differential Pavlovian bradycardiac conditioning in which one tone (CS+) was paired with the unconditioned stimulus, and another tone (CS-) was never paired with the unconditioned stimulus. All animals demonstrated differential conditioning during the first 3 days of conditioning. On days 4 - 7, however, auditory cortex lesioned animals did not exhibit significant differential heart rate conditioning; visual cortex lesioned animals showed no loss of conditioning during this period. The loss of differential conditioning in animals with lesions in the auditory cortex appears to be due to an increased response magnitude to the CS-. These data further support the hypothesis that auditory cortex plays a role in differential conditioning of bradycardia to acoustic stimuli (Jarrell et al., in press). It is suggested that a corticothalamic pathway is involved in the inhibition of the response to the CS-.

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181.10 THE PERIAQUEDUCTAL GRAY: PROJECTIONS TO CARDIOREGULATORY NUCLEI AND CONTRIBUTIONS TO CONDITIONED BRADYCARDIA IN THE RABBIT.
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Evidence suggests that the amygdaloid central nucleus (ACE) is involved in the acquisition (Kapp et al., 1979) and retention (Gentile et al., 1986) of vagus-mediated conditioned bradycardia in the rabbit, quite possibly via direct projections to the nucleus of the solitary tract (NTS) and dorsal vagal nucleus (DVN). The latter contains preganglionic cardioinhibitory neurons in this species. However, the ACE also projects to other regions which in turn project to the NTS/DVN complex, and which may also contribute to the conditioned response. One such region, the midbrain periaqueductal gray (PAG), has been implicated in cardioregulation and has recently been demonstrated to project to the NTS in the cat (Bandler et al., 1986). The present experiments were conducted to determine the extent to which projections from the PAG to the NTS/DVN complex exist in the rabbit and the degree to which lesions of the PAG affect the retention of conditioned bradycardia.

New Zealand rabbits received injections of Fast Blue (FB; 5%; 50-150 nl) along the rostral-caudal extent of the NTS/DVN complex. Two to three weeks later the animals were perfused and sections were examined using fluorescent microscopy for the presence of retrogradely labeled cells in the PAG. Labeled neurons demonstrated a predominantly ipsilateral distribution primarily within the ventrolateral region of the caudal PAG. A few were also present in the dorsolateral region.

Additional rabbits were assigned to one of three groups. Two groups (lesion and sham-lesion) were prepared surgically with bilateral lesion electrodes aimed at the ventrolateral PAG. The third group served as an unoperated control group. Following surgical recovery all animals received Pavlovian fear conditioning consisting of 20 conditioning trials during which a 5.0 second tone conditioned stimulus (CS) immediately preceded the presentation of a 0.5 sec, 2.0 mA shock unconditioned stimulus. Lesions were made 24 hours after conditioning and retention of the bradycardic response to the CS was tested 24 hours later by presenting 20 CS alone trials. Rabbits with lesions of the PAG demonstrated an attenuation of the magnitude of the conditioned bradycardic response when compared with sham-lesioned and unoperated control rabbits.

The demonstrated projections from the ACE to the ventrolateral PAG in rabbit (Kapp et al., 1986), taken together with the results of the present study, suggest that in addition to a direct pathway from the ACE to the NTS/DVN complex, an oligosynaptic pathway from the ACE to PAG to the NTS/DVN complex may contribute importantly to conditioned bradycardia in the rabbit. Supported by PHS NS16107 and a Grant-in-Aid from the American Heart Association.

181.11 MEDIODORSAL THALAMIC LESIONS IMPAIR DIFFERENTIAL PAVLOVIAN HEART RATE CONDITIONING.
S.L. Buchanan and D.A. Powell. WJB Dorn Veterans' Hospital and University of South Carolina, Columbia, SC.

We have previously demonstrated that midline prefrontal cortex, which receives projections from the mediodorsal nucleus of the thalamus (MD), is involved in the mediation of conditioned autonomic changes, and that electrical or chemical stimulation of MD evokes cardiovascular responses in the conscious rabbit. The present experiment examined the effect of ibotenic acid (IA) lesions of MD on differential Pavlovian heart rate conditioning. Bilateral ibotenic acid (Sigma) lesions were made by infusing .5 µl (10 µg/µl) at each of two injection sites per side. Control animals received injections of the phosphate buffer vehicle. After recovery, all animals received two 50-trial sessions of Pavlovian conditioning. CSs were 304 and 1216 Hz tones (4-sec duration); the US (on CS+ trials) was a .25 sec, 2.5-3.0 mA paraorbital shock. On each trial, heart rate was recorded for each interbeat interval (IBI) for 10 beats prior to tone onset and for the duration of the tone. On the day before Pavlovian conditioning was begun, the HR orienting response (OR) to tone-alone presentations was assessed in response to ten 4-sec presentations of one of the tones to be used as CSs. Histological analysis of brain sections of 8 animals with IA lesions revealed bilateral neuronal degeneration in MD in all animals. Anterior nucleus damage occurred in 1 animal. Anterior and medial portions of MD were destroyed in all animals, but more posterior portions of MD were intact in 4 animals. Both the HR component of the OR and the HR CR were in all cases decelerations from pre-tone baseline. The HR OR appeared to be slightly enhanced in IA-lesioned animals, although this effect was not significant. Ibotenic acid lesions significantly impaired HR discrimination, due to greater CS- responding in these animals, particularly during the first session. Moreover, these animals demonstrated somewhat larger CR magnitude to the CS+ early in training. These data suggest that MD does not participate in the "vagal drive" necessary for conditioned bradycardia, but may play a role in association formation per se.

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181.12 THE AMYGDALA, LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE RESONSE, AND FREEZING IN RATS.
R. N. Leaton and W. F. Supple, Jr. Department of Psychology, Dartmouth College, Hanover, N. H. 03755.

The amygdala often is associated with the central modulation of fear. Lesions of the central nucleus of the amygdala (CNA) block conditioned bradycardia in rabbits (Kapp, et al., 1979) and reduce many other signs of conditioned fear (Kapp, Pascoe, & Bixler, 1984). Amygdala lesions reduce both conditioned and unconditioned freezing in rats (Blanchard & Blanchard, 1972) and block potentiated startle (Hitchcock & Davis, 1986). We have shown significant correlations between freezing, potentiated startle, and startle amplitude generally (Leaton & Borszcz, 1985). Also we have found increased freezing over the early trials in acoustic startle habituation paradigms, freezing that is apparently conditioned to the contextual cues of the startle chamber (Borszcz, Cranney & Leaton, 1984). Since freezing, or the fear-state indicated by freezing, elevates startle amplitudes, it may mask the development of habituation. Given these various outcomes we hypothesized that any brain manipulation that reduced fear may produce what appears to be faster than normal habituation. We found that lesions of the ventral periaqueductal gray increased long-term habituation of the acoustic startle response, providing some support for this hypothesis (Borszcz, Cranney, and Leaton, 1984). We now report the effects of CNA lesions on long-term habituation of the acoustic startle response.

Acoustic startle response was measured to 118-dB, 100-msec, white-noise bursts in 120-day old Long-Evans rats. Each rat received 3 daily sessions in the startle chamber with 120 min between sessions. Each session consisted of 8 presentations of the startle stimulus on a 60-sec interstimulus interval. Rats were tested in this way every other day to complete 4 test days. Freezing behavior was monitored during the 10 sec preceding each startle stimulus. Two groups of 24 rats were tested; one following bilateral electrolytic lesions of CNA and one following sham operations.

Rats with amygdala lesions showed significantly greater reductions in startle amplitude within the first few sessions and across days than did the shams, appearing thus to habituate faster. Amygdala lesions did not affect initial response amplitude, and the group differences had disappeared by the last test day. The groups did not differ initially on freezing behavior, but the shams increased freezing significantly over trials during the first several sessions. After this initial increase, the freezing differences decreased over days as the shams reduced freezing behavior. By the last test day group differences in freezing were no longer significant. We conclude that fear, indexed by freezing behavior, was conditioned to contextual cues and produced elevated startle amplitudes which masked the development of habituation in the sham operated animals. Amygdala lesions, by reducing fear, reduced its response amplifying effects and therby enhanced the apparent development of long-term habituation.

181.13 EFFECT OF OLIVOCEREBELLAR TRACTOTOMY ON A CONDITIONED LIMB FLEXION RESPONSE IN THE CAT. T.J. Voneida, D. Christie-Jefford* and R. Bogdanski*, Department of Neurobiology, Northeastern Ohio Univ. College of Medicine, Rootstown, OH 44272.

This study represents part of a long term effort to identify those brain structures and pathways which are critical to the performance of a conditioned limb response. A limb flexion response (LFR) was conditioned to a tone conditional stimulus (CS) using shock to the limb as the unconditional stimulus (US). Thirteen cats underwent surgical section of the olivocerebellar tracts by a midline cut between the nuclei following attainment of criterion performance (2 scores of at least 80% and a third of no less than 75% on 3 consecutive days). Twelve of the 13 subjects retained the CR postoperatively, showing variable degress of loss, and all 12 were able to attain a criterion level of performance with training. The CR was abolished in one animal, though the unconditional response remained intact.

These results (with the exception of one subject) are counter to those reported by Yeo, Hardiman and Glickstein('86) following dorsal accessory olivary lesions in the rabbit following acquisition of a conditioned nictitating membrane response.

Possible discrepancies between the two studies will be discussed, including species differences, differences in training paradigms and operative procedures.

181.14 AGE OF LESION BUT ALSO AGE AT TESTING AND REWARD CONTINGENCIES DETERMINE DISCRIMINATION LEARNING AND TRIAL-UNIQUE OBJECT RECOGNITION IN NORMAL AND HIPPOCAMPECTOMIZED INFANT RHESUS MACAQUES. T. Alexinsky*, L. Rehbein* and H. Mahut, Psychol. Dept., Northeastern Univ., Boston, MA 02115

Five to six years following removal of hippocampus in infancy, monkeys were significantly impaired on a trial-unique delayed nonmatching-to-sample object recognition task (DNMS) but they were not impaired on the concurrent object discrimination task (COD) with repeated trials in which one member of each of the 8 pairs of objects remained the positive stimulus (Mahut and Moss, The Hippocampus, 4:241, 1986). Possibly, the functional dissociation of performance on the two types of tasks was due to practice with object discrimination tasks in the intervening years. Therefore, groups of 5 unoperated and 5 hippocampectomized infants were tested on both tasks close to the time of surgery.

One-stage, bilateral hippocampectomies were performed at 57, 66, 70, 77 or 81 days of age. Pre-training consisted of hue and five two-choice object discriminations. Testing on the COD task began when normal infants were 5-8 mos, and operated infants were 4½-5 mos of age. No significant group differences in performance were found. Unexpectedly, however, all infants performed better than did any of the 23 adult normal monkeys tested previously (Moss, Mahut and Zola-Morgan, J. Neurosci., 1:227, 1981) or 7 unimpaired adult monkeys after hippocampectomy sustained in infancy (Mahut and Moss, op. cit.). Thus, the capacity that mediates discrimination learning not only matures early but appears to function better early rather than later in life.

The trial-unique object recognition task was given when normal infants were 6-9 mos, and operated infants 5-7 mos. of age. Since infant monkeys are afraid of novelty and the capacity to learn object discrimination reversals does not fully mature until about 2 yrs of age (Mahut and Zola, Abst. Soc. Neurosci., 3:428, 1977; Mahut and Moss, op. cit.), the object recognition task was administered with the matching-to-sample method (DMS). Infants in both groups learned readily the basic task with 10 sec. delays between the presentation of the sample and its re-presentation with a novel object: 90% correct responses in 100 consecutive trials were made after means of 524 and 478 trials with 169 and 142 errors, for the two groups, respectively. For comparison, two normal control adult monkeys took 860 and 900 trials, with 241 and 260 errors, respectively. We are currently introducing longer delays (30-130 sec). This may reveal the state of maturation of mnemonic processes in unoperated infants and it remains to be seen whether delays will lead to a separation of performance between unoperated and operated infants.

181.15 DISASSOCIATION OF VISUAL AND OLFACTORY CONDITIONING IN THE NEO-STRIATUM OF RATS. Marc Viaud and Norman M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, H3A 1B1, Canada.

A role for the caudate nucleus in learning and memory has been established in studies using lesion (Dunnett & Iversen, Beh Br Res, 6: 213, 1982), stimulation (Wyers & Deadwyler, Physiol Beh, 6: 97, 1971) and neurochemical (Prado-Alcala & Cobos-Zapiain, Neurosci Lett, 14: 253, 1979) techniques. The topographical projection of cortex to striatum (Webster, J Anat, 95: 532, 1961) has led to the hypothesis that anatomically linked regions of the two structures may have similar functions. We made use of this hypothesis to study the involvement of two striatal areas in conditioning. We tested the effect of lesions in the posteroventral striatum (innervated by visual cortex) or in the ventrolateral striatum (innervated by olfactory cortex) on acquisition of a conditioned emotional response (CER) in the presence of a visual or an olfactory conditioned stimulus (CS).

Small bilateral electrolytic lesions were made either in the posteroventral (PV) or in the ventrolateral (VL) areas of the striatum. Sham operated rats were used as controls. Ten days after surgery the rats were water deprived and familiarized with a conditioning box containing a drinking tube for 15 minutes on three consecutive days. On day 4 each rat was placed into the conditioning box with no water available. For half the rats in each group a visual CS (a bright light) was present; for the other half an olfactory CS (a novel smell) was present. Each rat received 10 foot-shocks in the presence of the CS. For the next three days all rats were allowed to drink in the conditioning box. The sum of the latencies to begin drinking over the three days was taken as the measure of conditioning. In both the visual and olfactory conditioning situations the latency was significantly longer for sham-operated animals tested in the presence of the appropriate CS than it was for those tested in the absence of the CS, demonstrating that conditioned responses had been established.

Rats with PV lesions showed impaired acquisition of the visual CER compared to both rats with VL lesions and sham operated rats. In contrast, rats with VL lesions showed impaired acquisition of the olfactory CER compared to rats with PV lesions and sham operated rats.

These findings constitute a double disassociation: damage to the PV area of the striatum impaired the ability of rats to acquire sensori-motor associations involving visual but not olfactory information. Damage to the VL area of the striatum produced impairment of sensori-motor associative memory involving olfactory information but had no effect when visual information was involved. These findings support the hypothesis of regional heterogeneity in the striatum with respect to the nature of the information processed in learning and memory. They also suggest that the different areas of the caudate may perform similar learning-and-memory-related functions using information from a different modality in each case.

181.16 2-DEOXYGLUCOSE MAPPING OF BRAIN STRUCTURES ACTIVATED DURING IMPRINTING RELATED BEHAVIOR. S.C. Müller * and H. Scheich (SPON: ENA), Technical University, Institute of Zoology, Schnittspahnstr.3, 6100 Darmstadt, F.R.G.

The separation of a young chick from cage mates is a situation similar to the separation from the hen under natural conditions. A separated chick having visual and auditory contact to the group enters a state of arousal as indicated by EEG-recordings, while the vocalizations change to intense distress calling. The separated chick exhibits a specific pattern of 14C-2-deoxyglucose labeling in the brain compared to control chicks remaining in the group (Müller and Scheich, Behav Brain Res 19: 93, 1986). Enhanced labeling was found in three rostral forebrain areas, i.e. an area in the wulst (HAD), a lateral portion of neostriatum/hyperstriatum ventrale (LNH) and in a portion of median neostriatum/hyperstriatum ventrale (MNH). The same pattern of 2DG incorporation was described in young acoustically imprinted Guinea fowl chicks, when they heared the imprinting stimulus during 2DG (Maier and Scheich, Proc Natl Acad Sci 80: 3860, 1983). Therefore we conclude, that social separation is a useful paradigm for analyzing neuronal circuits also involved in filial imprinting.

In a quantitative densimetric analysis of 2DG autoradiographs 18 different brain areas were compared in separated chicks and control animals remaining in the group. In the forebrain of separated animals enhanced metabolic activity was found in addition to the three areas HAD, MNH and LNH in the lobus parolfactorius (LPO), in a dorsolateral area of the neostriatum caudale (Nd) and in an dorsolateral area of archistriatum ventrale (Av). Subtelencephalic structures with enhanced labeling were the central mesencephalic grey and the lateral habenula. Monocular chicks while separated develop pronounced asymmetric labeling in the visual nuclei and in HAD and LNH. The latter are in close topographical relation to visual projection areas and therefore assumed to be higher visual centers. In MNH auditory evoked potentials were recorded, characterized by latencies of about 25 ms and by habituation to pure tones.

Interestingly, comparable areas in songbirds were described to be involved in song learning, another imprinting like process. In contrast to the organization in chicks, these areas form discrete nuclei in songbirds. They are area X in the LPO, the magnocellular nucleus of the anterior neostriatum (MAN), HVc of the caudolateral neostriatum and the robust nucleus (RA) in the archistriatum intermedium.

We assume the involvement of similar neuronal circuits in early song learning and filial imprinting.

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181.17 LONG-TERM BEHAVIORAL AND HISTOLOGICAL EVALUATION OF THE CONSEQUENCES OF "TOTAL" CHOLINERGIC FOREBRAIN NUCLEUS LESIONS IN THE RAT. D.G. Spencer, Jr., E. Horváth*, T. Schuurman*, U. Benz*, and J. Traber*. Neurobiology Department, Troponwerke (Bayer), Neurather Ring 1, D-5000 Köln 80, FRG.

In a previous study, we found that rats that were pre-trained in a 4-hour delay version of the 8-arm radial maze task and then given multi-site ibotenic acid lesions of the nucleus basalis magnocellularis showed a subsequent deficit in this task. The deficit disappeared over the course of the next few weeks of retraining, however, as also reported for example by Bartus and colleagues (Pharmacol. Biochem. Behav., 23:125, 1985), but performance remained sensitive to disruption by scopolamine. A further group of rats was then trained to nearly perfect performance of the same task (4-hour delay interpolated between arm choices 6 and 7) and then given either sham surgery or a bilateral multisite ibotenic acid lesion along the entire length of the forebrain cholinergic nuclei: medial septum, nuclei of the vertical and horizontal limbs of the diagonal band, and substantia innominata/nucleus basalis magnocellularis. A total of 16 ibotenate injection sites were required, in which relatively low concentrations and volumes were used: ibotenate (1 mg/ml) was prepared in a saline/$NaHCO_3$ solution (pH 7.4), and volumes of 0.5- 0.6 µl, depending on site, were slowly injected. Rats so treated showed a large post-delay deficit in the 8-arm radial maze relative to controls, and this deficit did not recover over 4 months of retraining. Control percent accuracy post-delay during this period averaged approximately 70 % while that of the lesioned rats, about 45 %. Thereafter, a number of further behavioral tests were performed with the lesioned and control rats, along with an additional grow up of inexperienced age-matched subjects. In two tests of gross behavior and open-field performance, lesioned rats showed more locomotion and rearing and much less immobility and scratching-type grooming. Lesioned rats also displayed a large step-through passive avoidance deficit in relation to controls. In shuttlebox active avoidance, however, lesioned rats had a faster acquisition than controls, as well as showing increased inter-trial crossover activity. Learning performance in a water labyrinth, on the other hand, was disturbed in lesioned rats. In general, these data are very similar to the results of acute treatment with large doses of scopolamine. Histological examination and choline acetyltransferase activity determination confirmed the large extent of elimination of magnocellular acetylcholinesterase-positive forebrain cells and cholinergic innervation by the lesion. The present results support the use of animals so lesioned as a non-recovering model of human dementia.

181.18 THE EFFECT OF TRAINING SCHEDULE IN THE MORRIS WATER MAZE ON RATS WITH NUCLEUS BASALIS LESIONS. R.J. Mandel, F.H. Gage, and L.J. Thal. Dept. of Neurol., San Diego VA Med. Ctr. and Dept. Neurosci., Univ. of Calif., San Diego, La Jolla, CA 92093

Rats with excitotoxic lesions of the nucleus basalis (NBM) exhibit performance deficits compared to controls in the Morris Water Maze task. However, treatments designed to attenuate these impairments have had difficulty obtaining significant results. The present study was designed to examine whether different training regimens could further separate the learning curves of NBM-lesioned animals and nonlesioned controls, thus affording the task more sensitivity to potentially ameliorative treatments.

Thirty male F-344 rats received bilateral NBM lesions using 4.5 ug of ibotenate in 1 ul infused over 20 minutes. Thirty sham operated rats served as controls. The animals were divided into three experimental groups each consisting of 10 lesioned and 10 control rats. Training in the water maze consisted of: (1) 4 trials per day (standard) with no intertrial interval (ITI), (2) 4 trials per day with a 10 min ITI or, (3) 2 trials per day (2 trial) with no ITI. Each group was tested in the water maze for 5 consecutive days, followed by a 2-day rest, followed again by 5 additional consecutive testing days.

The two-trial paradigm resulted in an average difference in latency to find the platform between the control and lesioned animals of 22.2 sec/day. The standard paradigm resulted in an average difference in latency of only 9.8 sec/day which was not significantly different from escape latencies obtained in the 10 min ITI paradigm. In addition, the strategies of the animals as measured by percent time in the outer annulus supports the latency data. These data suggest that the two-trial regime is more difficult and may therefore be more sensitive to treatments designed to reverse the learning deficits caused by NBM lesions.

181.19 EFFECTS OF IBOTENIC ACID LESIONS OF THE HIPPOCAMPUS ON ACQUISITION OF A RADIAL MAZE MEMORY TASK. J.R. Leu[1], L.S. Johnson*[1] and L.E. Jarrard[2]. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100[1] and Department of Psychology, Washington and Lee University, Lexington, VA 24450[2].

Ibotenic acid, a neurotoxin which destroys neuronal cell bodies but spares fibers-of-passage, was used to destroy selectively the hippocampal cells of young adult male rats. This procedure utilizes multiple, bilateral, microliter injections of the neurotoxin to destroy preferentially the hippocampal cell bodies while avoiding collateral damage to adjacent structures. The resulting lesions include the dentate gyrus and the CA1-CA3 pyramidal cells, but avoid the subiculum.

Previous work by Jarrard has determined that ibotenate lesions of the hippocampus, unlike conventional lesions, do not permanently disrupt post-surgical performance in preoperatively trained rats. In this previous work no lasting differences were found between control and lesioned rats on retention of either a spatial or a non-spatial limited-baiting radial maze task. These results raise the question of whether ibotenate hippocampal lesions in rats cause any impairment suggestive of bitemporal amnesic syndrome in humans.

In the present study the primary question was whether naive rats with selective complete hippocampal lesions could learn a radial maze procedure, or whether learning would be prevented in a way suggestive of anterograde amnesia. Using an eight-out-of-eight arm baiting procedure in a radial maze, rats were allowed to explore the maze and consume food rewards. After a rat returned to the center of the maze following an excursion into an arm, the doors were closed for all eight arms to restrict the rat briefly to the center of the maze. This procedure disrupts patterned response strategies and increases the extent to which the animal must rely on memory to avoid re-entering previously visited arms. The lesioned animals (N=8) were markedly impaired relative to the control animals (N=8). Eighteen days after the operated and unoperated control groups had reached asymptotic performance averaging under one error (repeated arm entry) per trial, the hippocampal group still averaged approximately 8-10 errors per trial.

181.20 IMPAIRED MOTOR LEARNING IN HUNTINGTON'S AND PARKINSON'S DISEASES. W. Heindel*, D. Salmon, N. Butters, and C. Shults. Psychology Service and Depts. of Psychiatry and Neurosciences, VA Medical Ctr. and Univ. of Cal. Sch. of Med., San Diego, CA 92161.

A previous study in our lab found patients with Huntington's Disease (HD), but not patients with Alzheimer's Disease (AD), to be severely impaired in their ability to acquire a motor skill. Since HD patients at different stages of functional disability did not differ in their level of performance, their impairment was attributed to an actual learning deficit and not simply to a motor performance deficit associated with their chorea. These results provided support for a critical role of the basal ganglia in skill learning. The present study extends these results by comparing the performance of HD patients to that of patients with idiopathic Parkinson's Disease (PD) on the same motor task.

A pursuit rotor apparatus was used to assess motor skill learning in 14 HD patients, 16 PD patients, and groups of 10 and 15 control subjects age-matched to the HD and PD groups, respectively. Subjects were told to try to maintain contact between a stylus held in their preferred hand and a small metalic disk on a rotating turntable. The total time on target was recorded for each 20 sec. trial. All subjects were given 6 blocks of 4 trials each, spaced over a period of 2 hours. The initial level of performance was equated across all groups at about 5 sec. per trial by individually adjusting the turntable speed for each subject during a block of practice trials. All subjects were administered the Dementia Rating Scale (DRS), and the PD patients were also rated from 0 (absence of symptom) to 4 (greatest severity) on the classic PD symptom triad of tremor, rigidity, and bradykinesia.

As in our previous study, the HD patients demonstrated significantly less learning over the 6 test blocks than did their age-matched controls. The performance of the PD patients fell in between that of their controls and the HD patients, and did not differ significantly from either group. However, when a subset of HD and PD patients were matched for overall level of dementia, these 2 groups demonstrated similar amounts of learning and were both significantly impaired relative to their control groups. The performance of the PD patients on this task was not related to the severity of their tremor, rigidity, or bradykinesia, but was significantly correlated with their score on the DRS.

These results indicate that PD patients, like patients with HD, are impaired in the acquisition of motor skills, and that the severity of their learning deficit is correlated with their dementia but not with their primary motor dysfunctions. It appears, then, that PD and HD patients both differ from patients with AD in terms of their ability to acquire motor skills.

This study was supported by funds from the Medical Research Service of the VA and by NIA grant AG-05131.

182.1 GLUTAMATE RECEPTORS AND IRREVERSIBLE ANOXIC DAMAGE IN HIPPOCAMPAL SLICES: MECHANISMS OF INTERACTION. D. Lobner* & P. Lipton. (SPON: J. Hind) Dept. of Physiol., Univ. of Wisc. Madison, WI 53706.

There is irreversible damage to synaptic transmission in rat hippocampal slices following short periods of anoxia (Kass & Lipton, J. Physiol., 1982, 1986). Ischemia-induced cell necrosis in vivo and in culture is attenuated by NMA receptor antagonists suggesting that glutamate or aspartate release contributes importantly to anoxic/ischemic cell damage.

In the hippocampal slice model:

i) 10 minutes exposure to 7 mM glutamate irreversibly damages transmission in dentate gyrus and CA1; this damage is blocked in OCa/10 mm Mg buffer. 200 um DL-APV partially blocks the damage. There is no change in ATP or 45 Ca uptake in the neuropil of CA1 or dentate gyrus.

ii) Release of both endogenous aspartate and glutamate from the slices is enhanced by between 150 and 300% during 10 minutes anoxia.

iii) Irreversible damage to perforant path transmission following 10 minutes anoxia is attenuated approximately equally in both the dentate gyrus and CA1 by the following NMA antagonists: Ketamine (1 mM, 100% recovery); PCP (10 uM, 40% recovery); D-APV (50 um, 35% recovery), D-L APV (300 um, 40% recovery). Kynurenic acid (10 mM) allows complete recovery in CA1 but no recovery in dentate gyrus. The NMA antagonists do not prevent the population spikes which reappear in CA1 about 3-4 minutes after the onset of anoxia in CA1 ("reappearing spikes").

iv) In the absence of blockers ATP falls by about 75% and 45 Ca uptake increases by about 60% during 10' anoxia. In CA1 10 mM kynurenic acid attenuated the fall in ATP by 40% and did not affect the increase in 45 Ca uptake at all; 300 um D-L APV elevated control and anoxic levels of ATP by about 30% and also had no effect on the anoxic uptake of 45-calcium.

Thus: a) Exogenous glutamate can irreversibly damage transmission. b) Binding of glutamate/aspartate to NMA receptors contributes to irreversible anoxic transmission loss. c) These toxic effects of glutamate/aspartate are not due to an increase in neuropil calcium uptake.

There are two possibile actions of aspartate/glutamate during anoxia consistent with the proposal that increased cytosolic calcium is a major trigger of anoxic damage (Kass & Lipton, 1986). A) Binding to NMA receptors increases dendritic IP3 and leads to a release of stored calcium from the endoplasmic reticulum or B) increased Na influx resulting from NMA binding leads to a release of calcium from mitochondria. A kinetic model will be presented to show calculated effects of such actions on cytosolic calcium during anoxia.

182.2 ISCHEMIA INDUCES PROTEIN SYNTHESIS INHIBITION IN THE HIPPOCAMPAL SLICE- AN AUTORADIOGRAPHIC APPROACH. K.Raley and P.Lipton. Dept. of Physiology. Univ. Wisconsin, Madison, WI 53706.

Utilizing the rat hippocampal slice preparation, we have established that in vitro "ischemia" produces a profound and long-lasting inhibition of protein synthesis (Raley and Lipton, Neurosci.Abs.1986). In those studies, we measure radiolabelled lysine incorporations into PCA precipitates of tissue samples from the CA1 and dentate gyrus (DG) regions of the slice. It is not possible to localize the cell types in which protein synthesis is inhibited using this approach. Consequently, in the current study, we have developed an autoradiographic technique whereby we can actually visualize, at the light microscope level, the cell types affected by ischemia. Thus, it is possible to discern increases as well as decreases in protein synthesis in capillaries, glia, principal neurons and interneurons.

We prepare 500um transverse hippocampal slices from adult male rats using a vibratome (model G), and incubate the slices in 95%O_2-5%CO_2-buffered modified Ringers solution at 36°C. Experimental slices are rendered "ischemic" (95%N_2-5%CO_2-buffered Ringers without glucose) for 10 or 20 minutes and then allowed to recover for between 3 and 5 hours (in oxygenated buffer). All slices are exposed to ^{3}H leucine (6 uCi/ml) for the last 90' of this recovery period. Incorporation of label into protein is stopped by further incubating the slices for 60' in unlabeled buffer containing 100 ug/ml cycloheximide to inhibit further protein synthesis and wash out unincorporated label. Slices are fixed in an ice-cold 2% paraformaldehyde-2.5% glutaraldehyde fixative and later sectioned (40um) with a freezing microtome, mounted on slides, dipped in Kodak NTB2 emulsion, and developed for autoradiographic analysis one week later. Developed autoradiographs are analyzed by microspectrophotometry. 230 um diameter areas are observed under dark field illumination. The light reflected by silver grains is recorded on a chart recorder and the average output in a region is calculated. Differences due to the ischemic treatment are expressed as percent of the control light output for a cell region.

The distribution of label in sliced tissue is not entirely homogeneous but rather is often patchy. The CA1 pyramidal layer exhibits more patchiness than either the granule cell layer or the neuropil. In contrast, interneurons, glia and capillaries appear to be uniformly labelled.

	Light output per unit area		
	CA1 layer	DG layer	Molecular Layer (DG)
Control tissue (Arbitrary units)	27.9	29.8	22.0
3 Hr recovery from 10' ischemia (% control)	46%	22 %	36%

10' ischemia dramatically reduces the incorporated label, measured after 3 hr recovery , in the major neuronal cell layers of the slice. However, label incorporation remains in glial cells, interneurons and capillaries; in fact, incorporation may be increased in post ischemic endothelial cells. These results demonstrate a selective vulnerability of the principle cells in the hippocampal slice to 10' of ischemia. The degree of inhibition in the granule cell layer is more profound than that in the CA1 pyramidal cells, a difference which does not reflect the greater vulnerability of CA1 cells to ischemic damage in vivo. In vivo, this early inhibition is reversed in dentate granule cells but not in CA1 cells. An increased endothelial cell protein synthesis may indicate stress protein synthesis, which is known to become elevated for relatively prolonged periods following such injuries as heat shock, ischemia, and decapitation trauma (Kiessling et al, J.Cereb.Blood Flow Metab. 1986).

182.3 THE EFFECT OF BLOCKING SODIUM INFLUX ON ANOXIC DAMAGE TO THE DENTATE GYRUS AND CA1 REGION OF THE RAT HIPPOCAMPAL SLICE. I.S. Kass, J.A. Boening, J.E. Cottrell. Anesthesiology Department, State University of New York, Health Science Center at Brooklyn, Brooklyn, New York 11203.

An interruption of the brain's oxygen supply leads to the irreversible loss of brain function. We have investigated the mechanism of anoxic damage using the hippocampal slice preparation. Recent studies have implicated high intracellular calcium as an important event leading to anoxic and ischemic brain damage. Sodium is another ion whose intracellular concentration increases dramatically during anoxia and ischemia. We therefore examined the effect of blocking sodium channels on anoxic damage.

Hippocampal slices from adult rats (100-120 days old) were superfused with oxygenated artificial cerebrospinal fluid (ACSF). To generate anoxia the fluid superfusing the slice was switched to one preequilibrated with 95% N_2 - 5% CO_2.

Two regions of the hippocampus were examined. The population spike in either the dentate granule cell layer or the CA1 pyramidal cell layer was examined after stimulating either the perforant path or the Schaeffer collaterals respectively.

When the dentate granule cells were studied they were subjected to 10 minutes of anoxia. Untreated slices showed virtually no recovery from anoxia ($\bar{x}$ ± S.E.M.; 3±3%). If the slice was treated with Tetrodotoxin (TTX) (200 µg/l) during and 5 minutes before and after anoxia the evoked population spike measured in the dentate granule cell layer recovered (43±10%). Thus blocking sodium channels with TTX provides protection against anoxic damage.

The CA1 pyramidal cells are more susceptible to anoxic damage than the dentate granule cells. There was no recovery of the population spike from the CA1 pyramidal cells after 5 minutes of anoxia (0±0%). When the hippocampal slices were treated with TTX (200 µg/l) during and 5 minutes before and after anoxia the response recovered from 5 minutes of anoxia (69±20%). Thus as with the dentate granule cells the CA1 pyramidal cells are protected from anoxic damage when sodium channels are blocked with TTX.

Possible mechanisms by which reduced sodium entry could lead to reduced anoxic damage include: 1) reduced ATP utilization 2) reduced depolarization and depolarization induced calcium entry, and 3) reduced cytosolic calcium secondary to a decrease in sodium induced calcium release from intracellular organelles. We are currently investigating some of these mechanisms.

182.4 PURIFICATION OF BOVINE BRAIN CYTOCHROME OXIDASE FROM SYNAPTOSOMAL AND NON-SYNAPTOSOMAL MITOCHONDRIA. R.F. Hevner* and M.T.T. Wong-Riley. Dept. of Anatomy & Cellular Biology, Med. College of Wis., Milwaukee, WI 53226.

Cytochrome oxidase (C.O.) is a mitochondrial enzyme used as a marker of energy metabolism in brain. C.O. histochemistry shows that neuronal C.O. activity levels change in response to altered functional (synaptic) input. We hypothesize that such C.O. activity changes reflect changes in the amount of enzyme present. This hypothesis can be tested by C.O. immunocytochemistry. While such studies have been successfully pursued using antibodies prepared against C.O. from heart, the existence of tissue-specific C.O. isozymes suggests that antibodies to brain C.O. might be optimal immunocytochemical reagents. We have isolated the brain isozyme of C.O. for use as immunogen by an affinity chromatography procedure optimized to give high yields (>30%) with high purity (~7.75 nmol heme a/mg protein). This procedure uses a cytochrome c-linked thiol-Sepharose 4B matrix to bind C.O. at low ionic strength, with elution by a potassium phosphate gradient (modified from Broger et al., Meth. Enz. 126:64-72, 1986). C.O. was prepared from synaptosomal and non-synaptosomal mitochondria separately in order to examine possible heterogeneity of C.O. within brain tissue. For isozyme comparison, bovine heart C.O. was purified as well. Heart, brain synaptosomal, and brain non-synaptosomal C.O. were compared electrophoretically using a high-resolution urea-SDS-PAGE system (Kadenbach et al., Anal. Biochem. 129:517-521, 1983) which separates C.O. into 13 subunits. No differences in subunit mobilities were evident between synaptosomal and non-synaptosomal populations of brain C.O. This result suggests that the majority of C.O. in brain consists of a single isozyme. A clear difference was observed in the mobility of subunit VIII between brain (M_r = 9.7 kDa) and heart (M_r = 9.9 kDa) isozymes, whereas no other differences in subunit mobilities were apparent between those two tissue isozymes. In particular, subunit VIa, which, like subunit VIII, differs imunologically between heart and brain C.O. isozymes (Kuhn-Nentwig and Kadenbach, Eur. J. biochem. 149:147-158, 1985), displayed identical electrophoretic mobilities in those isozymes. These studies confirm the existence of tissue-specific C.O. isozymes based on subunit VIII electrophoretic mobility differences, but provide no indication for the presence of more than one isozyme in a single tissue (brain). Antigenic specificity of brain C.O. suggests that antibodies to the brain C.O. isozyme will have optimal specificity and, possibly, affinity for use as reagents in immunocytochemical studies of cytochrome oxidase in brain. (Supported by MCW MSTP Fellowship to RFH and NIH NS18122 and EY05439 to MWR.)

182.5 A RAPID EXTRACTION AND ION-PAIRING HPLRC METHOD FOR THE QUANTITATION OF [3H]-INOSITOL PHOSPHATES IN RAT BRAIN. P.J. Egofske* and D.L. Hammond (SPON: R. Kochman). CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077

A number of pharmacologically important receptor systems are coupled to phosphatidylinositol hydrolysis. This second messenger response is commonly monitored in vitro by quantitation of tritiated inositol phosphates ([^{3}H]-IPs) after tissue preparations have been prelabelled with tritiated inositol. Current methods for the quantitation of [^{3}H]-IP's in rat brain require numerous solvent extractions and an anion-exchange chromatography system which is either laborious, lengthy or inadequately separates [^{3}H]-IP's. In order to circumvent these problems, we have developed a novel extraction and ion-pairing high performance liquid radiochromatography (HPLRC) method for the quantition of [^{3}H]-IP's.

Rat brain cortex cubes were prelabelled in batch using a modification of the procedure described by Berridge et al. (Biochem. J. 206:587, 1982). Fifty-ul aliquots of the prelabelled cubes were incubated in 900 ul buffer for 10 min, then 100 ul of a test compound was added. The incubations were continued for specified times after which the reactions were terminated by replacing the buffer with 1 ml ice-cold 40% methanol:H_2O (0.7N HCl). The samples were then sonicated and centrifuged. The supernatants were dried and pellets reconstituted for protein determinations. Each supernatant residue was reconstituted in mobile phase A and an aliquot injected for HPLRC analysis. HPLRC conditions consisted of a 15 min 0 to 100% B linear gradient at a flow rate of 2 ml/min using a reverse phase column; where mobile phase A = 100% H_2O (Pic A^{TM}) and B = 40% methanol:H_2O (Pic A^{TM}, pH 2.0). The eluent was collected into scintillation vials at a rate of 10 drops/fraction. Five ml PCS^{TM} containing 2N HCl was added to each fraction and vials were counted by LSC.

In situ spiking experiments demonstrated that the recovery of tritiated inositol, Ins1P_1, Ins(1,4)P_2, Ins(1,4,5)P_3, and Ins(1,3,4,5)P_4 through the procedure exceeded 95% for each agent. Only 1-3% of each compound was lost to degradation after extraction and three days storage of dried extracts at -20 °C. The detection limit was 2.1 femtomoles/[^{3}H]-IP/100 ul injection. Stimulation with 100 uM carbachol, a muscarinic agonist, produced significant time-dependent differences in the amounts of [^{3}H]-IP's as compared to control values. Thus, this method for measuring [^{3}H]-IPs can be used to assess the activity of drugs at receptors coupled to this second messenger system.

182.6 **RECEPTOR-MEDIATED PHOSPHOINOSITIDE BREAKDOWN IN HUMAN OCULAR CILIARY EPITHELIUM.** Martin B. Wax, M.D. and Miguel Coca-Prados, Ph.D.*, Depts. of Ophthalmology and Pharmacology, Univ. of Pennsylvania School of Medicine, Phila., PA and Dept. of Ophthalmology and Visual Science, Yale Univ., New Haven, CT.

The hydrolysis of phosphoinositides (PI) in peripheral tissues can be stimulated by a number of putative neurotransmitters and this stimulation can be blocked by specific antagonists. PI breakdown was studied in cultured human ciliary epithelial cells similar to those cells previously described (PNAS 83: 8754). Epithelial cells derived exclusively from the non-pigmented layer of the ocular ciliary epithelium were transformed by simian virus 40 and grown in culture to semi-confluency. The cells were incubated in 3 µCi/ml of (^{3}H)-myoinositol for 2 days. The accumulation of inositol phosphates in response to several agonists (carbachol, 1 mM; ATP, 100 µM; arginine vasopressin, 1 µM; and phenylephrine, 100 µM) was determined at 37°C for times ranging from 5 sec to 15 min. In the presence of 10 mM $LiCl_2$, the maximum net production of the (^{3}H)-inositol phosphates (expressed as % of conversion of (^{3}H)-phospholipids) was approximately 5% for inositol-1 phosphate (IP1), 0.5% for inositol-1,4 bisphosphate (IP2), and 1.0% for inositol-1,4,5 trisphosphate (IP3). Carbachol elicited PI hydrolysis with an EC_{50} value of 39±9 µm. The EC_{50} values obtained for Arg-8 Vasopressin and ATP initiated PI breakdown were 32 ± 10 nM and 11.9 ± 1 µM respectively. Phenylephrine alone failed to stimulate the production of (^{3}H)-inositol phosphates in these cells. The production of all (^{3}H)-inositol phosphates in response to carbachol (1 mM) was inhibited by the selective muscarinic antagonists pirenzepine (K_i=148 nM) and AFDX-116 (K_i=1.49 µM). These observed potencies are consistent with those found in other peripheral secretory tissues such as the lacrimal gland. Phorbol 12,13 dibutyrate (1 µM) rapidly desensitized the production of (^{3}H)-inositol phosphates by carbachol to 25% of control values. Pertussis toxin treatment reduced the PI hydrolysis elicited by carbachol (40% of control), Arg-8 Vasopressin (15% of control) and ATP (50% of control). Thus we have demonstrated a role for G protein regulation in PI coupled, receptor-mediated mobilization of intracellular calcium in response to several drugs in these cells.

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182.7 **CNS EFFECTS OF THYROID HORMONE INVOLVE STIMULATION OF CYTOSOLIC PROTEIN KINASE ACTIVITY.** L. Kragie*, M. Schoenl*, W.D. Lawrence*, P.J. Davis* (Spon: L. Hershey), SUNY/Buffalo School of Medicine and V.A. Medical Center, Buffalo, NY 14215.

Changes in thyroid hormone homeostasis cause profound neurobehavioral symptoms through unknown mechanisms. In a previous report we described changes in synaptosomal Ca^{2+}-ATPase activity and neuronal intracellular Ca^{2+} concentration upon direct exposure of synaptosomes or cells, respectively, to iodothyronines in vitro (Soc Neurosci Meeting 1985, abstract 164.10). We presently describe significant stimulation of phosphatidylserine-dependent protein phosphorylation in synaptosomal cytosol when the latter is incubated in vitro with physiological concentrations of L-thyroxine (T_4) and L-triiodothyronine (T_3).

P_2 pellet from NZW rabbit cerebral cortex was obtained by the method of Hajos (Brain Res 93:485, 1975) and subjected to hypotonic lysis. The post-50,000 x g supernatant was diluted in assay buffer and protein kinase activity was measured as phosphorylation over 10 min at 30°C in the presence of 10 µM unlabeled ATP, tracer [γ-^{32}P]ATP, 25 mM Tris (pH 7.4), 0.25 mM Ca^{2+}, 6.25 mM Mg^{2+}, 0.25 mM EGTA, 0.01% leupeptin, 5 mM mercaptoethanol, 0.4 mg/ml calf thymus type III-S histone. Variables were presence and absence of phosphatidylserine, 1,2-diacylglycerol and thyroid hormone analogs. Analog incubations were carried out for 60 min at 37°C prior to protein kinase assay.

INCREASE IN PROTEIN PHOSPHORYLATION
(pmoles/mg/min, mean ± SEM)

Control		10^{-11}M	10^{-10}M	10^{-9}M	N	P (ANOVA)
433±100	T_3	+138±40	+263±51	+155±60	5	0.00078
283±107	T_4	-	+175±57	+159±56	7	0.00087

Maximal effect (+60% in enzyme activity) was seen at 10^{-10}M with both T_4 and T_3. There was no stimulation of phosphorylation with D-T_4 or D-T_3 (10^{-11} M - 10^{-9}M). Significant effects were obtained in both male and female brain preparations and in fresh and previously-frozen tissues. Thus, physiologically relevant levels of thyroid hormone regulate brain cytosolic phospholipid-dependent protein kinase activity in vitro. Hormonal activation of the enzyme(s) is non-nuclear and represents a novel, direct mechanism which is independent of signal transduction at the cell membrane.

182.8 QUANTITATIVE HISTOCHEMISTRY OF ENZYME CHANGES OVER TIME IN RAT SPINAL MOTONEURONS FOLLOWING AXOTOMY. Chr. Marx* and A.C.Nacimiento. Neurosurgical Research Laboratory, Saarland University Medical School, D-6650 Homburg/Saar, F.R.G.

Peripheral nerve injury brings about remarkable metabolic changes in the affected cells, as reflected by posttraumatic activity shifts in various intracellular enzymes. Relevant data available for spinal motoneurons (SMN), however, show considerable discrepancies, particularly regarding quantification of time dependence. To set a base-line for the evaluation of enzyme changes in SMN during regeneration after peripheral nerve graft repair, we obtained a time-activity profile after unrepaired sciatic nerve transection. At 2, 4, 6 and 8 weeks after axotomy SMN were examined in 20um cryostat sections processed for histochemistry of the following enzymes: glucose-6-phosphate-dehydrogenase (G6PDH), 6-phosphogluconate-dehydrogenase (6PGDH), cytochrome-oxidase (CO) and acetylcholinesterase (AChE). Microphotometric extinction measurements of enzyme activity were made at randomized 5-10 cytoplasmic spots in single SMN on both control and axotomized sides. The values obtained were averaged, and the results expressed as the ratio axotomy to control sides. Control measurements in 5 intact rats gave a ratio of 1.0±0.1.The measurements are summarized as follows:

	axotomy/control ratio			
	2 ws(n=4)	4 ws(n=5)	6 ws(n=4)	8 ws(n=4)
G6PDH	1.64±0.10**	0.82±0.10*	0.81±0.09*	0.40±0.13**
6PGDH	1.45±0.06**	1.13±0.17	0.75±0.11*	0.50±0.10**
CO	0.99±0.08	0.70±0.14*	0.54±0.16**	0.50±0.14**
AChE	1.00±0.06	0.92±0.07	0.78±0.08*	0.61±0.13**

* p 1%-5%; ** p 0.1%

These results suggest that the validity of any histochemical correlation between posttraumatic changes in neuronal enzyme activity and regeneration processes depends essentially upon a) adequate selection of the enzyme systems involved; and b) quantitative evaluation under standardized measuring conditions assuring a high degree of resolution and reproducibility.

182.9 LOCALIZATION BY IN SITU HYBRIDIZATION OF THE LIVER ISOZYME OF GLYCOGEN PHOSPHORYLASE WITHIN THE HUMAN CORTEX. F. Gorin, P. Ignacio*, R. Gelinas*, R. Mullinax*. Dept. of Neurology, Univ. Calif. Davis Sch. of Med., Sacramento, CA 95817

Brain glycogenolysis is critical in meeting the acute energy requirements of the CNS during hypoxic-ischemic events, hypoglycemia, and seizures. Glycogen phosphorylase is the rate-determining enzyme for the degradation of glycogen. Protein gel electrophoresis has previously demonstrated that there are at least three isozymes of phosphorylase in non-primates and that the brain expresses the muscle (M) and fetal (B) isozymes.

We have isolated and characterized cDNA encoding a portion of the liver (L) isozyme of glycogen phosphorylase from a human fetal brain cDNA library (Gorin et al., J. Neurogenetics-in press). Because the presence of this isozyme has not been previously described in the non-primate mammalian brain, we decided to investigate transcription of the L- phosphorylase gene in human brain and cytologically localize L- phosphorylase within the human and macaque cortex. RNA blot data indicates that the L- phosphorylase gene is transcribed in human fetal (19 week) and adult brain, whereas the M- phosphorylase gene is transcribed in adult but not fetal brain. Ochterlony data using sheep anti-rat L- phosphorylase polyclonal antibody (Osawa et al., FEBS 202:282, 1986) demonstrates the presence of the L- phosphorylase protein as well as transcription of the L- phosphorylase gene in human adult brain.

In situ hybridization using a 3' portion of the human L-phosphorylase cDNA demonstrates specific transcription of this isozyme in human and macaque cortex in white matter but not in grey matter. Counterstaining the tissue sections with a modified Bodian stain indicates that this white matter transcription occurs within glial elements present in white matter. (This work was supported by NIH grant NS 23644)

182.10 AUTORADIOGRAPHIC DEMONSTRATION OF GLYCOGEN IN THE RAT BRAIN, AND CHANGES INDUCED BY TACTILE STIMULATION. R.A. Swanson, F.R. Sharp and S.M. Sagar. Neurology Service, V. A. Medical Center and Department of Neurology, University of California, San Francisco CA 94143.

Glycogen is the single largest energy reserve in the brain, and under normal conditions brain glycogen turnover is several orders of magnitude faster than in liver. However, histochemical demonstration is not feasible due to rapidity of postmortem glycogenolysis and solubility in water-based fixatives and stains. Even by the use of microwave fixation and an ethanol-based PAS staining method, we found that normal brain glycogen levels were too low to stain. However, by the using [^{14}C] glucose or [^{14}C] 2-deoxyglucose precursors, we were able to demonstrate brain glycogen autoradiographically and detect local changes in glycogen metabolism in response to physiologic stimulation.

Rats were injected with 200 microcuries of [^{14}C] glucose or [^{14}C] 2-deoxyglucose and 20 minutes later sacrificed by microwave irradiation *in situ*, which abruptly stops glycogenolysis. Brains were removed, dehydrated in ethanol, delipidized in xylene, and embedded in paraffin. Twenty micron sections were cut and mounted on coverslips in an ethanol:glycerol bath. The sections were washed in hexane:isopropyl alcohol to remove paraffin and glycerol, and free glucose and 2-deoxyglucose were removed in washes of 80% ethanol. Autoradiographs were produced by exposing the sections to X-ray film for 5 days. The same or adjacent sections were then treated with amyloglucosidase to remove glycogen, and again exposed to film. The difference in optical densities of the images so produced were attributed to radiolabeled glycogen.

Differences in glycogen labeling were observed in response to physiologic stimulation. Rat whiskers were manually stimulated unilaterally during the 20 minute interval between radiolabel injection and microwave irradiation. Increased labeling was seen in the ipsilateral trigeminal sensory nucleus. This increased label may reflect either locally increased glycogen synthesis or increase flux of [^{14}C] labeled precursor during the stimulation. By correcting for changes in local glucose uptake, it may be possible to observe physiologic changes in glycogen metabolism at the cellular level.

182.11 RETINOIDS IN THE PINEAL BODY OF THE BOVINE BRAIN. A. T. C. TSIN AND T. S. WEBER*. The University of Texas at San Antonio, San Antonio, Texas 78285.

In mammals, the pineal gland is a brain structure of photoreceptor origin. The photopigment and retinoid contents of the bovine pineal gland have not previously been investigated. In the present study, bovine pineal glands (n = 10) were analyzed by high performance liquid chromatography for levels of all-trans retinol, retinal, and retinyl palmitate. These retinoids were extracted from all tissue samples by acetone, then purified by the use of an alumina column. The amount of all-trans retinol present was 58.71 pmole/gm pineal tissue, or 8.81 pmole/pineal gland. This is a small amount compared to the 4.84 nmole of all-trans retinol/gm tissue detected in fresh retina samples. However, it is approximately comparable to that in the muscle, ie: 32.66 pmole all-trans retinol/gm tissue. There was no all-trans retinal detected in the pineal glands. Of the different tissues assayed (retina, RPE, liver, muscle, brain cortex and subcortex), only the retina contained all-trans retinal, in the amount of 2.05 nmole/gm, or 1.34 nmole/retina. The level of all-trans retinyl palmitate present in pineal gland was 224.86 pmole/gm or 33.68 pmole/pineal gland, significantly higher than other brain tissue (with no detectable level of retinyl esters). The presence of a significant level of retinoids in bovine pineal glands may be cause to reevaluate our present understanding of pineal gland function in mammals. (Supported by Grant Numbers RR-08194-07 and GM-07717)

182.12 A HISTOCHEMICAL STUDY OF THE EFFECTS OF CHLORAL HYDRATE ANESTHESIA ON CEREBRAL METABOLIC ENZYMES. M.J. Ulissey* and M.E. Trulson (SPON: B.H. Rohde). Dept. Anat., Coll. Med., Texas A&M Univ., College Station, TX 77843.

Chloral hydrate is widely used as an anesthetic in neurophysiological experiments in animals. However, electrophysiological changes have been observed during anesthesia with the drug. Since many data have been collected from electrophysiological experiments in which chloral hydrate was used as an anesthetic agent, we examined the effects of chloral hydrate anesthesia on brain metabolism. Five key enzymes responsible for aerobic and anaerobic metabolism and the hexose-monophosphate shunt in the cerebral microvasculature and neuropil of rats were examined during chloral hydrate anesthesia. Male Sprague-Dawley rats were divided into 2 groups. Three rats served as control and received i.p. injections of physiological saline. The other 3 rats served as experimental animals and were anesthetized at surgical levels for 1 hour with chloral hydrate (400 mg/kg, i.p.). While the experimental animals were still anesthetized, all 6 animals were killed by decapitation. Forebrain tissue sections (including primarily the cortex and caudate nucleus) were immediately taken and frozen in cold isopentane. Sections were cut at 6 μm on a microtome-cryostat. Fresh frozen cryostat sections were then used to quantitate the following enzymes: glucose-6-phosphate-dehydrogenase (G6PDH), cytochrome oxidase (CO), lactate dehydrogenase (LDH), β-hydroxybutyrate dehydrogenase (βHBDH), and isocitrate dehydrogenase (ICDH). Enzymes were quantified using a Leitz-Data Acquisition and Display System. Ten arterioles and 10-15 μm x 15 μm sections of neuropil from each rat for each stain were analyzed. Significant decreases were observed in CO (-46%) and βHBDH (-28%) in arterioles, while G6PDH (+15%) and ICDH (+29%) showed a significant increase and LDH showed no significant change. In the neuropil, CO (+13%), ICDH (+12%) and G6PDH (+25%) showed significant increases following chloral hydrate while βHBDH and LDH showed no significant changes. The fact that rate-limiting enzymes of key metabolic pathways in brain were significantly altered during chloral hydrate anesthesia may have implications for electrophysiological changes that have been observed during chloral hydrate anesthesia, as well as responses to pharmacological agents.

182.13 ETHANOL ALTERS FOREBRAIN METABOLIC ENZYMES IN THE RAT. M.S. Cannon,* M.J. Ulissey,* and M.E. Trulson (SPON: M.S. Amoss). Dept. Anat., Coll. Med., Texas A&M Univ., College Station, TX 77843.

The potential effects of ethanol on cerebral metabolic enzymes have not been investigated. Therefore, in the present study we examined the effects of acute ethanol administration on enzymes involved in aerobic and anaerobic metabolism. Male Sprague-Dawley rats were used in all experiments. Three rats served as controls and were given physiological saline orally. The other 6 rats served as experimental animals and were administered a 50:50% solution (3 ml) of ethanol:physiological saline orally. After 1 hour the control animals and 3 of the experimental animals were killed by decapitation. Forebrain tissue sections were immediately taken and frozen in cold isopentane. The other 3 experimental animals were killed 48 hours after administration of ethanol. The sections were cut at 6 μm on a microtome-cryostat and fresh-frozen cryostat sections were then used to quantitate the following enzymes: glucose-6-phosphate-dehydrogenase (G6PDH), cytochrome oxidase (CO), lactate dehydrogense (LDH), β-hydroxybutyrate dehydrogenase (βHBDH), and isocitrate dehydrogenase (ICDH). The enzymes were quantified using a Leitz-Data Acquisiton and Display System. Ten arterioles from each rat for each stain were analyzed. The tissue sections obtained from the 1-hour treatment with ethanol showed the following statistically significant changes in arteriolar enzyme levels: CO was decreased by 26.3%, βHBDH was decreased by more than 98%, and G6PDH showed an increase of 79.9%. The animals that were allowed to recover for 2 days following ethanol administration demonstrated the following changes in enzyme levels: ICDH was increased by 41.6%, CO showed a further significant decrease to 63.2% below the control animal enzyme levels, βHBDH demonstrated an increase to 92.7% over control animal enzyme levels, G6PDH showed an increase of 115.0% over control animal enzyme levels and LDH showed an increase of 12.2% over control animal enzyme levels. These data suggest that the administration of ethanol impairs forebrain metabolism by attacking key enzymes within aerobic and anaerobic pathways. Arterioles play a critical role in adjusting perfusion to the metabolic requirements of tissues. The altered enzyme levels observed following ethanol may result in impaired vasomotor adjustments. These changes in enzymes involved in brain metabolism may account for some of the characteristic behavioral manifestations of ethanol intoxication.

HUMAN BEHAVIORAL NEUROBIOLOGY I

183.1 REGIONAL CEREBRAL BLOOD FLOW IN NORMAL AND BIPOLAR FEMALE SUBJECTS DURING A COGNITIVE ACTIVATION TASK. L. Flowers*, F. Wood*, and N. Gaby* (Spon: K. Campbell). Section of Neuropsychology, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Cortical responses to an induced behavioral state can be inferred indirectly by the regional cerebral blood flow method (rCBF). This method reliably reflects local metabolic changes during sustained sensory, motor, or cognitive activation.

Twenty-three normal and sixteen bipolar females engaged in three repetitions of a verbal recognition memory task. Subjects made a bimanual finger-lift response to indicate recognition of target words. rCBF was measured during the first and third trials by eight NaI scintillation detectors over each hemisphere. Presence and degree of manic and depressive symptoms in the bipolar group was determined on the day of testing. Eleven subjects were currently in a manic phase and five were mainly depressed.

Subjects differed in behavioral performance as well as blood flow, means, and variances. Normals performed more accurately and improved more over trials than did bipolars. On Trial 1, bipolar manics but not depressives differed significantly from normals in terms of behavioral performance. Trial 3 performances on this recognition memory task revealed a behavioral deficit common to both phases of bipolar affective disorder.

Normal subjects had the highest blood flow values at nearly all sites; bipolar depressives were next highest and bipolar manics had the lowest flow values. Bipolar flow, in general, was more variable on Trial 1 compared to normal flow. However, on Trial 3, the variability among bipolar depressives was similar to that of the normal subjects while the variability among bipolar manics remained high.

These findings suggest that the depressed phase of bipolar disease does not represent as severe a deficit in brain activation as is represented in the manic phase; rather, there appears to be a rough continuum along which depressives occupy an intermediate position between normals and manics. Among the manic subjects, there is a significant positive correlation between presence of depressive symptoms and mean flow values; that is, bipolar manics with some depressive symptoms had higher flow values, more similar to bipolars in the depressive state.

On the initial trial, bipolar depressives are more accurate than are manics. This suggests that, in the depressive phase, novelty may temporarily counteract the deleterious effects of depression on memory.

183.2 MODULATION OF THE ACOUSTIC STARTLE RESPONSE IN THE HUMAN BY TONE ONSET AND OFFSET. S.J. Lane, E.M. Ornitz*, D. Guthrie.* Department of Psychiatry, Division of Mental Retardation and Child Psychiatry, and Brain Research Institute, UCLA. L.A., CA, 90024

Modulation of the startle response (SR) is a polysynaptic brainstem function subject to mediation by cortical attentional mechanisms and intrinsic brainstem mechanisms (see Davis, 1984 and Bruno, 1985 for review). A predictable relationship exists between onset of auditory warning stimuli (WS) and the SR such that short discrete (SD) WS inhibit, while long continuous (LC) WS tend to facilitate SR magnitude. Latency influences are similar albeit more variable. Modulation in the human of auditory startle by offset of auditory stimuli has been less well defined and quantitative comparison of the effectiveness of onset vs offset is lacking. 3 experiments were designed to contrast onset and offset effects and to examine the influence of experimental context on SR modulation. Exp.1 examined modulation using both SD and LC tone onset trials. Exp.2 utilized a continuously present background tone (CT) and examined SD and LC tone offset in SR modulation. Exp.3 examined the influence of both tone onset and offset within the same experiment. Within each experiment responses to the various WS were compared to responses to unwarned startle stimuli. Results indicated that the unwarned SR, measured as eyeblink, was remarkably predictable on both amplitude and latency measures regardless of its occurrence within a background of CT or silence. Additionally, SD onset in Exp.1 and offset in Exps.2 and 3 consistently inhibited SR amplitude (Exp.1 $p<.0001$; Exp.2 $p<.0004$, Exp.3 $p<.007$). LC influences tended to facilitate SR amplitude somewhat more variably depending upon experimental context. Planned contrasts comparing onset and offset influences across experiments indicated that SD WS produced significantly greater SR amplitude inhibition in Exp.1 than in Exps.2 or 3. The trend toward amplitude facilitation following LC WS was significant only in Exp.3 ($p<.02$). Latency responses parallel those for amplitude. Onset of the SD tone significantly inhibited latency in Exp.1 ($p<.004$) and this response was greater than that seen in either Exps.2 or 3. Latency facilitation by the LC WS in Exp.3 was significantly greater than that observed in Exps.1 or 2 ($p<.05$). These results indicate that the SR is modifiable by antecedent WS of either onset or offset natures. Additionally, the degree of effectiveness in SR modulation is linked to experimental context. Finally, in contrast to Graham's suggestion of 2 separate neuronal systems mediating amplitude and latency modulation of the SR, the parallel relationship between these 2 SR parameters seen in our studies, regardless of the onset/offset characteristic of the WS, suggests that the same or similar neuronal mechanism(s) may mediate SR modulation.

183.3 TEMPORAL PERFORMANCE DEFICIT OF SCHIZOPHRENICS IN THE COLOR-WORD TEST (STROOP) - R. Hess, M. Seidlitz*, B. Reinhard*. Department of Psychiatry, University of Göttingen, FRG.

The peformance of schizophrenic patients and normal controls in a modified Stroop (color-word) test was investigated. The modification consisted in the potential to vary the frequency of presentation of the stimuli, such that a minimum presentation period could be determined for each person to fulfill the criterion of less than 10% errors during successive trials within 2 minutes.

Stimuli were the color words red, blue and yellow, each written in an ink different from its meaning. Two conditions were tested, namely 1. reading the word ignoring the color and2naming the color ignoring the meaning of the word. As further control served a series of 3-letter nonsense words written in the same colors and again tested in the 2 possible conditions (3 and 4). The different tasks were presented in the sequence 1 to 4 with brief breaks between successive tasks. Presentation time for a single stimulus could be varied from 800 msec. upwards with no interstimulus interval between two successive stimuli.

The results indicate that, in case of nonsense words, both tasks were performed about equally well, and there was no difference between schizophrenics and controls.

All persons had most difficulties in naming the color and ignoring the meaning of the color-word. This is consistent with previous reports and has been explained with an "overtraining" to perceive the meaning of a written word.

The only striking difference between schizophrenics and controls was in the most difficult task (name color, ignore word) where controls needed presentation times of 800 to 1000 msec. to perform at the level of criterion, while all schizophrenics needed presentation times longer than 1000 msec. some up to 2000 msec. So far there was no overlap between the 2 groups.

Hypotheses explaining this dysfunction in schizophrenics and its possible meaning to the pathogenesis of the disease will be discussed.

183.4 **CENTRAL MECHANISMS OF STEREOPSIS IN MAN AND CATS.** Maryse Lassonde and Maurice Ptito. Groupe de Recherche en Neuropsychologie expérimentale, Université du Québec à Trois-Rivières, Qué. G9A 5H7, Canada.

Stereopsis or binocular depth perception is usually thought to depend on the integration by cortical cells of the binocular information transmitted by two pathways: 1) the geniculo-striate pathway, via the **optic chiasm**; 2) the more indirect interhemispheric route, via the **corpus callosum**. The present set of studies investigated the selective effects of lesions of either pathway and assessed the consequences of striate cortex damage on binocular depth perception. **Human studies:** Four callosal agenesis subjects, five commissurotomized patients, three subjects with unilateral occipital damage and one chiasmatectomized patient were compared to 20 control subjects on random-dot stereoscopic tasks. Results indicate that patients without corpus callosum are able to succeed at most tasks although they show higher thresholds than IQ-matched controls. Similar results were obtained with occipitally-damaged patients when tested in their intact visual field; no stereopsis was found in their blind field Although midline stereopsis was observed in the chiasmatectomized patient, his stereoscopic abilities were very limited. **Animal studies:** Normal cats and cats with lesions of the optic chiasm, the corpus callosum or the striate cortex were required to discriminate random dot stereograms. Results parallel the findings obtained in the human studies, namely: a) callosal section produces little or no impairment in stereoperception; b) chiasmatectomy drastically reduces the ability of cats to perform the random-dot task whereas c) bilateral occipital removal abolishes binocular depth perception. Taken together, these results confirm that binocularity is dependent upon the integrity of the striate cortex and that the optic chiasm is the most important pathway in conveying binocular information.

183.5 CEREBRAL MORPHOLOGY ON MAGNETIC RESONANCE IMAGING IN DEVELOPMENTAL DYSPHASIA. T.L. Jernigan, P.A. Tallal*, and J. Hesselink* (SPON: D. Trauner). V.A. Med. Ctr., San Diego, CA 92161, Depts. of Psychiatry and Radiology, UCSD Sch. of Med., La Jolla, CA 92093.

A group of well-defined developmentally language disordered children, who have been followed longitudinally since age 4, were studied with magnetic resonance imaging (MRI) at the age of 8. At entry to the longitudinal study, all subjects had severe developmental receptive and expressive language delay that could not be attributed to hearing loss, mental retardation (Performance IQ exceeded 90), autism, or known damage to the CNS.

Subjects have received extensive behavioral evaluations annually to assess neuropsychological and linguistic development. In addition, academic achievement and social and emotional outcomes were assessed. At the time of the present study, all subjects continue to demonstrate severe developmental language delay.

Previous studies of these subjects (Tallal, P., Stark, R., Mellits, E.D. Neuropsychologia, 23:527-536, 1985) have revealed a specific defect in the processing of rapidly changing sensory stimuli, which predicts the degree of receptive language impairment (r=.89). The neurological substrate of this behavioral deficit is unknown.

Clinical neuroradiological evaluation of the images from the first 9 language impaired subjects revealed significant ventricular asymmetry in one (R)L), and parenchymal abnormalities in the deep white matter in two other of the subjects. No abnormalities were detected in the remaining six subjects. Quantitative measurements of cerebral size and hemispheric asymmetry have been completed and will be discussed in relation to the patterns of linguistic defects. Measures of signal values in white and gray matter areas have been made to attempt to assess degree of myelination. These results will also be presented and discussed, and contrasts between these children and globally retarded children will be made.

183.6 **CORTICAL PHYSIOLOGY DURING AUDITORY DISCRIMINATION IN SCHIZOPHRENIA: A REGIONAL CEREBRAL BLOOD FLOW STUDY.** K.F. Berman, S.C.W. Rosenbaum*, C.A. Brasher*, T.E. Goldberg*, D.R. Weinberger.
Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

We have previously investigated cortical physiology in schizophrenia using a variety of visually presented tasks, some linked to prefrontal cortex and some not, and have demonstrated prefrontal deficits primarily during specific prefrontal tasks. The present study was carried out to investigate regional cortical blood flow (rCBF) during auditory discrimination, a possible temporal lobe task.

The xenon133 inhalation technique was used to measure gray-matter rCBF, an indicator of cortical metabolism, in 15 normal subjects (mean age±SD 29.9±4.9 years) and 10 patients with chronic schizophrenia (age 30.5±6.9 years), all medication-free for at least four weeks. After an initial resting state rCBF procedure, which served to acclimate subjects to the testing procedure, two rCBF measurements were made during two different auditory tasks, which were presented in counterbalanced order. One task involved discrimination between pairs of sounds which consisted of rhythm patterns or speech sounds. For the other task, which served as a baseline control task, subjects heard the same sound pairs as in the discrimination task, but they were not asked to differentiate between them.

rCBF data are shown in the Table.

	PREFRONTAL	PRECENTRAL	TEMPORAL	PARIETAL	PARIETO-OCCIPITAL
Discrimination:					
normals	78±8	73±6	69±7	69±7	67±7
patients	70±8	68±9	62±8	64±9	61±7
p value	.05	.10	.05	.12	.04
Control Task:					
normals	79±7	75±6	70±6	70±6	67±6
patients	72±11	69±10	64±10	65±10	62±8
p value	.09	.11	.10	.11	.08

Patients tended to have lower rCBF in all regions during both conditions, and this reached significance at the $p<.05$ level in prefrontal, temporal, and parieto-occipital regions during the discrimination task. In normal subjects, proficiency on the discrimination task was unexpectedly associated with lower temporal rCBF, especially on the left ($r=-.57$, $p<.03$), as measured during performance of the task. Similar correlations, however, were also found with rCBF measured during the resting state.

These data suggest that, patients with schizophrenia may exhibit cortical functional abnormalities during an auditory dicrimination task. In contrast to our earlier finding with rCBF during a prefrontal task, the abnormalities do not appear to be regionaly specific or to be specific to cognitive state.

183.7 DECREASE IN SHIVERING BY MENTAL COMPUTATION. C. Klenow, F. Simmons, and R. S. Pozos. Dept. of Physiology, University of Minn., Duluth, School of Medicine, Duluth, MN 55812.

Shivering reflects the body's attempt to produce heat during cold exposure. This effector response is mediated via motor nerves and is thought to be initiated by central and peripheral thermal stimuli. Shivering as measured by electromyographic recordings demonstrates synchronized bursts of electrical activity over the body. Little research has been done on how to minimize the shivering except for the study by Martin and Cooper. They reported that shivering subjects who did mental arithmetic and isometric contractions were able to significantly decrease shivering (1). This study was undertaken to further analyze this phenomenon and quantitate any decrease in shivering that might be induced by having human subjects do mental computation.

Eight subjects (4 male and 4 female) who were lightly clad laid prone on a table and were individually subjected to a cold environment (30-32°F) for approximately 40-55 minutes. Electromyographic recordings were made using surface electrodes from five different muscle groups (masseter, trapezius, extensors and flexors of the wrists, and rectus femoris). After the onset of shiver, the subjects were asked to do simple arithmetic assignments. These consisted of having the subject hold a card in his hand and add four different sets of double digit numbers and call out the appropriate response. The control consisted of having the subject hold a blank card for the same period of time that he was holding the experimental card.

A decrement in the amplitude of shivering was seen in all eight subjects during the arithmetic task. Shivering returned when the task was completed. During the control studies, there was no decrease in the amplitude of shivering. Paired t-test analysis showed that there was a statistical difference between the RMS value before shivering and during the math tasks. In addition, there was a significant difference in the RMS value between math computation and the return of shiver. There was no difference between the shivering amplitudes before and after the math computation. This data indicates that the cerebral cortex must play some role in controlling the oscillator(s) responsible for the onset and maintenance of overall body shiver.

(1) Martin, S. and Cooper, K. 1981. Pflugers Arch. 391: 81-83.

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183.8 DISSOCIATION OF ABSTRACT REASONING ABILITIES BY LOCUS OF HEAD INJURY. A. Cronin-Golomb, W. A. Rho,* and S. Corkin. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139.

The ability to reason abstractly comprises multiple skills that may involve different brain regions. Twenty-seven veterans of the Korean Conflict were tested for the ability to reason abstractly, using two nonverbal tests that measure different aspects of this function. The subject group included 13 veterans with right-sided penetrating head injury (RHI), 9 with left-sided penetrating head injury (LHI), and 5 uninjured veterans who served as age-, education-, and experience-matched control subjects. Each subject performed two tests: the Hukok Logical Thinking Matrices Test (Daryn, 1977), which measures the ability to extend logically an existing pattern by incorporating new elements ("generational" ability); and the Abstract subtest of the Concept Comprehension Test, or CCT-A (Cronin-Golomb, 1986), which examines the ability to relate common objects on an abstract basis ("relational" ability).

The uninjured control subjects scored $\bar{X}$=6.6, sd=1.7 on the Hukok and $\bar{X}$=20.0, sd=1.4 on the CCT-A. The HI subjects as a group performed within the normal range, though not as well as the control group, on the Hukok ($\bar{X}$=5.7, sd=1.8) and the CCT-A ($\bar{X}$=19.1, sd=3.3). No significant differences were found between the RHI and LHI groups for either test. The HI groups were then subdivided by locus of lesion, as determined from medical report and CT scan for each subject. Of the original 22 HI subjects, 13 had injuries that were contained within a single lobe: frontal, parietal, temporal, or occipital. Subjects with exclusively frontal- (N=4) or occipital-lobe (N=1) lesions performed the two tests at the normal control level. The parietal-lobe group (N=5) performed the CCT-A at the normal level ($\bar{X}$=20.4, sd=2.7), but was impaired on the Hukok test ($\bar{X}$=5.0, sd=2.0). In contrast, the temporal-lobe group (N=3) outperformed the parietal-lobe group on the Hukok ($\bar{X}$=5.3, sd=1.5), but showed substantial impairment on the CCT-A ($\bar{X}$=15.0, sd=4.6). Small sample sizes precluded analysis of the data by side of injury for each lobe.

The results suggest that different abstract reasoning abilities may be localized to different brain regions. Specifically, optimal performance on a test of generational ability may require intact temporal and especially parietal lobes; achievement of normal scores on a test of relational ability appears to require intact temporal lobes exclusively.

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183.9 STIMULUS CANCELLATION PATTERNS IN MONKEYS WITH HEMINEGLECT. R.K. Deuel and S.P. Schaffer*. Departments of Pediatrics and Neurology, Washington University School of Medicine, St. Louis, MO 63110

We have previously found that monkeys with hemineglect always retire the ipsilateral of two simultaneously presented visual stimuli before beginning to address the one contralateral to their lesion (Deuel and Collins, 1984). It has recently been shown that humans with hemineglect after stroke fail to cross out stimuli (an array of lines on a piece of paper) contralateral to their lesions, but improve when the task is to eliminate (by erasing) the stimuli as they address them (Mark, Kooistra and Heilman, 1987). We therefore hypothesized that monkeys with hemineglect when presented with an array of visual stimuli, distributed equally to left and right of visual fixation, would address this array most efficiently if they used the strategy of starting in the ipsilateral field and eliminating stimuli already addressed. To test this hypothesis cynomologus monkeys were tested with a board encompassing an array of bait at six loci (in three columns of two) that was centered directly in front of the monkey at eye level, before and after frontal (FR, N = 11) or parietal (PA, N = 11) lesions. The hand ipsilateral to the lesion (or the future lesion) performed the motor response. Preoperatively animals showed no tendencies to start at any particular locus and the speed of board emptying was the same wherever emptying was initiated. Postoperatively both groups strongly preferred to begin bait retrieval in the ipsilateral column (and the column closest to the active hand). The FR group used this strategy significantly more frequently than the PA group (χ^2 = 4.27, df = 1, $p < .04$). Despite the frequency with which the frontal group used this strategy, it did not demonstrate greater efficiency at bait retrieval than the PA group, and in fact often left the contralateral side of the array unperturbed. As only the unaffected hand, ipsilateral to the lesion, was active and no eye movement deficits were noted, an explanation in terms of deployment of attention in space still seems likely, but data from human subjects suggests that the explanation of the different patterns of retrieval used by the two groups may differ according to separate pathophysiologies related to lesion locus.

183.10 WHERE DO HEMIANOPSIC PATIENTS FIXATE WHEN THEY LOOK STRAIGHT AHEAD? J. Sergent, G. Bertrand*, and J. G. Villemure*, Dept. of Neurol. Neurosurg., McGill Univ., and Montreal Neurological Inst., Montreal, QC, H3A 2B4, Canada.

Blindness in one visual field produced by destruction of the geniculo-striate pathways or striate cortex is a negative symptom due to absence of sensation. As a result, the patient may be unaware of the blind field and, despite the reduced area of vision, the whole visual field can still be scanned through head and eye movements. However, because of the hemianopsia, the area of highest acuity normally used for fixation falls just at the border of the blind field, which is not convenient to focus on a target. In the course of perceptual experiments on hemianopsics, considerable difficulty was encountered when the patients were requested to fixate a central point. The present study was designed to examine the accuracy of eye fixation in hemianopsics when they have to look straight ahead at a fixation point. The experiment consisted first in measuring Snellen acuity of each patient, then in constructing stimuli made of a row of single letters or digits of a size corresponding to each patient's Snellen acuity, and finally in presenting these stimuli tachistoscopically. Given that Snellen acuity corresponds to the resolving power of the fovea, by requesting the patient to look straight ahead and by presenting, on the horizontal meridian, alphanumeric characters that only the fovea can resolve, the location of the identified character with respect to the center of the visual field would indicate where the patient is actually fixating. While control subjects always identified the character the closest to fixation, there was marked deviation of the eyes toward the blind field in all hemianopsic patients, irrespective of the side of the lesion and of the presence of unilateral neglect. The extent of eccentric fixation ranged from 0.9° to 3.6°, with a mean of 2.5°. In order to correct for this deviation when accurate fixation is required, a simple method was devised whereby the right and the left visual fields were colored in pale blue and pale green of equal luminance, respectively, along with a small black square located at the border of the vertical meridian in the intact field, 3° above and below the central fixation point. When instructed to fixate the central point so that only one color and the two squares were seen, all but one patient reported the character the closest to fixation on the identification task. Eccentric fixation in hemianopsics does not reflect a so-called pseudo-fovea, but an uncontrolled adaptive response to the deficit so that the object of attention is perceived within its surrounding context.

183.11 PERCEPTUAL AND MOTOR SET EFFECTS ON VERY SHORT-LATENCY COMPONENTS OF VERs. Marta Oakley & Robert G. Eason, Department of Psychology, UNCG, Greensboro, N. C. 27412.

Evidence that the behavioral state of an individual can influence short-latency components of visual, auditory, and somatosensory EPs continues to mount (Oakley, et al, NS. Abstr., 1985, 1986; Woldorff, et al, NS Abstr., 1986; McCarthy & Wood, NS Abstr., 1986). However, the question of whether behavioral influences on these components involves precortical gating mechanisms remains unresolved.

The present study was undertaken to provide further information on this issue, and also to ascertain whether motor set, along with perceptual (or sensory) set, contributes to the responsivity of subcortical elements prior to the arrival of poststimulus information at the cortex. Anatomical and physiological data are suggestive of this possibility (e.g., Hammond, J. Physiol., 1956; Evarts & Tanji, Br. Res., 1974; Robinson, et al, J. NS, 1985; Schlag & Schlag-Ray, J. Neurophysiol., 1984; Wurtz & Goldberg, J. Neurophysiol., 1972).

Using the paradigm of Eason, et al (Phy. & Beh., 1969), subjects were required to respond to target stimuli (doublet flashes) appearing 30° peripherally in the relevant (i.e., attended) visual field by (1) making an eye movement to that location, (2) making a foot-lift response, or (3) keeping a silent count of the doublets.

VERs were obtained at frontal and parietal sites (F3,4 and P3,4) of each hemisphere to 100 single flashes presented randomly from within each visual field (one attended, the other not) during which time subjects were set to make one of the types of responses to doublets which were randomly interspersed among the single flashes. The flashes subtended a visual angle of 35' and were about 2.7 log units above threshold. VERs were obtained only to the single flashes to which an overt response was not required.

An early segment of the VERs lying within a latency range of 40-70 msec poststimulus was significantly affected both by the relevancy of the visual field and by the type of response the subject was set to make. When set to count doublets, the segment was relatively more positive when a given visual field was being attended to than when it was not. When set to make eye movements, the segment was relatively more negative under the attend than under the unattend condition. Non-significant results were obtained for the foot-lift condition.

The short latency, low amplitude, and homogeneous distribution of the polarity of the segment across recording sites suggests it was generated within subcortical structures of the visual system. The observations suggest that both selective attention (perceptual set) and motor set can influence the responsivity of the visual system prior to the arrival of poststimulus information at the cortex.

183.12 **BIOBEHAVIORAL RELATIONSHIPS IN DYSLEXIA.** D.W. Shucard, K.R. Cummins* and J.L. Shucard*. Department of Neurology, State University of New York at Buffalo, School of Medicine, Buffalo, NY 14222.

Dyslexia is a disorder characterized by difficulty in learning to read despite conventional instruction, adequate intelligence and socio-cultural opportunity. Evidence derived largely from behavioral studies suggests that dyslexia is most likely a heterogeneous disorder that, in at least some reading-disabled (r-d) children, may be related to a disturbance in the normal functional organization of the brain (e.g. a disturbance in right-left cerebral organization, a failure to develop adequate left hemisphere specialization for language, a disturbance in normal inter-hemisphere transfer of visual-phonemic information). The purpose of the present study was to investigate hemispheric patterns of auditory probe event-related potentials (AERPs) in a large sample of r-d and control subjects in order to determine: 1) if differences in functional cerebral organization exist between normal and disabled readers, and 2) if it is possible to identify different subtypes of individuals within the r-d population through the combined analysis of electrophysiological and behavioral data. Previously, we reported significant differences in the patterns of AERP amplitude asymmetries between 12 - 16 year old normal and disabled readers matched for age, sex and handedness. In this report we describe relationships obtained between electrophysiological and behavioral measures. For example, a significant relationship was found between AERP measures of cerebral asymmetry and the discrepancy between spatial and sequential abilities, derived from the WISC-R, in the r-d subjects ($r = -.42$, $p < .01$). These findings indicate that the greater the spatial score compared to the sequential score, the higher the amplitude of the left temporal AERPs compared to the right in r-d subjects. Normal controls matched for sex, age and handedness did not show any significant correlation between these two variables. These findings suggest that the AERP asymmetry may reflect a neurophysiological basis for reading disability in at least one group of children studied. These results may be indicative of a reduced capacity for sequencing skills in r-d children due to a disturbance in left hemisphere functioning.

183.13 ENCOUNTERING VIOLATIONS IN MEANING: THE N400 AND TEXT COMPREHENSION Banghart, J.G., Danks, J.H., and Vardaris, R.M., Dept. Psychology, Kent State University, Kent, OH 44242

The results of several studies (Kutas & Hillyard, 1980a, 1980b, Neville, 1986) have led resarchers to postulate that the N400 ERP component is an index of semantic integration. Psycholinguistic theorists hold that text consists of large units of meaning, called macrostructure or propositions. Manipulations of these larger units result in changes in meaning of the text by, for example, contradicting some previously stated information. Behavioral studies (e.g Danks & Hill, 1981; Danks & Rittman, in prep.) have shown that factual inconsistencies produce behavioral disruptions (e.g. longer oral reading times) at the end of the sentence containing the critical word. This finding has important implications for psycholinguistic theory in terms of whether meaning is integrated immediately or delayed (clausal integration hypothesis). In the present study we investigated N400 amplitude during text comprehension when semantic anomalies and factual inconsistencies were encountered.

Methods:Strongly right-handed males pre-tested for adequate reading comprehension were assigned to one of four groups: factual inconsistency-behavior, factual inconsistency-ERP, semantic anomaly-behavior, semantic anomaly-ERP. The two texts contained a total of 24 violations and 24 controls. ERPs (Wl, Fz, and Pz) or behavioral latency to move the text across the screen were collected on critical words and final words in critical sentences.

Results:Behavioral results showed that semantic and factual violations disrupted performance relative to control words at the critical and final word locations. Semantic anomalies were associated with a larger N400 than controls at both word positions, and this N400 was greater at the critical word than the final word. This trend was reversed for control words in the semantic group. The N400 for the factual words was not different from the control words at either the critical or the final word, and the amplitude was the same for critical and final words for factual inconsistencies. For control words, N400 was larger in the final position.

Conclusion:N400 for factual inconsistencies does not behave like the N400 for semantic anomalies or control words. The factual inconsistencies may disrupt normal processing of sentences from intermediate or final word positions, confirming previous behavioral findings, and further supporting the clausal-integration hypothesis.

183.14 ANTEROGRADE AND RETROGRADE AMNESIA IN PATIENTS WITH CHRONIC PROGRESSIVE MULTIPLE SCLEROSIS. W.W. Beatty, D.E. Goodkin*, N. Monson* and P.A. Beatty*. Dept. of Psychology, North Dakota State Univ., Dept. of Neuroscience, Univ. of North Dakota School of Medicine, and Fargo Clinic-Meritcare, Fargo, ND 58105.

To better characterize the memory disturbance in chronic progressive multiple sclerosis (MS), 38 MS patients and 26 age-and education-matched controls were administered the Minimental State Exam (MMSE, Folstein et al., 1975) and a battery of tests of information processing speed, language, egocentric orientation, short-term and long-term episodic memory and remote memory. Patients were divided into subgroups of cognitively impaired (MMSE < 28) or cognitively "unimpaired" (MMSE: 28-30, the range for controls). Patients in the cognitively impaired group were seriously impaired on all measures of cognitive function including the Boston Naming Test (a measure of anomia). Patients in the cognitively unimpaired group, although less severely affected, performed significantly below controls on all measures of anterograde and remote memory, verbal fluency, egocentric orientation and the Symbol Digit Modalities Test (which measures speed of information processing), but they were not anomic. Deficits on memory tests were observed using both recall and recognition procedures and the proportion of patients who scored below the 5th percentile for controls exceeded 35% on all measures of recall or recognition memory. The cognitive deficits displayed by these patients could not be attributed to affective illness, concurrent use of medications for depression, spasticity or problems with bladder control, poor visual acuity, or to prior treatment with immunosuppressants.

The pattern of memory failure observed in patients with normal MMSE scores closely resembled that seen in subcortical dementias such as Huntington's Disease. These patients exhibited deficits on the remote memory battery which were equally severe for all past decades and they did not show increases in the proportion of perseverative responses on either the Brown Peterson STM test or any of the measures of verbal fluency. In general, the pattern was similar for the patients with low MMSE scores, but these patients were anomic and showed modest increases in perseveration on some of the verbal fluency tests.

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183.15 N100 AND P300 TUNING EFFECTS DURING AN ATTENTION SWITCHING TASK. Erik Sirevaag* and Arthur Kramer* (SPON: L. Malmgren). Dept. Psych., University of Illinois, Urbana-Champaign, Il 61820. The purpose of this experiment was to examine the relationship between components of the Event-Related Brain Potential (ERP) and processes involving selective attention. Tones (1000 and 1400 hz) were presented randomly to the left and right ears. An arrow presented visually before each sequence of twelve auditory stimuli indicated which ear was to be attended to. Prior to the experiment a target frequency was designated for each subject. If a given tone was presented to the correct ear and was of the target frequency a button press response was required. The interval between tone presentations varied from 550 to 850 msec. Subjects performed 50 blocks of these 13 trial sequences followed by a 5 min rest period. In a single session, 7 groups of 50 blocks were performed. Electroencephalographic (EEG) data from three electrode positions were recorded (Fz, Cz, and Pz). The ERP data were analyzed by examining the amplitudes and latencies of a variety of components as a function of the ear, frequency, and target/non-target classification of the ERP eliciting tones. The ERP components were also examined as a function of the temporal position of the stimulus following a switch in attentional locus (early, middle, and late temporal positions). Larger P300s were associated with tones of the target frequency presented to the attended ear. The difference in amplitude between P300s associated with target and non-target stimuli increased as a function of temporal position -- evidence of a tuning effect upon P300 amplitude. Reaction time (RT) did not vary as a function of sequence. A non-parametric estimate of sensitivity (a') indicated that the increases in P300 amplitude were associated with increases in operator sensitivity. The N100 component was significantly larger for tones of the correct frequency, independent of ear of presentation. This finding may indicate that subjects first selected the tones on the basis of frequency with further selections (as indexed by the P300) occurring on the ear dimension. N100 amplitude also displayed a sensitivity to the sequential position of the stimuli within the temporal sequence. Larger N100s were elicited by the first stimuli in the sequence than in the two latter segments. The frequency and sequence effects on N100 amplitude did not interact indicating a general decline in the N100s to all stimuli. These results indicate that following an attentional switch, the first few stimuli elicit large N100s which decline rapidly in amplitude and then remain constant. In contrast, target P300 amplitudes gradually increase as subjects become more familiar with the response requirements of the given trial sequence.

183.16 NEUROPSYCHOLOGICAL AND NEUROBIOLOGICAL ACCOUNT OF A DISORDER. U. Bellugi, S. Marks*, A. Bihrle*, T. Jernigan, F. Culler*. Cognitive Neurosciences, The Salk Institute, P.O. Box 85800, San Diego, CA 92138-9216.

This paper presents a line of investigation which forges links between a specific metabolic disorder, an unusual neuropsychological profile, abnormal brain organization, and a newly discovered neuropeptide as the basis for the disorder. We have undertaken an investigation of the neurobiological and neuropsychological features of Williams Syndrome, a rare clinical disorder of hitherto unknown etiology which appears to affect behavior, language and learning in highly specific ways. A psychophysical result of this metabolic disorder is an unusual sensitivity to classes of sounds, not characteristic of other syndromes. Our neurobehavioral studies of adolescent Williams children indicate that there is a marked discontinuity between linguistic functions and other cognitive functions -- an island of sparing of language capacities in the face of general mental retardation. This extreme dissociation between language and cognitive development is rare and important to an understanding of language and its functional organization in the human brain.

Several lines of evidence will be reviewed suggesting that the cerebral hemispheres may not be organized in the normal fashion in Williams Syndrome children. Results of Magnetic Resonance Imaging techniques will be reported in Williams children, indicating abnormalities in specific brain structures which relate to the unusual neurobehavioral profile. Studies of brain function, including electrophysiological measures, also implicate abnormal brain organization underlying this disorder. We investigate a hypothesis which relates CNS dysfunction and abnormalities of calcium and calcintonin metabolism in children with Williams Syndrome. We hypothesize that these children may have a single underlying genetic disturbance which results in the abnormal production of a specific neurotransmitter. This hypothesis will be linked to the unusual neurobehavorial profile found across domains.

LEARNING AND MEMORY: PHARMACOLOGY I

184.1 POST-SESSION NALOXONE ADIMINISTRATION IMPAIRS AUTOSHAPED LEVER RESPONSE LEARNING. R.B. Messing, S. Allen*, L. Aanonsen* and S.B. Sparber. Dept. of Pharmacology, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

Naloxone (Nx) given after training enhances memory consolidation (performance in a retention test) in aversively motivated tasks (Gallagher & Kapp, Life Sci. 23 [1978]: 1973; Messing et al, Behav. Neur. Biol. 27[1979]: 266), but it impairs performance if given prior to training (Izquierdo, Psychopharm. 69[1980]: 111). Posttrial Nx also enhances memory-related performance in a food motivated spatial memory task (Gallagher et al., Science 221[1983]:975). The present work further assessed the effects of Nx on appetitive learning.

Food deprived rats were autoshaped in Skinner boxes to touch a retractable lever; delivery of a food pellet followed lever retraction. Intertrial intervals (ITI's) between lever presentations varied between 22 and 68 s. The lever was retracted immediately if a touch occurred within 15 s or automatically after 15 s. In an initial experiment, male Sprague-Dawley (SD) rats were given saline or Nx (2 mg/kg) i.p. 5 min before a training session of 12 trials. Two days later they were tested, in the absence of drug, in a session of 36 (3 blocks of 12) trials. Repeated measures ANOVAs were computed for touches of the extended lever (maximum of 12/block), nose-pokes (touches) directed at the retracted lever during ITI's (a measure less constrained by ceiling effects than extended lever touches), and unconditioned expoloratory rearing activity, measured as touches of a metal strip mounted above the grid floor on two walls of the Skinner boxes. Nx depressed basal (training) levels of lever responding, in addition to slowing acquisition rate (i.e. there was an effect of Treatment on both measures of lever responding, and a Block x Treatment interaction for nose-pokes). No effect of Nx was observed on rearing activity, which declined across trial blocks. Additionally, injection of saline 5 min before behavioral testing increases the rate of autoshaping compared to injections 30 min before (Messing & Sparber, Trends Pharmacol. Sci. [1984]5: 149). These results suggest that effects of Nx on measures of learning may be confounded by both behavioral depressent effects and by an injection effect such a short time prior to testing.

Accordingly, a second experiment was carried out wherein Nx (0.5 or 2 mg/kg) was injected after 5 of 7 training sessions (12 trials each) to male and female SD rats. Also, a 6 s delay of reinforcement was inserted between lever retraction and food delivery, lengthening the acquisition process and making it less likely that ceiling effects would prevent observation of drug-induced facilitation. The low dose retarded acquisition of both lever-directed behaviors. There was no effect of Nx on rearing activity. The results support the notion that low doses of Nx have direct agonist action or stimulate release of endogenous opioids (Levine et al, Nature [1979]278: 740). The higher dose may, in addition, block opioid effects or convey mainly the antagonist component of a mixed agonist-antagonist drug.

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184.2 DESGLYCINAMIDE-8-ARGININE VASOPRESSIN REVERSES A TRIMETHYLTIN-INDUCED LEARNING DEFICIT. S.B. Sparber, C.A. Cohen*, and R.B. Messing. Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Trimethyltin (TMT) is an organometal neurotoxin which produces lesions primarily in the limbic system. Selectivity seems to depend upon the dose, but the hippocampus and related entorhinal cortical structures, of importance for learning and memory, are most often described as target sites (Dyer *et. al. Neurobeh. Tox and Teratol.*, 4, 141-48, 1982). We have previously demonstrated that subjects treated with a moderate dose of TMT prior to acquisition sessions, are unable to learn a forward autoshaping task with a 6 sec delay of reinforcement, but are capable of acquiring the same task when no delay of reinforcement is used (Cohen *et. al., Neurosci Abs,* 10, 1205, 1984). These data suggested that the performance deficit is one of learning (i.e. consolidation) rather than of memory (i.e. storage), retrieval, or sensorimotor impairment.

To more rigorously test this, we determined if performance of a task already learned would be impaired by the neurotoxin. Subjects were exposed to Skinner boxes, equipped with retractable levers that were presented on a random time 45 sec schedule, with intertrial intervals ranging from 22 to 68 sec. Once the lever was extended, it remained out until a lever touch occurred or 15 sec had elapsed, after which the lever was retracted and a food pellet was delivered 6 sec later. Adult male Long Evans rats were given 10 acquisition sessions of 24 trials, following which TMT (6.0 mg/kg, p.o.) was administered. One month later, these rats performed the lever-touching behavior as well as controls, despite the fact that the same dose of TMT interfered with learning if given one month prior to acquisition sessions, thus confirming our hypothesis.

In a second experiment we determined if the peptide analog of vasopressin, desglycinamide-8-arginine vasopressin (DGAVP), could reverse the learning deficit caused by pretreatment with TMT. Rats treated with TMT one month prior to acquisition training in the autoshaping task, and which did not learn to associate the lever with delivery of a reinforcer after 10 sessions of 12 trials each, were treated with saline or DGAVP (7.5 ug/kg, s.c.) 1 hr before sessions 11-13; treatment was discontinued prior to sessions 14 and 15. Peptide treated subjects showed evidence of acquisition and exhibited higher levels of lever-directed behavior than controls. Performance was maintained after DGAVP treatment was discontinued, indicating that the learning-enhancing action of DGAVP was not transient or state-dependent.

Thus, the automated autoshaped task with various delays of reinforcement, and TMT-induced or other CNS lesions of varied severity, may provide a useful animal model of human cognitive impairments, as well as a tool for investigation into treatment of such disorders.

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184.3 **NITROUS OXIDE'S EFFECTS ON HUMAN LEARNING AND MEMORY.** R.J. Leonesio*, D.S. Ramsay*, P. Weinstein*, H.H. Samson III* and B. Jones* (SPON: A.C.N. Chen) Department of Psychology, University of Washington, Seattle, Washington 98195.

Nitrous oxide (N_2O) is a commonly used analgesic and anxiolytic drug. Although it is dentistry's most common form of conscious sedation, little is know about its effects on learning and memory. The effects of nitrous oxide on the acquisition and retrieval of number-word pairs were examined in 95 male subjects (ages 18-35). Using the method of adjusted learning, subjects were asked to acquire the pairs to either of two learning criteria while breathing either "air" (30% N_2 and 70% O_2) or "nitrous oxide" (30% N_2O and 70% O_2). Subjects who received air during the acquisition session acquired (to a criterion of 1 correct response) all 18 pairs with an average of 23.32 trials (SEM = 1.80) whereas subjects receiving nitrous oxide required an average of 48.17 trials (SEM = 3.61). 30% nitrous oxide slowed the acquisition of number-word pairs ($t = 6.12$, $df = 93$, $p < 0.05$).

Two week later, half of the subjects in each of the previous two groups were administered either air or nitrous oxide and were asked to recall the word that accompanied each number cue. There was no evidence of any state-dependent effect of nitrous oxide on recall. Although nitrous oxide inhalation impaired retrieval during the retention session, multiple regression analyses suggested that learning while under nitrous oxide may actually have enhanced recall. The observation that subjects can acquire and store information while breathing nitrous oxide suggests the possibility that psychological techniques that rely on the subject's ability to learn may be used during nitrous oxide conscious sedation.

This study was supported by NIH grants DE 00161, DE 07150 and RR 05346 with additional support from the Alcoholism and Drug Abuse Institute of the University of Washington.

184.4 CALCIUM INFLUX AND CALCIUM-CALMODULIN ACTIVITY ARE NECESSARY FOR FORMATION OF SHORT- AND INTERMEDIATE-TERM MEMORY IN THE CHICK. E.L. Bennett, T.A. Patterson, M.C. Alvarado*, A. Khorram*, & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Research in our laboratory indicates that memory formation in the chick involves at least three stages, distinguishable by their underlying neurochemical mechanisms and temporal parameters (Patterson et al., 1986). Formation of long-term memory is dependent upon protein synthesis. Experiments described below examined the role of calcium influx and calcium-calmodulin activity in formation of short-term memory (STM) and intermediate-term memory (ITM).

Two-day-old male Dekalb-Warren chicks were trained in a one-trial taste avoidance task. In Experiment 1, dose response functions were determined for lanthanum chloride ($LaCl_3$), an inhibitor of calcium influx, and trifluoperazine (TFP), a calcium-calmodulin inhibitor. Injections (10 µl/hemisphere) of saline or drug were made into the medial hyperstriatum ventrale 5 min (for TFP) or 10 min (for $LaCl_3$) pretraining and chicks were tested 24 hr posttraining. 10.0-40.0 mM $LaCl_3$ and 1.2-9.8 mM TFP produced significant amnesia.

Experiment 2 showed that $LaCl_3$ (10.0 mM) produced amnesia when given 30, 15, or 10 min pretraining. $LaCl_3$ was not amnestic when given earlier than 30 min before training, when given 5 min pretraining, or at any time posttraining. TFP (4.9 mM) produced amnesia when given 30, 15, or 10 min pretraining or when given 5 min after training. TFP was not amnestic when given earlier than 30 min pretraining or later than 5 min posttraining.

Experiment 3 determined the time courses of amnesia development following 10 min pretraining injection of $LaCl_3$ or TFP. $LaCl_3$ (10.0 mM) produced amnesia that developed by 5 min posttraining. TFP (4.9 mM) produced amnesia that developed 15-20 min posttraining. Amnesia produced by these agents is permanent. Similarities of the effects of $LaCl_3$ and TFP to other inhibitors of formation of STM and ITM (Patterson et al., 1986) indicate that $LaCl_3$ interferes with formation of STM, and TFP interferes with the formation of ITM.

These results suggest that neurochemical systems involving calcium influx mediate formation of STM and systems involving calcium-calmodulin activity mediate formation of ITM. Hypotheses about the possible roles of these systems in memory formation will be presented.

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184.5 IMMATURE PROCESSES OF SPATIAL LEARNING IN HOODED RATS. A.-F. Chevalley and F. Schenk (SPON. C. Marchand). Institute of Physiology, University of Lausanne, 1005 Switzerland.

Previous studies have shown that young hooded rats under approximately 32 days of age do not develop systematic, direct approaches to the invisible platform during training in the Morris place navigation task. During probe trials, however, they show significant discrimination of the training position. We have further developed studies of such immature forms of spatial learning in various homing experiments using a hole board on which rats are trained to find one hole out of many which is connected with their home cage.

Even in the absence of proximal cues, 21-day-old rats show a significant discrimination of the training sector during a probe trial with no connected hole, yet in reaching the goal, even when clearly indicated by an object, their performances lack in precision. In addition, their behaviour is characterized by a stereotyped scanning in the starting area. After 22 days of age, rats run straight to a cued hole and show organized scanning behaviour. However, if it is only possible to locate the training hole with the help of distal room cues, the systematic use of direct approaches is not observed until after the age of 30 days. Until around 42 days, rats rely on proximal, contextual cues (if available inside the enclosure) in combination with distal ones to discriminate the training position, while older rats rely predominantly on distal room cues.

In order to further study the immature processes of spatial learning in rats, young subjects of various ages were trained to find the connected hole surrounded by a configuration of objects dispersed within the enclosure. This procedure has allowed us to assess the variability in routes leading to the goal at different ages, and to estimate the importance of intra-table cues versus distal ones in conflicting situations created by a table rotation. These results will be discussed from the aspect of the sensitivity to steroïd hormones in puberty of the responsiveness to catecholaminergic drugs.

On the whole, our results confirm that young animals are quite capable of place recognition at least one week before they develop their abilities to aim and head straight for a goal from any starting position which represents the criterion for true place learning. The two structures which seem indispensable for this aspect of spatial learning could be the subiculum and/or the parietal cortex, as indicated by a recent lesion study (Kolb and Walkey, 1987).

184.6 THE EFFECTS OF ALCOHOL AND TIME ON DIFFERENT FORMS OF MEMORY. B.L. Schwartz*, E.S. Parker, and E .Tulving . Psychiatry, Georgetown Univ. and VAMC Washington, DC 20422, NPI, Univ. of California, Los Angeles, CA 90024, Dept. of Psychology, Univ. of Toronto, Toronto, Canada M5S 1A1.

A current issue in the science of memory and learning is the distinction among multiple forms of memory . Evidence for the distinction comes from dissociations among performances on various memory tasks. The question addressed here is whether two types of impaired memory function (acute alcohol amnesia and forgetting over time) have dissociable effects on 3 forms of memory (episodic memory, repetition priming, and skill). We examined whether there are differences in the patterns of memory failure produced by alcohol intoxication and the natural passage of time.

In the alcohol experiment, subjects participated in 2 sessions a week apart. In the first session, 32 subjects drank alcohol (1 ml/kg) and studied material on the rising and falling blood alcohol curve. In the second session, memory was tested under the same alcohol conditions. The control group of 32 subjects had placebo drinks instead of alcohol in both sessions. In the time experiment, 32 subjects studied the same materials as subjects in the alcohol experiment. They were tested 3 hrs, 1 week, 1 month, and 4 months later.

A modification of the word fragment completion task was used (Tulving, E., Schacter, D.L., & Stark, H.A., Journ. of Expt. Psych.: Learn., Mem. and Cog., 8:336, 1982). At study, subjects completed word fragments with the aid of successively less fragmented cues (e.g., a--a--in, as-a--in, assa--in) until the entire word was presented. At test, the same successive cuing method was followed using a mixture of studied (old) and unstudied (new) words. After completing the fragment cue, subjects made a yes/no recognition judgment about the word. The recognition test assessed episodic memory. Priming was measured by the facilitation in completing old versus new word fragments, and skill was measured by improvement in the completion of new word fragments with practice.

Alcohol and time both impaired recognition performance. In the case of alcohol, recognition changed as a function of blood alcohol at study. In the case of time, recognition declined as retention interval increased, with a reduction of 70% over a 4-month period. Time, but not alcohol, affected priming. Skill of completing word fragments was resistant to both the effects of alcohol and time.

The different patterns of memory failure produced with alcohol intoxication and the passage of time may serve as experimental models of chronic amnesic disorders such as Korsakoff syndrome and Alzheimer's disease.

184.7 CLONIDINE REVERSES LEARNING DEFICITS AFTER PARTIAL FORNIX SECTION. SJ. Sara, C. Maho* and M. Ammassari*. Lab. de Physiologie Nerveuse, CNRS, Gif-sur-Yvette, 91190 FRANCE and Ist. di Psicobiol.,CNR,Rome.

Studies of memory disorders associated with aging have focused on the cholinergic (ACh) system because of: -massive deficits seen in the brains of Alzheimer's patients, -relative ease of producing learning and memory deficits in animals with ACh dysfunction. The recent report that α2 adrenoceptor agonist clonidine (CLO) improves cognitive function in aged monkeys (Arnsten & Goldman-Rakic, Science, 230, 1273-1276), has prompted interest in the role of the noradrenergic (NE) system in memory dysfunction associated with aging. Our present studies are addressing the possible interaction of these two systems implied at a behavioral level.

Partial section of the fornix produces a consistent deficit in radial arm maze learning and an increase in the dominant theta frequency in the hippocampus. The ACh agonist, oxotremorine restores normal theta as well as behavior, suggesting that the deficit is due to decrease in ACh input to the hippocampus, (Maho et al, in press). Thus a convenient model is provided to study NE-ACh interaction.

Rats received knife cuts to the dorsal fornix or sham operation. They were trained in an 8 arm radial maze to collect food pellets at the end of each arm. They were treated with CLO (.01mg/kg) before each daily trial; controls were injected with saline. The number of repeated choices out of 8 and the number of choices before a repetition were used as performance indices. Lesioned rats were significantly impaired in the acquisition of this task, but did approach the performance of controls on the tenth trial. CLO-treated rats, lesioned or not, had an acquisition profile indistinguishable from that of sham operated saline injected rats. Rats were retested after one week with no drug treatment before the daily trials. There was a performance deficit in all groups on the first test trial, with the lesion-saline group making more errors than the other groups. This lesion group approached the performance level of the others only after 5 retraining trials. Lesioned rats treated with CLO during the 10 original training days, maintained a "normal" performance during the 5 day retraining phase without CLO.

We are presently studying the effect of CLO on reinstatement of normal theta frequencies in the hippocampus to determine if this is a necessary condition for alleviating the lesion-induced behavioral deficit. It is unlikely that the effective site of action of CLO is in the hippocampus, since NE inhibits release of ACh, both directly through α2 receptors and through a GABA-linked mechanism. A more likely mediating mechanism might be through CLO-induced inhibition of locus coeruleus cells with consequent decrease in release of NE, thus permitting an increase in ACh release from spared fiber terminals.

184.8 PREEXPOSURE TO THE PASSIVE AVOIDANCE PARADIGM ALTERS SCOPOLAMINE'S AMNESIC EFFECT IN MICE. M.J.Benvenga and T.P.Jerussi. Pharmacology Group, Anaquest/BOC Health Care, Murray Hill, New Jersey, 07974.

The one-trial passive avoidance paradigm in mice is widely accepted for studying the amnesic effects of drugs. Footshock paired with entering a darkened chamber during a training trial, produces a significant increase in the latency to enter the chamber on a subsequent test trial. However, the latency can be reduced by administering an antimuscarinic, such as scopolamine, 10-30 minutes before the training (Buresova, et.al., Psycopharm., 1964; Bohdanecky & Jarvick, Int. J. Neuropharm., 1967). In the present study we assessed the amnesic effect of scopolamine administered to mice that were initially exposed to a sham passive avoidance procedure (i.e. without footshock).

Male Swiss-Webster mice (25-30g.) were injected with 0 (vehicle = distilled water), 0.5, 1.0, 2.0, or 4.0 mg/kg. of scopolamine 30 minutes prior to one-trial passive (dark) avoidance training (N = 12/dose). Other mice (N = 12/dose/time interval) received a sham passive avoidance trial 30, 60, or 120 minutes, or two sham trials 30 and 60 minutes prior to the dosing regimen (above). Thirty minutes later these groups received passive avoidance training. All mice were then tested 24 hours later and the latency to enter the darkened chamber was recorded. Median latencies were compared by Mann-Whitney U-tests.

The results indicated that the latencies of scopolamine treated mice that did not receive a sham trial were significantly ($p < 0.05$) shorter than vehicle control animals. However, there was a shift of the dose-response relationship for animals that received a single sham trial. Moreover, larger doses of scopolamine were needed to produce an amnesic effect the closer the sham trial was to the training trial. On the other hand, the dose-response shift was not as pronounced when two sham trials were given. Therefore, it appeared that experience with the apparatus and/or procedure mitigated against scopolamine's amnesic effect.

184.9 RETURN OF THE AMOUNT OF MUSCARINIC RECEPTOR TO NORMAL VALUES IN DIFFERENT RAT BRAIN AREAS DURING THE EXTINCTION OF A LEARNED OPERANT BEHAVIOR. A. Ortega*, V. Alemán, A. Meneses* and A. Oscós. Departamentos de Neurociencias and Farmacología, Centro de Investigación y de Estudios Avanzados del IPN, México, D.F. 07000.

Thirty female 90-day old rats were divided ramdomly in 6 groups of 5 animals each. Groups were arranged as follows: active control (AC) group, a maximal learning acquisition (MA) group, a group with 2 extinction sessions (E_{48}) one every 24 hours, one group with 3-extinction sessions (E_{72}) and another group with 4 extinction sessions (E_{96}), finally a group with maximal learning acquisition -- (MA_{96}) but 96 hours later received the last training session in - order to determine its level of learning and to be used as the -- control of the E_{96} group. Active extinction was carried out with - one dayly session of a nonpaired conditioned (CS) and unconditioned (UCS) stimuli. The learning paradigm we used was an "autoshaped" - version of an illuminated press lever associated to an UCS of --- Pavlov's type. The specified brain areas, were dissected, homogenized and the P_2 fractions were obtained in the usual manner. Incu - bations were carried out one hour at 30°C, containing 0.1 mg of -- protein and (-) [N-methyl-^{3}H] scopolamine was used as the labelled ligand. We did not find at any of the extinction days, a signifi - cant change on the number of muscarinic (M) receptors neither in - the frontal cortex nor in the septum nucleus as compared to the -- control group. On the other hand, caudate and temporoparietal cortex ($T\text{-}P_c$) receptor population showed a tendency to increase, that is to return to normal values, as the extinction progresses. The same but less marked tendency, was observed in the hippocampus of these groups. An opposite tendency of the M receptors, was observed in amygdala of these groups, as extinction progresses. In the MA-group, we only observed an increase in the M receptor population of the $T\text{-}P_c$ and a decrease in hippocampus. In other analized brain areas no change was found. When the MA_{96} group was compared against the MA group, we did not detect any change in the M receptor population from amygdala, septum, T-P cortex, hippocampus or frontal - cortex. However, the receptor population from caudate nucleus decreased significantly. In a previous abstract we have demonstrated that the M receptor population in caudate, amygdala, $T\text{-}P_c$ and frontal cortex were increased during the acquisition of learning. These findings and those here reported during extinction, suggest both a cause-effect and a time relationship between the M receptor po - pulation of some brain areas of the brain and the evolving and extinction of a learning process.

184.10 BLOCKADE OF RADIATION-INDUCED CONDITIONED TASTE AVERSIONS AFTER THE DEVELOPMENT OF ETHANOL TOLERANCE. Walter A. Hunt* and Bernard M. Rabin (SPON: John McDonough). Behavioral Sciences Department, Armed Forces Radiobiology Research Institute Bethesda, MD 20814-5145 and Department of Psychology, University of Maryland Baltimore County, Catonsville, MD 21228.

A conditioned taste aversion (CTA) can develop to a novel tasting, but normally preferred solution after it is paired with a toxin. Previous research has demonstrated that a variety of toxins, including ionizing radiation, lithium chloride, WR-2721 (a radioprotectant), copper sulfate, and cisplatin, act similarly through the area postrema (AP) to induce CTAs. An exception is an ethanol-induced CTA, which is not blocked in AP-lesioned animals. Additionally, prior administration of one toxin that is AP-mediated can block the acquisition of a CTA to another AP-mediated toxin. However, this procedure is not successful when ethanol is the toxin. The present research demonstrates that although short-term ethanol preexposure does not block a radiation-induced CTA, the development of tolerance to ethanol will do so. Male Sprague-Dawley rats were rendered ethanol-tolerant (as revealed as shorter sleep-times after a 3-g/kg, i.p. dose of ethanol, compared to nontolerant animals) by administering two doses of ethanol/day (4-6 g/kg, p.o.) for 5 days. The animals were then water deprived for 23.5 hrs/day for the next 5 days. After that time, they were presented with a 10% sucrose solution and the amount consumed in 30 min was recorded, followed by irradiation with 1 Gy (100 rads) of gamma radiation. Two days later, the sucrose was presented again and the amount consumed in 30 min was recorded. A radiation-induced CTA was observed in control animals. However, a CTA was not acquired by ethanol-tolerant animals. Experiments where animals were pretreated with ethanol on 3 days prior to irradiation did not block the development of a CTA. These data suggest that it is not the ethanol itself that provides protection against radiation-induced CTAs, but rather it is the animal's response to the chronic presence of ethanol. Also, they provide evidence that cellular desensitization in general might be an effective approach to radioprotection in order to reduce toxicity associated with exposure to radiation on long-term space flights and during cancer therapy.

184.11 ASYMMETRICAL EFFECTS OF UCS PREEXPOSURE ON TASTE AVERSION LEARNING FOLLOWING TREATMENT WITH IONIZING RADIATION AND OTHER TOXINS. B. M. Rabin and W. A. Hunt*, Behavioral Sciences Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145, and Dept. of Psychology, University of Maryland Baltimore County, Catonsville, MD 21228.

A conditioned taste aversion (CTA) is produced when a novel tasting solution is paired with unconditioned stimuli such as ionizing radiation, lithium chloride (LiCl) or ethanol. Preexposing an organism to a toxin blocks CTA learning when that toxin is subsequently used as a UCS. If the same humoral factor mediates both radiation- and LiCl-induced CTA learning (Rabin & Hunt, Neurosci. Biobehav. Rev., 1986, 10: 55), preexposing the organism to one UCS should disrupt the acquisition of a CTA to the other UCS. In contrast, preexposure to ethanol should have no effects on radiation- or LiCl-induced CTA learning since different mechanisms, not involving the area postrema, mediate the acquisition of an ethanol-induced CTA. Rats were placed on a 23.5-hr water deprivation schedule for 10 days. On days 2, 5, and 8 they were preexposed to one UCS, either radiation (100 rad), LiCl (3.0 mEq/kg, i.p.) or ethanol (4.0 g/kg, p.o.). On the conditioning day (day 10) the rats were given a choice between water and a 10% sucrose solution followed by treatment with either the same or a different UCS. On the test day (day 12), all animals were again given a choice between sucrose and water. The results showed that preexposing rats to LiCl prevented the acquisition of both an LiCl- and radiation-induced CTA. Preexposing rats to radiation blocked the acquisition of a radiation-induced CTA, but not the LiCl-induced CTA. Preexposing rats to ethanol did not affect the acquisition of a CTA following treatment with either radiation of LiCl. In contrast, both radiation and LiCl preexposure disrupted the acquisition of an ethanol-induced CTA in intact rats, but not in rats with lesions of the area postrema. The observation of asymmetrical preexposure effects between the various stimuli indicate that the relationships between these stimuli are more complex than originally hypothesized. In addition, the results with the ethanol-induced CTA suggest that these relationships may somehow involve the area postrema even when the area postrema is not involved in the acquisition of the original CTA.

184.12 AF102B: A NEW M1 AGONIST WITH POTENTIAL APPLICATION IN ALZHEIMER'S DISEASE (AD). A. Fisher, R. Brandeis*, Z. Pittel*, I. Karton*, M. Sapir*, S. Dachir*, A. Levy, F. Mizobe# and E. Heldman*. Israel Inst. Biolog. Res., Ness- Ziona 70450, ISRAEL; # Snow Brand Milk Products, Tokyo, JAPAN.

AD, a disorder characterized by a central presynaptic cholinergic deficit, has no rational and effective treatment as yet. As postsynaptic muscarinic receptors (mostly M1 receptors) are relatively intact in AD, M1 agonists should improve cognitive dysfunctions associated with the cholinergic hypofunction in this disease. In this regard, a novel muscarinic agonist of utmost rigidity, cis-2-methyl spiro (1,3-oxathiolane-5,3')quinuclidine, AF102B, was reported by us recently (Israel Patent, May 10, 1985; Soc. Neurosci. 1986). AF102B induced atropine-sensitive contractions in the guinea-pig ileum and trachea preparations at an $EPMR_{ACh}$ of 100 and 1, respectively. Binding to muscarinic receptors in rat brain homogenates showed: a) the order of potency- oxotremorine> AF102B= pilocarpine> cis-AF30= McN-A-343~ arecoline~ trans-AF30= AF102A (the trans isomer)> methacholine~ carbachol (CCh)= bethanechol [(-)-^{3}H-QNB displacement, cerebral cortex)]; b) that AF102B was more selective for M1 receptors than oxotremorine, AF102A, cis- or trans-AF30 (Eur. J. Pharmacol. 37, 329, 1976) and McN-A-343 [forebrain and cerebellum, (-)-^{3}H-QNB assay)] or by differential displacements of (-)-^{3}H-QNB, ^{3}H-cis-Dioxolane and ^{3}H- Pirenzepine in forebrain]. The agonistic feature of AF102B was also shown by a shift to the right of (-)-^{3}H-QNB displacement curve (forebrain homogenates) in presence of GppNHp. In contrast to CCh, AF102B did neither potentiate phosphoinositides (PI) hydrolysis nor did it inhibit adenylate cyclase (AC) activity (cerebral cortex and cerebellum). However, AF102B blocked CCh-induced PI hydrolysis, without effecting CCh- induced inhibition of AC activity. In a workable animal model for AD, [the AF64A-treated rat (3nmol/2ul/side, icv)], AF102B (1 mg/kg, ip or po), AF102A (1mg/kg, ip), cis-AF30 (1mg/kg, ip) and physostigmine (0.06mg/kg, ip) reversed a cognitive impairment in a step-through passive avoidance task; both AF102B and AF102A (1mg/kg, ip), unlike physostigmine (0.1 mg/kg, ip), were positively active in the Morris water maze (MWM), but only AF102B (5mg/kg, ip) was beneficial in the 8-arm radial maze. Single or repetitive administrations of AF102B (1mg/kg, ip) improved also AF64A-induced working memory deficits in the MWM, but did not effect open field behavior. Restoration of AF64A-induced cognitive impairments by low doses of AF102B, lack of adverse side-effects up to high doses, a wide therapeutic index, and the M1 selectivity -- all indicate that this compound can be considered as a promising candidate drug for the treatment of AD. Supported by SNOW BRAND, JAPAN.

184.13 EFFECTS OF PICROTOXIN INJECTIONS INTO DIFFERENT REGIONS OF THE STRIATUM ON RETENTION OF PASSIVE AVOIDANCE. Rigoberto Salado-Castillo* and Roberto A. Prado-Alcalá. Dept. of Physiol., Sch. of Med., Natl. Univ. of México, P.O. Box 70-250, México, D.F., 04510, México.

Interference with neural activity of the striatum, induced by electrolytic lesions, electrical stimulation, local anesthetics, potassium chloride and neurotoxins produces significant deficits in the retention of passive avoidance. The same is true when cholinergic and dopaminergic synaptic activity is altered. Since these transmitters are functionally related to striatral GABAergic activity it was postulated that direct application of a GABA antagonist would also result in a memory deficit. Male Wistar rats were trained, in one trial, to avoid the darker compartment of a two-compartment box. Independent groups were trained and two minutes later unilaterally injected with picrotoxin (1 ug/1uL) into one of the quadrants of the anterior striatum (medial-dorsal, medial-ventral, lateral-dorsal or lateral-ventral) or in one of three regions of the posterior striatum (medial-dorsal, lateral-dorsal or postero-ventral); a group of intact rats was also studied. Retention of the passive avoidance task was tested 24 h after training. The antero-medial-ventral group did not differ from the control group; the rest of the groups had significantly lower retention scores than the latter groups, and the postero-ventral and postero-lateral-dorsal groups had lower retention scores than the rest of the groups. These data suggest that striatal GABAergic activity is differentially involved in memory processes, since picrotoxin produced a greater effect when injected into the posterior regions of the striatum than when injected into other regions of this structure.
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184.14 LIMBIC SYSTEM UNIT ACTIVITY AND BEHAVIOR ALTERED DURING LEARNING BY LOCAL DEPLETIONS OF NOREPINEPHRINE IN RABBITS. S. Sparenborg and M. Gabriel. Dept. Psychol., U. of Illinois, Champaign, IL, 61820.

Based on past studies, we have proposed a model (Gabriel, Sparenborg & Stolar, in The Hippocampus Vol. IV, Plenum, 1986) which explains functional relationships between behavior and neural activity, in relation to discriminative avoidance training, in the anteroventral nucleus of the thalamus (AVN), the cingulate cortex (Brodmann's Area 29), and the hippocampus. The dense innervation of these structures by the locus coeruleus suggests that norepinephrine (NE) may play an important role in the learning-related functions of this system.

To examine this role, localized depletions of NE were induced in either the AVN (n=7 rabbits) or Area 29 (n=7 rabbits) by the microinjection of 6-hydroxy-dopamine (2 ug 6-OHDA in 2 ul saline, 1% ascorbic acid). All subjects, including 7 vehicle-injected control rabbits, received systemic injections of the dopamine uptake inhibitor GBR-12909. Multi-unit activity was monitored through electrodes implanted in the AVN and Area 29. Avoidance training and extinction was given in a rotating-wheel conditioning apparatus 10-20 days after the injections. Positive (CS+) and negative (CS-) conditional stimuli (1 and 8 kHz tones) were each presented for 60 trials in daily sessions. The unconditional stimulus was a footshock delivered five seconds following the onset of the CS+ on trials in which no avoidance response occurred. After training, NE levels in the AVN, Area 29, and neighboring structures were measured with HPLC.

Both cortical and thalamic NE depletions increased AVN CS-elicited unit firing. Thalamic depletions also increased CR performance on the first day of training and during extinction. Viewed in the context of our theoretical model, these results suggest that both cortical and thalamic NE is involved in the attention-related limiting of thalamic activity and behavior in intact animals exposed to unexpected training contingencies. Cortical depletions decreased the rate of CR performance and the frequency of unit firing in Area 29. These results suggest that NE enables the flow of thalamically driven excitation in Area 29 to motor structures (e.g., the striatum) involved in CR output. (Supported by NIMH Grant 37915 to MG.)

184.15 ASSESSMENT OF REFERENCE AND WORKING MEMORY DURING ACUTE AND CHRONIC EXPOSURE TO ANTI-CHOLINERGIC AGENTS. K. Raffaele, D. Olton, and Z. Annau, Johns Hopkins University, Baltimore, MD 21205.

Many studies have evaluated the effects of repeated exposure to drugs or toxins by monitoring physiological and sensory-motor systems (e.g. temperature, rotorod performance, weight gain, etc.) at many time points both during and after the exposure. Few studies have monitored the performance of learning and memory tasks in a similar way. We have developed a task that enables the assessment of both reference memory and working memory on a daily basis. The task uses a cued non-spatial serial reversal with repeated stimuli.

The apparatus had a stem and two interchangeable goal boxes which were painted with different patterns of black and white paint (stripes or spots). After criterion was met (8 correct responses in 10 consecutive trials), the correct goal box was reversed. The correct goal box at the start of each day's testing was the same one that was correct at the end of the previous day's testing (reference memory assessment). The reversal (working memory assessment) was given only after criterion had been met in the reference memory assessment.

Following two to three weeks of training, rats achieved a stable performance level, which was dependent on the length of the delay between trials. The sensitivity of performance on the task to acute or chronic disruption of the cholinergic system was then tested. Performance was disrupted by scopolamine given 20 minutes before testing in doses ranging from 0.25 to 1.0 mg/kg. Chronic exposure to DFP (injected following testing, 1.0 mg/kg on the first day and 0.5 mg/kg every third day thereafter) also disrupted performance on this task.

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184.16 EXCITATORY AMINO ACIDS AND MEMORY PROCESSING. J.L. Davis and J.F. Flood, Office of Navel Research, Arlington, Life Sciences Directorate, VA 22217 and GRECC, VA Medical Center, Sepulveda, CA 91343.

Certain of the amino acids, L-glutamate and L-aspartate, are rapidly gaining the status of excitatory neurotransmitters in the central nervous system. Excitatory amino acid receptors are classed as N-methyl-D-aspartate (NMDA) and non-NMDA types. Among the non-NMDA type receptors kainate and quisqualate subtypes are recognized. Glutamic, aspartic and kainic acid simulate both NMDA and non-NMDA type receptors. In spite of the potent effects on neural activity, little is known about their effects on memory processing.

In this study, we found that glutamic, aspartic, and kainic acids administered intracerebroventricularly after footshock avoidance training in a t-maze enhanced retention test performance measured one week after training. In an effort to determine whether enhancement of memory selectively involved NMDA or non-NMDA receptor subtypes, we tested the effects on retention of (-)APV, APH, and GAP, all NMDA antagonists. These were compared to (+)APV (inactive) and GAS (specific non-NMDA kainate type receptor antagonist).

At 25 pg administered icv, (-)APV, APH and GPA all caused amnesia (20% recall score) compared to saline-injected controls (86% recall score). The inactive (+)APV had no effect on retention test performance (86% recall score), but GAS, an antagonist of the kainate receptor subtype impaired retention (25% recall score).

It is interesting that microgram amounts of glutamic, aspartic and kainic acid were needed to improve retention but only pg gram amounts were needed to cause amnesia. Microgram quantities of (-) APV, APH and GAS caused amnesia as well but these doses caused severe and prolonged motor convulsions. Based on these preliminary studies, NMDA and non-NMDA receptors may both be involved in memory processing but this does not rule out subtle differences using different types of training.

ABBREVIATION:
(-)APV, D(-)-2-amino-5-phosphovaleric acid
(+)APV, D(+)-2-amino-5-phosphovaleric acid
APH, DL-2-amino-7-phopnonoheptanoic acid
GAP, gamma-D-gluamylaminomethyl phophonic acid
GAS, gamma-D-gluamylaminomethyl sulphonic acid

184.17 SPATIAL WORKING MEMORY IN C57BL/6 MICE. M. B. Upchurch. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Work in this laboratory has indicated that C57BL/6Ibg mice exhibit spatial learning in the Morris water task. These data are in contrast to those reported by Reinstein et al. (Brain Res., 1983, 263, 172), who found that mice of a closely related substrain, C57BL/6J, were incapable of spatial learning in a radial arm maze. The radial arm maze and the Morris water task differ in several major respects. The radial maze is appetitively motivated, while the Morris task is aversively motivated; the radial maze evaluates spatial working memory, while the Morris task examines spatial reference memory; and the radial maze requires the animal to develop a win-shift strategy, while the Morris task requires a win-stay strategy. In addition, the correct form of response in the radial arm maze is more complex than that in the Morris water task, and animals of some strains may require an extensive number of trials in order to achieve criterion levels of performance. It is well documented that C57 mice can learn appetitively motivated operant tasks; thus, it seems unlikely that their failure to learn the radial arm maze was caused by lack of appropriate motivation. This study investigates the possibility that C57BL/6 mice are poor at win-shift learning or lack ability to use spatial working memory.

C57BL/6Ibg mice were trained in a water escape task in which they were required to swim to a slightly submerged platform in a goal arm of a T-maze. Each learning trial consisted of two parts. First, the animal was given a run in which only one goal arm was open. The order of goal arm blocking was pseudo-random, with the restrictions that each arm was blocked for half the trials and that neither arm was blocked more than three times in a row. Upon reaching the platform, the animal was allowed to rest for 30 sec and was then placed in a holding cage for 20 sec. The mouse was then returned to the T-maze for the second part of the trial, in which both goal arms were open and the animal was required to make a choice between the two. For half the mice, the correct response was to choose the arm that was blocked during the first part of the trial. The remaining mice were required to return to the previously open arm. The mice were given ten trials per day for ten consecutive days.

The initial response of the mice was to select one goal arm to visit consistently during the free-choice portion of the trial. After sixty to ninety trials, however, the mice began to develop response strategies based on the position of the open goal arm during the first part of the trial. There was no evidence that mice in the win-shift group were less capable of learning the task than mice in the win-stay group. The data suggest that C57BL/6 mice can learn spatial working memory tasks, but that extensive training is required before they overcome an initial tendency to perseverate and instead exhibit responses based on working memory. (Supported by training grant HD07289.)

184.18 INTRAVENTRICULAR ACETYLCHOLINE REVERSAL OF T-MAZE DEFICITS FOLLOWING LESIONS OF THE NUCLEUS BASALIS OF MEYNERT. J. Mastropaolo and J.N. Crawley. Unit on Behavioral Neuropharmacology, NIMH, Bethesda, MD 20892.

Lesioning of the cholinergic nucleus basalis of Meynert (NBM) produces a deficit in memory for recent events concomitant with a decrease in CAT activity in rats, and has been proposed as an animal model of Alzheimer's disease (Hepler, Olton, Wenk and Coyle, Neuroscience, 5: 866-873, 1985; Flicker, Dean, Pontecorvo, Figueiredo and Fisher, Pharmacol Biochem Behav 23: 125-135, 1985).

To test the ability of centrally administered acetylcholine to reverse these memory deficits, male Sprague Dawley rats received lesions of the NBM and the medial septal area (MSA) by the microinfusion of 4 ng ibotenic acid into each of 5 anatomical sites. Control rats were identically infused with saline. In addition, an indwelling 24 gauge stainless steel cannula was stereotaxically implanted in the lateral ventricle of all rats. After 7 days of postoperative recovery, all rats were food deprived to 85% of their free feeding weight. Behavioral testing was conducted in a T-maze, using a food-reinforced alternation task with a 2 minute delay. When performance was stable at this delay, control rats averaged 9/12 correct, while lesioned rats averaged 6/12 correct responses. Groups of rats were microinjected with either 1, 5 or 10 ug of acetylcholine (ACH) into the lateral ventricle. On the next day, baselines were obtained in which animals were run in the T-maze but received no drug treatment. On the following day, groups of rats received either a) 5 mg/kg of the muscarinic antagonist atropine (i.p.) and 10 ug ACH (ivt), b) 3 mg/kg of the nicotinic antagonist mecamylamine (i.p.) and 10 ug ACH (ivt), c) or saline (i.p.) and 10 ug ACH (ivt). Following another baseline day of testing without drug treatment, groups of rats were injected (ivt) with either d) 10 ug atropine and 10 ug ACH, e) 20 ug mecamylamine and 10 ug ACH, or f) saline and 10 ug ACH.

Control animals showed no significant change in performance with any of the drugs tested. Lesioned animals showed a significant increase in choice accuracy, relative to baseline performance on the preceding day, following the administration of 10 ug of ACH. Increase in choice accuracy produced by ACH was significantly inhibited by atropine but not by mecamylamine when the antagonists were administered either peripherally or centrally. These data suggest that intraventricularly administered acetylcholine can reverse the memory deficit produced by lesions of the NBM and MSA. Further, since this effect was blocked by atropine but not mecamylamine, this effect of acetylcholine appears to be mediated by a muscarinic cholinergic mechanism.

184.19 POLYUNSATURATED FAT DIETS IMPROVE SPATIAL MEMORY IN RATS. G. Winocur* and C.E. Greenwood (SPON: P. Garfinkel). Dept. Psychology, Trent Univ., Peterborough, Ontario K9J 7B8 and Dept. Nutritional Sciences, Univ. of Toronto, Tor., Ont., Canada M5S 1A8.

Our previous studies indicate that alterations in the type of dietary fats fed to rats influence a variety of behaviours, including cognition (Life Sci. 38:1789) measured using a Morris Water Tank thought to reflect spatial learning and memory skills. That is rats fed a semi-synthetic diet containing 20% (w/w) polyunsaturated fat in the form of soybean oil (SBO) learned the task faster and showed a transient increase in resistance to extinguish this response in comparison to rats consuming a saturated fat source, lard. To extend these observations, similar studies were performed using the radial arm maze as a further test of spatial memory, and examining the interaction of dietary fat and the cholinergic antagonist atropine on the rat's performance. Young, rapidly growing rats were fed the SBO (n=21) or lard (n=24) diets for three months prior to behavioural testing. Animals were familiarized with the task by allowing pairs of rats to explore the maze for 15 minutes daily for three days. Performance was then recorded on the next 8 consecutive days. Dietary fat source influenced task acquisition such that the SBO fed rats showed superior performance on the first day of testing (total number of errors/trial = 1.8±0.3; mean±SEM) in comparison to the lard fed animals (# errors = 2.9±0.4). The influence of dietary fat on performance was no longer apparent by day 4 (# errors = 0.9±0.2 vs 1.4±0.2 for SBO and lard, respectively) and remained similar through to day 8 (# errors = 0.8±0.3 vs 1.1±0.3). A subset of animals from each diet (n=9 and 8 for SBO and lard) was further tested following drug challenges and their performance compared with standard laboratory chow fed rats (n=10) of similar age. Drugs were administered intraperitoneally 60 minutes prior to testing. Drug-free performance of these animals was 0.3±0.3, 1.1±0.5 and 1.3±0.5 errors for SBO, lard and chow fed rats respectively. Atropine sulphate (2.0 mg/kg) impaired performance such that lard (2.9±0.9) and chow (2.8±0.9) fed rats made significantly more errors than SBO (1.1±0.3) fed animals following drug administration. This effect of atropine on maze performance appears to be attributable to central cholinergic blockade since neither atropine methyl nitrate (2.0 mg/kg), which does not cross the blood brain barrier, nor amphetamine (0.5 mg/kg) impaired performance. The results of these studies further support our observation that polyunsaturated fat diets improve spatial learning in rats and furthermore suggest that the effect of dietary fat on cognitive performance may be mediated via the cholinergic system since behavioural response to central cholinergic antagonists is influenced by dietary fat fed. (Supported by grants from NSERC and MRC).

MONOAMINES AND BEHAVIOR IV

185.1 THE MASSETERIC REFLEX: A MODEL SYSTEM FOR STUDYING MONOAMINERGIC NEUROTRANSMISSION IN BEHAVING ANIMALS. D.A. Morilak, I.L. Stafford, and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Fuller understanding of the functional roles of brain monoaminergic neurons would be aided by the study of a simple neuronal system whose circuitry received dense monoaminergic input. In addition, it would be desirable if this system could be studied: in vivo; in intact animals; in the absence of anesthesia or restraint; and under physiological conditions. The brainstem monosynaptic masseteric (jaw closure) reflex is such a system. The reflex can be elicited by activation of stretch receptors in the masseter muscle or by phasic electrical stimulation of the mesencephalic tract of the trigeminal nerve (Mes5). Mes5 neurons make monosynaptic connections with neurons in the motor nucleus of the trigeminal (Mo5), which directly innervate masseter muscles. Mo5 neurons also receive dense input from both noradrenergic (NE) and serotonergic neurons. Reflex amplitude can be measured by means of bipolar recording electrodes placed in the masseter muscles. Previous studies by Chase and colleagues demonstrated that the reflex response was dramatically modulated by changes in state across the sleep-wake cycle (Experientia, 24 (1968) 47-48). In addition, in studies carried out in anesthetized rats, we previously reported that NE exerted a facilitatory effect on this reflex, which was mediated directly in Mo5 (J. Neurosci., 5 (1985) 1300-1306). We now report on our studies of this reflex in behaving cats. This abstract describes the basic methodology and changes in the reflex that are produced by a variety of environmental manipulations, while the following abstract describes our studies of the influence of NE upon this system. The reflex is elicited at each of 3 different current levels ($\sim$100 - 300 μA) by delivering square wave pulses (0.4 msec duration) to Mes5 once every 6 sec, for a total of 8 trials. A mean reflex reponse amplitude is then calculated for each current level. Under quiet waking conditions, the resulting current-response curve represents the baseline condition. All of the following manipulations, which are known to activate brain NE neurons (Prog. Neurobiol., 27 (1986) 183-194), significantly increased the amplitude of the masseteric reflex response in behaving cats: exposure to 15 min of 100 dB white noise; restraint lasting for 15 min; and 5 min exposure to an adult dog. In addition, single clicks presented 100 - 250 msec prior to the test stimulus augmented the reflex, whereas clicks presented either earlier or later failed to do so. These studies demonstrate the utility of this reflex in reflecting changes in response to environmental manipulations. Experiments currently in progress are examining whether this reflex facilitation by environmental stimuli is mediated by NE neurons.

185.2 NORADRENERGIC FACILITATION OF THE MASSETERIC REFLEX IN BEHAVING ANIMALS. I.L. Stafford and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

We previously proposed the masseteric reflex as a model system to index functional changes in brain noradrenergic (NE) activity (see previous abstract; J. Neurosci., 5 (1985) 1300-1306). We also reported that the amplitude of the reflex response was modulated by environmental conditions known to affect the activity of NE neurons. This suggested that the observed changes in the reflex might be attributable to changes in NE activity. The present studies directly examine the effects of NE upon the amplitude of the masseteric reflex in behaving cats. As described in the preceding abstract, cats were implanted with masseteric stimulating and recording electrodes. In addition, 22 ga stainless steel guide cannulae were permanently placed in the motor nucleus of the trigeminal (Mo5). This allowed us to infuse NE (or NE agonists) directly into Mo5 by means of 28 ga injection cannulae, and to examine any resulting changes in the amplitude of the elicited reflex response. During experiments, the injector was prefilled with a test drug, inserted into the guide cannula, and connected to a microsyringe driven by a remotely controlled syringe pump set to deliver 500 nl over a 1 minute period. Following baseline current-response determination, test doses were delivered and current-response curves were determined 1, 5, 15, 30, and 60 min later. Microinfusions of NE (0.125 - 5.0 μg) produced dose-dependent changes in the amplitude of the elicited reflex responses. The peak increase ($\sim$200 - 400% above baseline) occurred in response to infusion of 1.0 μg NE and was observed at all current levels tested. Increases were evident 1 min post-infusion, reached maximal levels at 5 min post-infusion, and returned to baseline levels by 30 min. The increase seen in response to 1.0 μg NE was blocked by pre-treatment with the α_1-adrenergic antagonist prazosin (5 mg/kg, i.p.) but not by pre-treatment with the serotonin (5-HT) antagonist methysergide (0.5 mg/kg, i.p.). (This dose of methysergide did, however, completely block the increase in the reflex seen in response to 1.0 μg 5-HT.) Infusions of the α-adrenergic agonist phenylephrine (0.5 - 5.0 μg) also produced dose-dependent increases in the amplitude of the masseteric reflex. By contrast, infusions of the β-adrenergic agonist isoproterenol (0.5 - 5.0 μg) had no effect on the reflex. Neither saline nor pH control infusions had any effect on the amplitude of the reflex. In summary, these data indicate that NE modulates the amplitude of the masseteric reflex, probably at α-adrenergic receptors, by a direct action in Mo5. Experiments currently in progress are investigating whether activation of the NE input to Mo5 under physiological conditions is responsible for augmentation of the reflex response.

185.3 SYSTEMIC NALOXONE ADMINISTRATION ACTIVATES LOCUS COERULEUS NEURONAL ACTIVITY UNDER STRESSFUL BUT NOT NON-STRESSFUL CONDITIONS. B.L. Jacobs and E.D. Abercrombie. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544 and Dept. Behav. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15260.

We reported previously that the activity of noradrenergic neurons in the locus coeruleus (LC) of freely moving cats is tonically increased by stressful conditions [Soc. Neurosci. Abstr., 12 (1986) 1134]. Since endogenous opioid peptides are known to be released during stress and because there is a dense population of opioid receptors in the LC, we were interested in determining the degree to which opioid receptor blockade would affect the stress-related activation of noradrenergic LC neurons. The present study examined the effects of naloxone (1 mg/kg i.v.) on LC noradrenergic neuronal activity when administered at the 15 min point of a 30 min duration restraint stress. For restraint, animals were placed in a canvas handling bag and then wrapped with an elastic bandage about the torso thus precluding movement of the limbs. Neurons in the area of the LC were identified as noradrenergic by physiological and neurochemical criteria which included: a) slow discharge rate ($\sim$1.0 spike/sec); b) long duration action potential; c) complete suppression of activity during REM sleep; and d) complete suppression of activity following systemic administration of the alpha-2 agonist clonidine (25 µg/kg i.p.). During the first 15 min of the 30 min restraint period, LC noradrenergic unit activity was increased by 95% above baseline. Following naloxone infusion at 15 min, the firing rate of these neurons showed a further increase to 270% of baseline (n=6). This second increase was not observed during 30 min restraint in the absence of naloxone administration. Naloxone administration during stress also increased signs of distress as evidenced by increased frequency of struggling and vocalization. In contrast, when naloxone was administered to non-stressed animals during quiet waking behavior, no change in LC noradrenergic neuronal activity was observed (n=7). Neuronal activity under this condition was no different from that observed following i.v. infusion of saline (n=6). Although these results cannot be ascribed positively to a direct effect of naloxone upon opioid receptors in LC, a moderating role for endogenous opioids in the responsiveness of LC noradrenergic neurons to stressful stimuli is suggested. Experiments in our laboratory are currently examining whether this activation of LC neuronal activity is attributable directly to the blockade of opioid receptors in the LC. These results also support the idea that the endogenous opioid system is not tonically active during non-stress conditions.

185.4 ACTIVITY OF LOCUS COERULEUS NORADRENERGIC NEURONS DURING DEFENSE REACTION IN BEHAVING CATS. E.S. Levine, E.D. Abercrombie, R.B. Bandler, and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Locus coeruleus noradrenergic neurons (LC-NE) have been hypothesized to play a role in the response to stress, including fear- and anxiety-producing situations. We have previously reported that the activity of these neurons, recorded in behaving cats, is generally correlated with the level of behavioral arousal. In addition, LC-NE neurons showed a sustained increase in firing rate during the presentation of both environmental and physiological challenges (J. Neurosci., in press; Brain Res., in press), as well as during a conditioned emotional response (Brain Res., 371 (1986) 335-344). In the present study, we further examined this issue by studying the response of LC-NE neurons in behaving cats during exposure to a dog, a naturalistic fear-producing situation. LC-NE units were identified by their a) slow firing rate (<2 Hz), b) long duration action potential (>2 msec), c) complete suppression of activity during REM sleep, d) complete suppression following administration of the $\alpha 2$ adrenoceptor agonist clonidine (25 µg/kg, i.p.), and e) histological localization to the LC. During the recording session, cats were exposed to a dog through a steel mesh screen for five minutes. We have previously shown that this exposure produces a marked increase in heart rate and plasma NE, indicative of strong autonomic activation (Soc. Neurosci. Abstr. 12: 1134, 1986). In response to the dog, cats typically moved to the rear of the booth and showed a strong feline defense reaction characterized by piloerection, arched back, hissing, growling, and broadside stance. LC-NE unit activity was significantly elevated ($\sim$180%) above spontaneous active waking baseline during the entire trial. When the dog was removed, unit firing and heart rate rapidly decreased and returned to baseline within five minutes. These data should be contrasted with our previous findings that exposure of cats to either inaccessible rats or another cat did not increase LC-NE firing above active waking baseline levels (Brain Res., 371 (1986) 324-334). In addition, we have also reported that serotonergic neurons in the dorsal raphe nucleus were not specifically affected by the presentation of a dog (Soc. Neurosci. Abstr. 12: 1134, 1986). In a continuation of this research, we have conducted pilot studies which indicate that LC-NE neurons are dramatically activated during the defense reaction elicited by excitatory amino acid stimulation of the periaqueductal grey. Taken together, these data indicate that stressful or fear-producing situations are particularly effective in activating LC-NE neurons, and that these neurons may play a role in the responses to these challenges.

185.5 STRAIN SPECIFIC BEHAVIORAL EFFECTS OF INESCAPABLE SHOCK AND DESMETHYLIMIPRAMINE. N.Shanks and H. Anisman. Psychology Dept., Carleton University, Ottawa, Ont. CANADA. K1S 5B6

In an attempt to relate the impact of stressors in animals to affective disorders in humans, a series of experiments was conducted to assess the effects of acute inescapable shock on the performance of several strains of mice (A/J, Balb/cByJ, C57BL/6J, DBA/2J, C3H/HEJ, CD-1) in a shuttle escape, forced swim, and exploratory task. Wide ranging differences of performance were noted across strains as a function of inescapable shock treatment. Interestingly, however, behavioral disturbances engendered by the stressor in one particular task did not necessarily result in behavioral disruption in the same strain when performance was assessed in a different task. In effect, it appeared that although uncontrollable stressors may elicit behavioral disturbances in numerous paradigms, an impairment in a particular task was not predictive of impairments in other tasks.

Repeated treatment with desmethylimipramine (DMI) (5mg/kg, 2 X daily) was also found to have strain specific effects in alleviating the stressor induced behavioral disturbances. For instance, in the A/J strain DMI applied chronically either before or after inescapable shock treatment prevented the appearance of shuttle escape deficits. In the CD-1 and Balb/cByJ strains, however, the drug treatment was ineffective in altering performance, while in the C57BL/6J mice, DMI had a prophylactic effect, but not a therapeutic effect. It will be noted as well, that these particular strain specific effects were not evident when other antidepressant agents were employed. Thus, repeated administration of amitriptyline and bupropion were found to prevent behavioral disturbances in the CD-1 strain, where it will be recalled DMI was without effect. It is suggested that genetic factors may influence the behavioral symptoms associated with uncontrollable aversive events as well as the effectiveness of antidepressant drugs ameliorating these symptoms.

185.6 THE EFFECTS OF CHRONICALLY ELEVATED LOCOMOTOR ACTIVITY ON BRAIN DOPAMINERGIC MARKERS. S.G. Speciale, T. Ryschon* and G. Ordway*. Depts. of Psychiatry & Physiology, Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235-9070 U.S.A.

There is considerable evidence of a role for brain dopamine(DA) pathways in motor function. A number of laboratories have reported enhanced caudate nucleus (CN) DA metabolism after locomotion in animals, while Parkinson's disease is accompanied by motor impairment and prominent post-mortem reductions in CN DA and its innervating substantia nigral neurons. We reported recently that acute running wheel activity (0.5m diameter, motorized 4rpm for one hour) significantly elevated the DA metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in rat CN (Speciale et al., Br. Res. Bull. 16:33, 1986). DA and serotonin (5HT) metabolism were relatively unchanged.

We wanted to explore this problem further by examining the effects of longer increased locomotion on metabolism in specific monoamine pathways and correlate their receptor properties. Adult Sprague-Dawley rats ran voluntarily in a freely-moving wheel attached to the home cage with ad libitum food and water, for 1, 4 or 7 weeks. Control (kept in home cages) and experimental animals were anesthetized (pentobarbital, 50mg/kg, i.p.), decapitated, the brains dissected out rapidly, and frozen whole on dry ice. Serial coronal sections were cut in a cryostat: 20um for histology, 20um for in vitro autoradiography, and 300um for neurochemical analysis. Autoradiography for DA D2 receptors was carried out using 3H-spiperone (Herkenham & Pert, J. Neurosci. 2:1129, 1982). NE, DA, HVA, DOPAC, 5HT and its metabolite, 5-hydroxyindoleacetic acid (5HIAA) were analyzed in tissue micropunches, using HPLC with electrochemical detection. In contrast to the acute running wheel paradigm, in the CN from animals running for 4 weeks, both DA and DOPAC concentrations, as well as the DOPAC/DA turnover ratio were decreased significantly from controls. NE, HVA and the HVA/DA ratio, as well as 5HT, 5HIAA and the 5HIAA/5HT ratio were reduced in the experimental group, but fell short of statistical significance. D2 receptor density and affinity will be correlated with monoamine metabolism over the increasing duration of locomotor activity.

185.7 AMYGDALA AND NUCLEUS ACCUMBENS LESIONS IMPAIR COCAINE SENSITIZATION. R.M. Post, S.R.B. Weiss*, J. Smith Jr*., T.L. Sullivan* and A. Pert. (SPON: J. Frascella). Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

In many species, psychomotor stimulants can produce progressively increasing behavioral responses with repeated administration (behavioral sensitization). These effects can be long-lasting and are affected by dose, gender, and intermittency of administration. In addition, behavioral sensitization has a conditioned component and is environmentally context-dependent, e.g., sensitization occurs in rats that are pretreated and tested in the same environment, but not in rats that are given equal doses of cocaine in a different environment. This aspect of cocaine sensitization led us to examine the role of certain brain areas in this phenomenon which have traditionally been associated with conditioning, memory, or stimulant-induced locomotor activity. Electrolytic lesions of the amygdala, dorsal hippocampus, ventral hippocampus, and cerebellum, and 6-OHDA lesions of the nucleus accumbens were examined for their effects on behavioral sensitization to cocaine. The sensitization paradigm employed is one in which rats receive a single high dose of saline or cocaine (40 mg/kg) in the test cage and are tested the following day with lower dose cocaine 10 mg/kg). Locomotor activity and stereotypy were evaluated. This paradigm reliably produces sensitization which is completely context-dependent.

Lesions of the nucleus accumbens and the amygdala blocked the sensitization to cocaine while the other lesions were without effect. The lesions of the nucleus accumbens produced approximately a 60% depletion of dopamine in this area, and while preventing the sensitization , these lesions did not prevent the hyperactive response to the high-dose cocaine on the pretreatment day.

Pathways involved in cocaine-induced sensitization may involve the amygdala and the dopaminergic components of the nucleus accumbens. The amygdala has also been implicated as a substrate in conditioned fear and in other types of learning and memory paradigms (particularly when lesions are combined with that of the hippocampus). Lesions of the nucleus accumbens have also been shown to impair amphetamine conditioning (Gold et al., 1986). Behavioral sensitization and conditioning may be relevant to the development and evolution of psychopathology in human cocaine users, therefore, further analysis of the neurohumors and pathways related to these phenomena seem warranted.

185.8 THE FUNCTIONAL OUTPUT OF THE NUCLEUS ACCUMBENS AS REVEALED WITH 2-DEOXYGLYCOSE AUTORADIOGRAPHY. A. Pert and L. Estall*. (SPON: T. Bevan). Biological Psychiatry Branch, NIMH, Bethesda MD 20892

It is certain that the nucleus accumbens represents a critical focus for the excitatory effects of sympathomimetic compounds. Injections of indirectly- as well as directly-acting dopaminergic agonists into this structure increase locomotor output, while 6-OHDA lesions attenuate the excitatory effects of amphetamine and cocaine. Recently, Swerdlow et al. (Brain Res., 306, 1984, 141-143) have demonstrated that the efferent pathway from the NA to the subpallidal region appears to serve as one important output of mesolimbic activity for the expression of locomotor behavior. Whether the pallidal output is solely involved in translating the sympathomimetic activation of the NA into locomotor excitation remains to be determined. The purpose of this investigation was to assess the functional outputs of the nucleus accumbens following direct activation with sympathomimetics using 2-deoxyglucose autoradiographic procedures. Rats were implanted with unilateral cannulae guides aimed for the n. accumbens. Following recovery, the animals were injected in the NA with either 15 nmoles of d-amphetamine sulfate or 50 nmoles of cocaine HCl. Three minutes following NA injections, the rats were administered 100 µCi/kg of [^{14}C]-2-deoxyglucose (2DG) through indwelling jugular catheters. Forty-five minutes following 2DG injections, the animals were sacrificed and their brains removed. The brains were prepared for autoradiographic analyses using standard procedures. Injections of amphetamine and cocaine were found to decrease metabolic activity in the head of the caudate nucleus, olfactory tubercle, cingulate cortex, as well as the NA ipsilateral to the injection. Significant increases, however, were found in the ipsilateral ventral pallidum and the striatal fundus. The activity in some thalamic nuclei decreased ipsilateral to the injection. In the hindbrain, increases in activity were found in the zona reticulata and ipsilateral medial and lateral olivary complex. Combined with lesioning procedures, the 2DG methodology may allow a more comprehensive analysis of the functional outflow from behaviorally relevant neural systems.

185.9 INTRAVENTRICULAR (ICV) NOREPINEPHRINE (NE) STIMULATES LOCOMOTION IN NUCLEUS ACCUMBENS (NAC) DOPAMINE-DENERVATED RATS. N.R. Swerdlow and G.F. Koob. Dept. of Psychiatry, UCSD Medical Center and Division Neuroscience and Endocrinology, Scripps Clinic and Research Foundation La Jolla, CA 92037

Locomotor activation in rats following ICV infusion of NE is greatly potentiated by depletion of whole brain catecholamines by ICV injection of 6-hydroxydopamine (6OHDA). This "supersensitive" locomotor response to NE has been suggested to result from the action of NE on supersensitive denervated NE receptors. In a series of experiments, we examined the neural substrates for this NE-stimulated locomotion.

In the first experiment, rats were surgically implanted with ICV cannulae after ICV injection with 6OHDA (n=5) or vehicle (n=5). Beginning 10 d later, all rats were tested for their locomotor response to 4 doses of NE (0,0.2,2.0 or 20 µg ICV) in a randomized design on four test days, with each test day separated by one non-test day. Dose-dependent locomotor activation was observed in ICV 6OHDA -injected rats; no significant locomotor activation was noted in ICV vehicle - injected rats. Biochemical analysis revealed that these 6OHDA infusions significantly depleted brain NE and dopamine (DA) from all brain areas assayed, including the cortex, hippocampus, striatum and hypothalamus.

A second group of rats were surgically implanted with ICV cannulae following pretreatment with desmethylimipramine and infusion of 6OHDA (n=5) or vehicle (n=5) into the NAC. Beginning 10 d later, all rats were tested for their locomotor response to ICV NE as above. Dose-dependent locomotor activation was observed in NAC 6OHDA-injected rats; no significant locomotor activation was noted in NAC vehicle-injected rats. NE-stimulated locomotion in NAC 6OHDA-injected rats was indistinguishable in amplitude and time course from NE-stimulated locomotion in ICV 6OHDA-injected rats. NAC 6OHDA-lesioned rats also demonstrated a supersensitive locomotor response to the DA receptor agonist apomorphine. The NAC 6OHDA lesions significantly depleted NAC DA; NE was not significantly depleted from brain regions assayed.

These results suggest that exogenous NE stimulates locomotor activation in ICV and NAC 6OHDA-injected rats through activation of supersensitive NAC DA receptors. These findings do not support the hypothesis that brain NE systems normally are an independent neural substrate for locomotor activation in the rat, but do suggest that NE might stimulate locomotor activation through an action at NAC DA receptors in states of DA receptor supersensitivity.

185.10 6-OHDA LESIONS OF THE VENTRAL TEGMENTAL AREA AFFECT SIGNALLED ESCAPE RESPONDING, BUT NOT CLASSICALLY CONDITIONED RESPONSES IN THE RAT. W. Jeffrey Wilson & Jennifer C. Hall*, Department of Psychological Sciences, Indiana University - Purdue University at Fort Wayne, Fort Wayne, IN 46805, USA.

Electrolytic lesions of the ventral tegmental area in the rat enhance signalled active avoidance performance by facilitating classically conditioned fear responses to the signal (Wilson & Baeske, Acta Neurobiol. Exp., 1987, 47). We have proposed that this effect was due to damage to the mesolimbic dopamine system; the present experiment tested this hypothesis.

Lesions of the mesolimbic dopamine system were produced in 16 rats by the infusion of 2 µg 6-OHDA in a volume of 1 µl isotonic saline + 1 mg/ml ascorbic acid. Vehicle alone was infused into 16 additional rats. Beginning at least 1 week after surgery, 8 Lesion and 8 Control rats received 8 daily Classical Conditioning sessions in which a 5 sec tone was paired with a 1.5 mA scrambled foot shock in a rectangular plastic shuttlebox. The remaining rats received Pseudoconditioning sessions in which the tone and shock were explicitly unpaired. Each session consisted of 50 presentations of both tone and shock, with tones occurring randomly every 10 to 40 sec. The shock remained on until the rat shuttled, or for a maximum of 5 sec in the absence of a shuttle response. Responses during the tone and during the inter-tone interval were recorded, as was the latency to escape the shock.

The 6-OHDA lesion failed to enhance responses during the tone, i.e., classically conditioned responses, and had no effect on overall responsiveness in the inter-tone interval. Classically Conditioned Control rats tended to escape the shock more rapidly than the Pseudoconditioned Control rats, indicating that the tone came to control their escape responses as a result of its pairing with the shock. This effect was absent in the Lesioned rats: the Classical and Pseudoconditioned rats escaped equally slowly, with a latency comparable to that of the Pseudoconditioned Control rats. Thus the 6-OHDA lesion disrupted the rats' ability to respond to the signal for shock by preparing to escape.

These results are of interest for two reasons. First, they indicate that the enhancement of classically conditioned responses following electrolytic lesions of the ventral tegmental area is not due to damage to the mesolimbic dopamine system, as we thought. Second, the results indicate that the ability of a signal to facilitate escape responses is disrupted by this lesion. Thus, although Koob et al. (Brain Research, 1984, 303) have suggested that lesions of both the mesolimbic and nigrostriatal dopamine systems are necessary to disrupt avoidance performance, damage to the mesolimbic system alone is not without its effect in aversively motivated learning situations.

185.11 GENETIC DETERMINANTS OF SUSCEPTIBILITY TO COCAINE-INDUCED SEIZURES AND DEATH. T.W. Seale and J.M. Carney*. Departments of Pediatrics, Pharmacology and Biochemistry, Univ. of Oklahoma Health Sci. Ctr., Oklahoma City, OK 73190.

Significant inter-individual heterogeneity in susceptibility to (-) cocaine-induced seizures, life-threatening cardiovascular effects and psychological dependency occurs in man. It is unclear whether this variability in responsiveness results from intrinsic, inherent differences in susceptiblity of peripheral and central targets to this drug or from environmentally-induced acquired effects. To examine the role of genotype in the determination of *susceptibility to selected actions of cocaine, we have investigated the* occurrence of variation in susceptibility to acute cocaine-induced seizures and death among 9 strains of inbred mice. All mice used in this study were 8 week old males obtained from the Jackson Laboratory. These strains were chosen for study on the basis of their genetic divergence from one another, not upon previously defined neuropharmacological or behavioral criteria. One strain, DBA/2, was chosen as a prototype strain, against which the responses of the other strains were compared. Dose response curves established a seizure threshold dose of 70 mg/kg ip and a CD_{50} dose of 85 mg/kg ip in DBA/2 mice. Clonic but not tonic seizures occured. Lethal effects were observed at higher doses of (-) cocaine (LD_{50} 135 mg/kg ip). These responses were stereospecific; a (+) cocaine dose of 150 mg/kg ip was without effect. Seizures, but not lethality, were blocked by clonazepam pretreatment (0.5 mg/kg ip). Screening for increased susceptibility, carried out at a dose of 70 mg/kg ip (the threshold dose for DBA/2 mice), identified 4 strains -- AKR, C3H/He, C57BL/6 and SWR -- in which a significantly increased frequency ($p < 0.005$) of animals convulsed after this dose of cocaine. The CD_{50} dose for these strains was approximately 60 mg/kg ip. These inherent differences in susceptibility to cocaine-induced seizures were cocaine-specific, rather than generalized differences in convulsant sensitivity. These strains were not uniquely sensitive to other chemical convulsants such as picrotoxinin or N-methyl-D-aspartate. Relative susceptibility to the convulsant action of cocaine did not correlate with inherent relative susceptibility to the lethal effects of cocaine nor other cocaine-induced behavioral changes. Four phenotypic classes of cocaine responsiveness were identified among the strains -- low susceptibility to both seizures and lethality (DBA/2, BALB/cBy, C57BL/6By strains), low susceptibility to seizures but high susceptibility to death (A, CBA strains), high susceptibility to seizures but low susceptibility to death (AKR, C57BL/6 strains) and high susceptibility to both actions of cocaine (C3H/He and SWR strains). From these data we conclude 1) genetically determined differences in inherent sensitivity to the actions of cocaine do occur, 2) susceptibilities to the convulsant and lethal actions of cocaine are under separate genetic control, i.e., inherent alterations in cocaine responsiveness can be behavior- or target organ-specific, 3) the observed differences in cocaine responsiveness between strains do not apear to reflect pharmacokinetic differences, 4) selected strains of inbred mice offer valuable tools to obtain further insight into the pharmacogenetics of cocaine's actions.

185.12 EFFECTS OF ACUTE AND CHRONIC COCAINE ON SPONTANEOUS LOCOMOTOR ACTIVITY IN RATS. A. Markou. Department of Psychology, University of California, San Diego, La Jolla, CA 92093.

The present study was designed to characterize the effects of acute and chronic cocaine on spontaneous locomotor activity. Thirty Sprague-Dawley rats were housed individually in activity chambers. Several behavioral measures (e.g. crossovers, rearings, eating) were continuously monitored. Animals were assigned randomly to three groups. Group 1 was a saline-control group. *Group 2, after a three day habituation period, was injected (IP)* with 20.0 mg/kg cocaine for seven consecutive days. *Group 3* received only one 20 mg/kg cocaine injection after a nine day habituation period. The experiment was also designed to test for the possible role of conditioning factors in the behavioral augmentation observed after chronic cocaine administration. Twenty-four more animals were used. Animals housed four per cage in the animal room were injected with saline or cocaine for 6 days. All rats were transferred to the individual activity chambers 15 hours before challenge with saline or cocaine on the seventh day.

Analyses of the data indicated that acute administration of 20 mg/kg cocaine resulted in a brief stereotypy phase in which little locomotor activity occurred, followed by a sustained increase in locomotor and rearing activity. Activity levels returned to baseline levels within three hours of injection. The response to acute cocaine was not affected by different pre-injection habituation periods (i.e. 15 hours, 3 or 9 days). When cocaine was administered chronically to animals in the individual activity chambers, the initial stereotypy phase became more pronounced and increased in duration. By contrast, there was no evidence of an enhanced responsiveness to repeated cocaine when animals, injected in their home cages were tested in the experimental chambers. The above finding implies a role for conditioning factors in the changes in the response to cocaine after chronic administration.

185.13 SIMULTANEOUS ANALYSIS OF SEROTONIN NEURONAL ACTIVITY AND RELEASE IN BEHAVING ANIMALS. L.O. Wilkinson, K.M. Martin, S.A. Auerbach, C.A. Marsden, and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

A large body of evidence suggests a specific role for serotonin (5-HT) in various behaviors and physiological processes. However, work in our laboratory and elsewhere has indicated that 5-HT neuronal activity remains unchanged in a number of behavioral and physiological conditions. The present experiments were designed to examine how changes in the neuronal activity of 5-HT cells correlate with extracellular 5-HT levels in the terminal fields of these neurons.

Extracellular 5-HT was measured in the striatum of the awake, freely moving cat using the dialysis technique of Hernandez et al. (1986). In brief, a concentric probe with a permeable tip was lowered through an implanted guide cannula at the time of the experiment. A modified Ringers solution containing 10 µM of the specific 5-HT reuptake inhibitor fluoxetine was pumped through the probe, collected, and injected into an HPLC with EC detection for analysis of 5-HT. Extracellular 5-HT was examined during: 1) electrical stimulation of the dorsal raphe nucleus, 2) systemic administration of 8-OH-DPAT, which acts on the cell body autoreceptor to decrease neuronal firing, and 3) fluctuations in unit activity observed during changes in the behavioral state of the animal during the sleep/wake cycle.

The following generalizations can be made. Low frequency stimulation of the DRN produced an increase in extracellular 5-HT that was directly related to stimulation intensity. Administration of 8-OH-DPAT (250 µg/kg, sc.), produced a 60% decrease in extracellular 5-HT and the duration of this effect approximately paralleled the decrease in unit activity seen after administration of this dose. In one instance of simultaneous 5-HT unit recording and measurement of extracellular 5-HT, the correlation between these 2 measures was 0.85. 5-HT efflux also seems to parallel changes in behavioral state across the sleep/wake cycle. These data demonstrate the coupling of neurotransmitter release to action potential generation under these conditions, and substantiate the validity of the dialysis technique.

185.14 5-HT DEPLETION FAILS TO INHIBIT METHYSERGIDE-INDUCED SUCKLING IN WEANLING-AGE RATS. A. H. Lichtman*, C. R. McLaughlin, & C. P. Cramer. (SPON: H. C. Hughes). Dept. of Psychology, Dartmouth Coll., Hanover, NH 03755.

The hypothesis that an emerging serotonergic (5-HT) inhibitory mechanism is involved in the process of weaning is supported by the finding that acute administration of several 5-HT antagonists, including methysergide, facilitate nipple attachment in weanling-age rats (Williams, Hall, & Rosenblatt, 1979). In addition, several 5-HT agonists inhibit nipple attachment, and pretreatment of methysergide reverses these inhibitory effects. If methysergide facilitates nipple attachment exclusively via a 5-HT system, then depletion of 5-HT should attenuate this effect.

In Experiment 1, we administered the long term 5-HT antagonist, parachlorophenylalanine (PCPA, 300 mg/kg, ip.) or saline to pups at either Days 20 or 22. In Experiment 2, we administered on Day 20 an intraventricular injection of either the 5-HT neurtoxin, 5,7-dihydroxytryptamine (5,7-DHT, 150 µg in 3 µl of isotonic saline with 0.5% ascorbic acid), or an equivalent volume of the vehicle. In addition, all subjects were pretreated with pargyline (50 mg/kg, ip.), an MAO inhibitor, to prevent destruction of noradrenergic neurons (Breese, Vogel, & Mueller, 1978). In both experiments, animals in each group were administered either methysergide (20 mg/kg, ip) or saline 20 minutes prior to a 1 hour attachment test at Day 25. Both the latency to attach to a nipple and the total time attached were recorded.

Methysergide facilitated nipple attachment on both dependent measures in Experiment 1. Suckling was also facilitated in rats treated with PCPA at Day 20, but not at Day 22.

In the second experiment, the animals injected with 5,7-DHT actually attached with longer latencies and for shorter periods of time than the sham-lesioned animals. Again, methysergide facilitated attachment independent of neurotoxin exposure. Another unexpected finding was that the sham-lesioned animals spent more time attached than expected, suggesting that the pargyline pretreatment may have facilitated nipple attachment.

These data suggest that 5-HT may not be the only system involved in methysergide-induced suckling during the weaning period.

185.15 TIME COURSE OF NEUROPHARMACOLOGICAL REORGANIZATION OF STRIATAL FUNCTION FOLLOWING UNILATERAL 6-HYDROXYDOPAMINE LESIONS. R.J. Carey and M.R. Lynch. Psychiatry Department, SUNY Health Science Center at Syracuse, and VA Medical Center, Syracuse NY 13210.

Extensive unilateral destruction of nigral dopamine neurons in adult rats by unilateral intranigral injection of 6-hydroxydopamine produces persistent movement asymmetries. A salient motoric manifestation of this disturbance is a tendency of animals to rotate in a direction ipsilateral to the lesion hemisphere. This ipsilateral turning can be greatly exaggerated by amphetamine treatment. In the present study, the postoperative time course of the effects of a low dose of d-amphetamine sulfate (1.0 mg/kg) on rotational behavior in 6-OHDA treated rats was examined. Separate groups of rats (N=6) given unilateral 6-OHDA intranigral injections (4 ul of 3 ug/ul) were tested for locomotor and rotational behavior at 1, 3, 6 and 9 days postoperative. Following 1.0 mg/kg amphetamine injections the 6-OHDA rats exhibited rotational behavior which was related to the time postoperative. At one day postoperative low rates (0.2/min) of ipsilateral and contralateral rotational responses were observed. On days 3 and 6 vigorous contralateral rotation occurred (6.0/min). On day 9 and all subsequent test days ipsilateral rotation was observed (3.0/min). Vehicle injected and control animals did not exhibit rotational behavior to amphetamine. Measurement of striatal dopamine and dopamine metabolites HVA and DOPAC indicated that the direction of rotational behavior was not always directly related to hemispheric dopamine content. At day one postoperative, dopamine in the 6-OHDA hemisphere was increased but at days 3 and 6 postoperative the level of dopamine was reduced to 30% and 3% of the intact hemisphere respectively. On day 9 postoperative the striatal dopamine level was also at 3% of the intact hemisphere. Increases in HVA but not DOPAC activity in the 6-OHDA hemisphere were closely correlated with the contralateral rotation induced by amphetamine. These findings indicate that a dynamic reorganization of striatal dopamine function occurs during the first week following a 6-OHDA lesion and this process may provide a fruitful model for assessing neuropharmacological plasticity associated with brain injury.

185.16 STIMULATION OF SEROTONIN-1A RECEPTORS PREVENTS CISPLATIN-INDUCED EMESIS IN CATS. J.B. Lucot and G.H. Crampton. Dept. Pharmacol., Wright State Univ., Dayton, OH 45435.

Emesis elicited by cancer chemotherapeutic agents is a significant clinical problem. The novel anxiolytic, buspirone, has been reported to block emesis elicited in cat by motion and by xylazine, which presumably use different neural pathways to trigger the emetic reflex. Buspirone also blocks cisplatin-induced emesis in cat. The mechanism by which buspirone exerts this effect is unknown because it stimulates serotonin-1A (5-HT_{1A}) receptors and at higher doses, blocks presynaptic dopamine receptors. Accordingly, the selective 5-HT_{1A} agonist, 8-hydroxy-2 (di-n-propylamino)tetralin (8-OHDPAT) was evaluated for its antiemetic effects and found to be effective against motion sickness and xylazine-induced emesis in cats. This led to the hypothesis that 8-OHDPAT would block cisplatin-induced emesis.

Jugular catheters were implanted unilaterally under ketamine and pentobarbital anesthesia and sterile conditions, threaded under the skin and externalized through the back of the neck. Patency was maintained by drawing blood, flushing with 50 U/ml heparin and filling with 1000 U/ml heparin after surgery and every other day thereafter. Experiments were conducted 72-96 hr after surgery. All cats received 7.5 mg/kg cisplatin (Sigma) in a solution of 2 mg/ml infused over 4-5 min. Experimental cats received 0.16 mg/kg 8-OHDPAT (Research Biochemicals Inc., n=8) or 0.64 mg/kg (n=6) SC immediately before cisplatin. Controls were done to verify the efficacy of each bottle of cisplatin and added to historical controls (n=10). All emetic events were recorded over a 6 hr observation period, after which the cats were euthanized with T-61 (Hoechst) administered IV.

The dose of 0.16 mg/kg increased the latency to emesis and the dose of 0.64 mg/kg decreased the number of emetic episodes ($P<.05$, Wilcoxin's test). The dose of 0.16 mg/kg produced no nonspecific effects, while the dose of 0.64 mg/kg elicited strong defensive behaviors. These data demonstrate that stimulation of 5-HT_{1A} receptors blocks emesis elicited by a variety of emetic stimuli and that this action is adequate to explain the antiemetic effects of buspirone.

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185.17 SEROTONERGIC MECHANISMS OF AGGRESSIVE AROUSAL M. Potegal, L. Haimes and L. Skaredoff* New York State Psychiatric Institute, New York, NY, 10032

"Priming" a female hamster by allowing it one biting attack on a drug-treated target hamster reduces the latency of the subsequent attack. "Satiating" a hamster by allowing it to attack a series of targets until it meets a criterion of 3 successive target presentations without attack increases the latency and reduces the number of subsequent attacks. The half life of the priming effect is approximately 12 hr, that of the satiation effect is 24 hr (Potegal, Agg. Behav,10,303,1984; Potegal and Popken,Behav Process,11,199,1985) Within the N. accumbens/preoptic area there are bidirectional priming/satiation-associated changes in both ^{3}H-5HT uptake and ^{3}H-ketanserin binding (Barkai et al, Neurosci. Abst,10,1170,1984; Potegal and Wagner, Neurosci Abst,11,1176,1985). The finding that 5,7-DHT injections into the POA alter attack satiation is complementary evidence for a serotonergic regulation of aggressive arousal (Potegal et al, Neurosci Abst,10,1170,1984).

We now report two further experiments in this series. In the first of these, radiofrequency lesions were placed in the dorsal or median raphe of female hamsters using movements elicited by stimulation of the III, IV and VII cranial nerves and descending pathways to the limbs for localization of lesion sites. Animals were tested once preoperatively and 3 times postoperatively on a combined priming/satiation test. Dorsal raphe lesions increased aggression relative to controls, median raphe lesions reduced it. The difference between the lesion groups was statistically significant.

In the second study we have found that the $5HT_2$ antagonist ketanserin prevents in dose-related fashion the reduction in attack latency which would otherwise follow the (initial) priming attack. A dose of 150 nmol/kg preceding the test is sufficient to maintain the probe attack latency equal to that of the priming attack. In the dose range where this selective effect can be obtained there appear to be no major effects on the initial attack latency itself nor on the number of attacks in the test. This rules out interpretations such as a generalized decrease in motor function or motivation.

The results of the first study strengthen the evidence for a serotonergic regulation of aggressive arousal. If the results of the second study are confirmed by ongoing work with the specific $5HT_2$ antagonists ritanserin and pizotifen, it will suggest a specific role for $5HT_2$ receptors in this regulation.

185.18 EVIDENCE FOR ADRENERGIC INVOLVEMENT IN FEEDING-INDUCED BEHAVIORAL STEROTYPIES (FIBS) IN PIGEONS.
I. J. Goodman (Spon. C. Craig) Department of Psychology, West Virginia University, Morgantown, WV 26506

In weight reduced pigeons, a restricted daily feeding may induce a variety of behavioral sterotypies (FIBS) (e.g. repetitive pecking) for hours thereafter on such diet days. This phenomenon is suggested to be associated with a functional interaction of brain dopaminergic (DA) and noradrenergic (NA) systems, based on forebrain transmitter assays (Goodman et al, 1983). While a current report (see Fisher & Goodman, 1987) finds that administered adrenergic agents significantly influence apomorphine-induced behavior sterotypies, the present study reports the effects of these agents on FIBS activity.

Adult, male pigeons were observed in their home cages .5 hr before and .5, 1.5 and 2.5 hrs following feeding, and scored for the presence of FIBS on these 4 occasions. Pre-feeding FIBS occurred rarely. On selected days birds were systemically injected with an adrenergic agonist or antagonist. Behavioral comparisons were made with previous non-drug day results. A second test was used to measure peck frequencies for 1 min. epochs over a 2 hr period with drugs that did not suppress FIBS scores in the first test.

Clonidine, an alpha agonist acting at pre- and postsynaptic receptors, significantly decreased FIBS at all doses tested (.005-.2 mg/kg, im). Yohimbine, a presynaptic alpha antagonist, did likewise (.1-1.0 mg/kg, im). Propranolol, a beta antagonist, also suppressed FIBS (1-5 mg/kg, im). Prazosin, (.1-.5 mg/kg, im), a postsynaptic alpha antagonist, and isoproterenol (.03-.1 mg/kg, im), a beta agonist, did not reduce FIBS. In this test, a ceiling effect prevented a FIBS score from indicating true facilitation. In peck frequency testing, thus far, isoproterenol has failed to facilitate pecking; whereas, prazosin caused a significant rise in pecking. These findings support the view that FIBS are dependent upon actions in the adrenergic as well as dopaminergic systems. It appears that the latter system facilitates FIBS (Goodman et al, 1983) and that the former one inhibits it. This is not unlike the circumstance described for drug-induced (APO) sterotypies. In both cases the bridging mechanism(s) betweem DA and NA actions remains to be elucidated in the context of relevant sensorimotor neural circuitry.

186.1 NICOTINIC RECEPTOR ACTIVATION IN RAT SUPRAOPTIC NEUROSECRETORY CELLS IN VITRO : EFFECTS OF MECAMYLAMINE, HEXAMETHONIUM AND ALPHA-BUNGAROTOXIN. C.W. Bourque and D.A. Brown. MRC Neuropharmacology Research Group, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, England.

The neurohypophyseal release of vasopressin is regulated by the electrical activity of magnocellular neurosecretory cells (MNCs) located in the supraoptic and paraventricular nuclei of mammals. Previous studies in the rat have shown that acetylcholine (Ach) excites putative vasopressin-secreting MNCs (Hatton, G.I. et al, J.Physiol. 345; 297, 1983) and that nicotinic Ach receptor antagonists can block the vasopressin response of hypothalamic explants exposed to an increase in osmolarity (Sladek, C.D. and Joynt, R.J., Endocrinol. 105;367, 1979). In apparent agreement with the presence of nicotinic receptors on these cells, a high density of binding sites for radiolabelled alpha-bungarotoxin (α-BuTX) has been reported in the supraoptic and paraventricular nuclei (e.g. Meeker, R.B. et al, J.Neurosci. 6; 1866, 1986).

In the present experiments the characteristics of these nicotinic Ach receptors have been examined in MNCs impaled in the supraoptic nucleus of perfused explants of rat hypothalamus (c.f. Bourque, C.W. and Renaud, L.P., J.Neurosci.Meth. 7; 203,1983). Bath application of Ach (5-100μM; n=9 cells) caused a depolarization and an increase of membrane conductance. This effect could be observed in the presence or absence of 0.5μM tetrodotoxin suggesting that the origin of the response was post-synaptic. Identical responses were also obtained by applying nicotine (5-100μM; n=13 cells) or dimethylphenylpiperazinium (DMPP, 5-100μM; n=4 cells). Bolus infusions of nicotine or DMPP achieving similar concentrations yielded consistently reproducible responses and were therefore used to examine the effects of various antagonists. Bath application of hexamethonium (20-200μM : n=4 cells) or mecamylamine (5-50μM; n=3 cells) both caused a dose-dependent and reversible reduction of agonist-induced responses. In contrast, 8-40 minute applications of 1μM α-BuTX (purified preparation kindly provided by Dr J.O. Dolly) had no measurable effect on responses observed in any of five cells.

These results further suggest that MNCs in the rat supraoptic nucleus are endowed with nicotinic Ach receptors. Nicotinic responses in these cells resemble those in autonomic ganglia in being blocked by mecamylamine or hexamethonium but not by α-BuTX, the significance of the α-BuTX binding sites present in the supraoptic nucleus is therefore unclear. (Supported by the MRCs of Canada and the U.K.).

186.2 MODULATION OF ADRENERGIC CONTROL OF VASOPRESSIN RELEASE BY HYPOTONICITY IN ORGAN-CULTURED HYPOTHALAMO-NEUROHYPOPHYSIAL EXPLANTS. Celia D. Sladek and Richard W. Clough. Dept. Neurology and Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Previous studies in this laboratory and others have demonstrated both stimulation and inhibition of vasopressin (VP) release by norepinephrine (NE). One hypothesis advanced to explain these findings is that both alpha and beta adrenergic receptors participate in the regulation of VP release. This hypothesis was addressed in the present study by evaluating the effects of receptor-specific adrenergic agonists on VP release from organotypic explants of the hypothalamo-neurohypophyseal system (HNS) in tissue culture. Phenylephrine (10^{-5}M), an alpha$_1$-adrenergic agonist, increased VP release by 455 ± 198% over basal release ($p<.025$, n=7) from HNS explants maintained in static organ culture for 48 hrs prior to the experiment. A beta adrenergic agonist, isoproterenol (10^{-5}M), did not alter VP release, and clonidine, an alpha$_2$ agonist, was ineffective at 10^{-5}M, but stimulated VP release at 10^{-4}M (428 ± 174%, $p<.025$, n=7). These results confirm our previous observation that alpha$_1$ agonists excite VP neurons (Br. Res. 365:192, 1986), but do not provide evidence for an inhibitory action of either beta or alpha$_2$ agonists. Thus, the differential effects of NE on VP release cannot be accounted for solely on the basis of excitation of alpha$_1$ receptors and inhibition by either beta or alpha$_2$ receptors.

A second hypothesis which was evaluated, is that the effect of NE on VP release depends on the other regulatory signals which are simultaneously affecting the HNS. This hypothesis is supported by the observation that NE (10^{-5}M) delivered into the culture medium in isotonic saline stimulated VP release from HNS explants maintained in either static ($p<.005$, n=11) or perifusion culture ($p<.05$, n=5), but NE (10^{-5}M) delivered in 10 ul of dH_2O inhibited VP release from explants in static culture ($p<.025$, n=9). This volume of dH_2O alone did not significantly alter VP release, but it did decrease the osmolality of the culture medium by 3-5 mosmol/kg H_2O. In order to determine if this reflected differential activation of alpha and beta receptors, the effect of hypotonicity on the response to phenylephrine and isoproterenol was evaluated. Use of dH_2O as the vehicle for delivery of phenylephrine (10^{-5}M) eliminated the stimulation of VP release observed when isotonic saline was used as the vehicle, but inhibition of VP release was not observed. Isoproterenol (10^{-5}M) remained ineffective in altering VP release when delivered in either saline or dH_2O. These observations support the hypothesis that hypotonicity modulates the effect of NE on VP release and may represent the in vitro equivalent of the in vivo observation that small decreases in osmolality inhibit stimulation of VP release by hypovolemia (Stricker and Verbalis, Am. J. Physiol. 250:R267, 1986).

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186.3 PREGNANCY AND LACTATION ALTER SPINAL CORD LEVELS OF OXYTOCIN AND VASOPRESSIN. C. Miaskowski*, G.L. Ong*, and J. Haldar (SPON: A. Sinha). Dept. of Biological Sciences, St. John's University, Jamaica, NY 11439.

Previous work from our laboratory has shown a cyclic variation in spinal cord levels of oxytocin (OT) and vasopressin (VP) during the stages of the rat's estrous cycle (Miaskowski, C., et al., Endocrinology, 120: 1685, 1987). The purpose of this study was to determine if pregnancy and lactation produced alterations in the levels of these neurohypophyseal peptides. Sprague Dawley rats were sacrificed at 10 and 20 days of pregnancy and lactation. The spinal cords were segmented into cervical, thoracic, and lumbosacral (L/S) regions, homogenized in 0.1 N HCl, and extracted using the Sep-pak method. Hormone content was determined by radioimmunoassay and compared to proestrus and diestrus control values. The table summarizes the increases in OT and VP content in the various segments of the spinal cord during pregnancy and lactation:

		% INCREASE COMPARED WITH DIESTRUS		% INCREASE COMPARED WITH PROESTRUS	
		10 DAYS	20 DAYS	10 DAYS	20 DAYS
PREGNANCY					
Cervical	OT	35.5^{++}	-----	295.7^{++}	87.4^{++}
	VP	-----	-----	-----	-----
Thoracic	OT	33.7^{*}	25.6(NS)	122.5^{++}	109.7^{++}
	VP	5.6(NS)	-----	105.6^{++}	88.3(NS)
L/S	OT	157.0^{++}	47.8(NS)	449.2^{++}	215.8^{++}
	VP	102.9^{+}	50.0(NS)	221.4^{+}	139.3(NS)
LACTATION					
Cervical	OT	33.8(NS)	116.6^{++}	188.4^{+}	366.8^{++}
	VP	-----	31.3(NS)	-----	94.5(NS)
Thoracic	OT	186.2^{++}	113.3^{+}	376.5^{++}	255.1^{++}
	VP	49.4(NS)	-----	190.6^{++}	53.9^{*}
L/S	OT	207.0^{++}_{**}	184.1^{*}_{*}	556.1^{++}_{**}	507.1^{**}_{**}
	VP	151.1^{**}	93.8^{*}	297.7^{**}	206.9^{**}

Statistical significance was determined using a Student's t-test ($*p<0.05$, $**p<0.02$, $+p<0.01$, $++p<0.001$ and NS = not significant). The data demonstrate that pregnancy and lactation produce changes in spinal cord levels of OT and VP with the most dramatic increases occurring in the L/S segment. The exact physiological role for OT and VP within the spinal cord during pregnancy and lactation remains to be elucidated.

186.4 CHANGES IN OXYTOCIN AND VASOPRSSIN LEVELS IN THE RAT SPINAL CORD FOLLOWING HYPERTONIC SALINE ADMINISTRATION. D.Lukic*, J. Vrba* and J. Haldar. Department of Biological Sciences, St. John's University, Jamaica, NY 11439

On the basis of immunohistochemical studies and radioimmunoassay (RIA) data it is well established that nerve endings containing vasopressin (VP) and oxytocin (OT) are present in numerous extrahypothalamic areas and the spinal cord in addition to the classical hypothalamo-neurohypophysial system. It has yet to be determined, however, what physiological role these extrahypothalamic hormones serve. Work from our laboratory documented for the first time that spinal cord OT and VP levels change during different stages of the estrous cycle (Endocrinology 120:1685-1687,1987). Recently we demonstrated that immobilization stress is a powerful stimulus for increasing spinal cord OT level while VP levels were not altered (Fed.Proc.:46,Abs.3068,1987). The purpose of this study was to determine whether increased plasma osmolality is an effective stimulus for altering spinal cord OT and VP levels.

All experiments were conducted with male Long Evans rats (240 + 5g). Rats were injected ip either with isotonic (0.85%, 2 ml/Kg) or with hypertonic saline (1M, 2 ml/Kg). Rats were either sacrificed 15 min. or 3 hours after treatment. The pituitary, hypothalamus, pons-medulla, and cervical, thoracic and lumbosacral segments of the spinal cord were isolated. All samples were homogenized in 0.1N HCl, and centrifuged. The supernatant was separated for protein estimation and the remainder was extracted using C-18 Sep-pak cartridges. OT and VP levels were then determined by RIA. Results obtained from these experiments show (1) hypertonic and isotonic saline-induced changes for both OT and VP are more effective in rats sacrificed at 15 min. as compared to 3 hours (2) At 15 min., OT increased significantly in the pons-medulla and in all segments of the spinal cord following hypertonic saline administration. (3) VP levels decreased in 15 min. with hypertonic saline treatment in both the cervical and thoracic segments of the spinal cord.

In conclusion, these data indicate (1) increased osmolality is an effective stimulus for the alteration of extrahypothalamic OT and VP levels (2) changes observed at 15 min. maybe due to a stress rather than an osmotic effect (3) spinal cord OT levels and not VP levels are more susceptible to stressful stimululi and, (4) spinal cord OT and VP levels are under different control mechanisms.

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186.5 REGIONAL HYPOTHALAMIC AND NEUROHYPOPHYSEAL BLOOD FLOW DURING HYPERCAPNIA IN THE RAT: N-ISOPROPYL-P-IODOAMPHETAMINE [ISOPROPYL-2-^{14}C], A NEW TRACER FOR MEASURING CEREBRAL BLOOD FLOW. C.L. Myers*, R.B. Page, and R.M. Bryan. Departments of Surgery (Neurosurgery), Physiology, and Anatomy, M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033.

Previous studies have shown that blood flow in the neurohypophysis [median eminence (ME) and neural lobe (NL)] does not increase during hypercapnia as it does in other brain regions. The ME and NL consist of nerve terminals whose cell bodies lie in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. It is not known if blood flow to the cell bodies in the PVN and SON fails to respond to hypercapnia as it does to their nerve terminals in the ME and NL or if their regional blood flow increases during hypercapnia like other brain regions. We measured regional cerebral blood flow in the ME, NL, PVN, SON, and other brain regions using a new tracer, n-isopropyl-p-iodoamphetamine [isopropyl-2-^{14}C] (IPIA). IPIA is completely extracted during a single pass through cerebral capillary beds. This property provides several advantages over diffusible tracers for measuring flow in regions such as the ME and NL which have high rates of flow. Rats were surgically prepared with catheters in one femoral artery and vein using nitrous oxide and halothane. A plaster cast was fitted around the hip and groin area to restrain the rats and to protect the catheters. Hypercapnia was produced by increasing the partial pressure of CO_2 in the ventilatory gas mixture. Blood flow during normocapnia and hypercapnia was as follows:

	Regional Cerebral Blood Flow (ml/100g/min)	
	Normocapnia ($PaCO_2$ = 42 + 2 mm Hg)	Hypercapnia ($PaCO_2$ = 66 + 4 mm Hg)
ME	624 ± 95	589 ± 61
NL	954 ± 66	862 ± 75
PVN	310 ± 14	497 ± 31 ($p<.05$)
SON	339 ± 43	484 ± 13 ($p<.05$)

Blood flow in the ME and NL was not affected by hypercapnia while flow in the PVN and SON increased significantly. Not only did blood flow in the PVN and SON increase during hypercapnia but flow in these regions was more sensitive to the increases in $PaCO_2$ than any other brain region studied. We conclude that blood flow in the cell bodies of the PVN and SON are regulated differently during hypercapnia than their nerve terminals in the ME and NL. [Supported by PHS grants NS19341 (RMB) and NS15926 (RBP).]

186.6 DIETHYLSTILBESTEROL TREATMENT INCREASES RAT NEUROINTERMEDIATE LOBE OXYTOCIN AND VASOPRESSIN BUT NOT METHIONINE ENKEPHALIN. J. A. Schriefer* (SPON: A. Hassen). Dept. of Pharmacology, West Virginia School of Osteopathic Medicine, Lewisburg, WV 24901.

Immunohistochemical evidence suggests that a number of peptides may be co-localized with oxytocin (OT), perhaps in the same granules, in the magnocellular neurons of the hypothalamo-neurohypophysial system (HNS). These peptides may have a role in the feedback regulation of OT release. Changes in oxytocin content might be expected to be accompanied by similar changes in content of the co-localized peptides. One peptide thought to be co-localized with OT and to have an inhibitory effect on oxytocin release is methionine enkephalin (MENK). Estrogen treatment is known to increase neurointermediate lobe OT. The estrogen stimulation paradigm was used to determine whether MENK changes in parallel with OT.

Female Sprague-Dawley rats (200-250 g) were treated with diethylstilbesterol (70 µg/day, sc) for two days. On the third day, the rats were decapitated, the neurointermediate lobe removed and homogenized in 0.25 % acetic acid. The neurointermediate lobe content of OT, vasopressin, and MENK were determined by specific radioimmunoassays. The OT and vasopressin content of the lobes was increased 1.5-2.0 fold in diethylstilbesterol-treated rats but the MENK content was unchanged. The basal and K^+ - stimulated release of OT from isolated, perfused HNS explants and neurointermediate lobes from diethylstilbesterol-treated rats was examined and found to be increased compared to that from controls. In the DES-treated rats, increased OT release rates in the presence of a decreased MENK/OT ratio are consistent with an inhibitory role for MENK in OT release. The effect of diethylstilbesterol on the MENK/OT ratio of the neurointermediate lobe implies that a means for independent regulation of OT and MENK synthesis in the same neuron must exist or that the two peptides are not located in the same neurons.

Supported by NIH grant HD 22362.

186.7 CRH NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS: VASOPRESSIN CONTAINING AND VASOPRESSIN DEFICIENT SUBPOPULATIONS. M.H. Whitnall, S. Key* and H. Gainer. Lab. of Neurochemistry, NINCDS, NIH, Bethesda, MD 20892

Half of the CRH axons projecting to the portal capillary system in normal rats contain vasopressin (AVP), co-packaged into the same secretory vesicles as CRH, while the remaining CRH axons contain no immunocytochemically detectable pro-AVP-derived peptides (Whitnall et al., Nature 317:248, 1985; Neuroendocrinol. 45:420, 1987). These axonal subpopulations respond differently to adrenalectomy (Whitnall et al., Endocrinol. 120:2180, 1987), suggesting that the levels of CRH and AVP in portal blood may be modulated by independent regulation of separate populations of CRH neurosecretory cells. This hypothesis predicts that two sub-populations of CRH-containing perikarya exist, regulated differentially by hormones and/or axonal inputs. We have tested this hypothesis using post-embedding E.M. immunocytochemistry in the nucleus of origin of these CRH fibers: the paraventricular nucleus (PVN). Male, 200 g rats were injected with 10 µl of 1% colchicine into the lateral ventricle 24-48 h before perfusion with 4% glutaraldehyde. Hypothalami were embedded in LR White, and serial ultrathin sections were immunoperoxidase-stained for CRH, pro-AVP-derived peptides, and pro-oxytocin (OT)-derived peptides. Very few CRH-positive cells were found in untreated rats, but many CRH-positive cells, both AVP-containing and AVP-deficient, were found in the PVNs of rats treated with colchicine. Three rostro-caudal levels in the caudal portion of the PVN have been analyzed in a 48 h colchicine-treated rat. Of the 192 CRH-positive perikarya found, 4 were OT-containing, 120 were AVP-containing, and 68 were unstained for pro-AVP- or pro-OT-derived peptides. The AVP-containing CRH neurons were concentrated in the dorsal part of the medial parvocellular region of the PVN, while the AVP-deficient CRH neurons were preferentially located in the ventral part of the medial parvocellular region and in the lateral parvocellular region (defined according to Swanson and Sawchenko, Ann. Rev. Neurosci. 6:275, 1983). We are presently mapping the distributions of the CRH neuronal subtypes in normal and adrenalectomized rats. The ability to identify AVP-containing and AVP-deficient subpopulations of CRH perikarya in the PVN strengthens the hypothesis of functionally independent sub-populations of CRH neurosecretory cells, and will allow us to determine what specific receptors and axonal inputs might differentially control the activity of the two components of the CRH neurosecretory system.

186.8 A COMPARISON OF SYNAPTIC AND INTRINSIC PROPERTIES OF MAGNOCELLULAR NEUROENDOCRINE CELLS IN KITTEN AND RAT. M. Fagan and R.D. Andrew. Anatomy Dept., Queen's University, Kingston, Ontario K7L 3N6.

Both rat and kitten magnocellular neuroendocrine cells (MNCs) display firing patterns in hypothalamic slices that reflect activity recorded in the respective intact animal. Specifically, 50% of MNCs recorded intracellularly from supraoptic nucleus (SON) in rat can burst repetitively (35 of 70 cells), a pattern diagnostic of vasopressinergic MNCs in the intact rat. Even though the majority of MNCs in cat SON are vasopressinergic, phasic firing is rarely observed in vivo (Koizumi and Yamashita, J. Physiol. 285 341, 1978) or in coronal slices from kitten. Of 201 MNCs in kitten SON recorded extracellularly to date only 7 were phasic, the remainder being continuous, irregular or silent. Moreover unlike rat, kitten MNCs rarely fired an afterdischarge upon brief orthodromic stimulation. Intracellular recordings (n=14) have revealed that action potentials of kitten MNCs lack the depolarizing afterpotential (DAP) generated intrinsically in rat. Since summation of DAPs cannot occur, a regenerating plateau potential which supports orthodromically triggered or phasic bursts in rat MNCs is missing in kitten.

Given this dramatic difference in the capability of generating bursts intrinsically, it was of interest to compare synaptic properties of kitten MNCs with their rat counterpart. Similar to rat, both inhibitory and excitatory postsynaptic potentials (IPSPs and EPSPs) occurred spontaneously and a compound IPSP or EPSP (40-80 ms) could be evoked by stimulation 0.5 mm dorsal to SON. Unique to kitten however in 3 of 9 cells tested was a fast (approx. 5 ms) depolarizing (3 - 6 mV) event that preceded the evoked PSPs. The origin of the fast event is currently under study.

Longer duration synaptic responses, not exclusive of the first two, were also observed in kitten. A prolonged (up to 20 s) excitatory response was most clearly observed following 10-15 Hz orthodromic stimulation for 1 s, as previously described in rat (Gribkoff and Dudek, Soc. Neurosci. Abstr. 11 1236, 1985). In one cell the amplitude of the response was large enough that a single stimulus supported overriding spikes for 13 s. In addition, extracellular recordings from 6 cells showed an inhibition of spontaneous firing for up to 10 s following a single orthodromic stimulus. Thus, although kitten MNCs normally lack an intrinsic bursting mechanism, the long-lasting effects of brief synaptic input may promote or inhibit firing over many seconds.

186.9 EXCITATORY ACTION OF OXYTOCIN ON PUTATIVE OXYTOCIN NEURONS IN THE SUPRAOPTIC NUCLEUS OF RAT SLICE PREPARATIONS. H. Yamashita, K. Inenaga, S. Okuya and H. Kannan. Dept. of Physiology, Univ. of Occupational and Environmental Health, Sch. of Med., Kitakyushu, 807 Japan.

Oxytocin (OXT) plays an important role in lactation and parturition by releasing into the bloodstream from the neurohypophysis. On the other hand, OXT may also be released centrally to modify the neuronal activity in the central nervous system, including that of OXT neurons themselves. An immunohistochemical study has revealed that OXT-immunoreactive terminals synapse on OXT neurons in the supraoptic nucleus (SON). Moreover, it has been shown that exogenous OXT enhances OXT release from isolated magnocellular nuclei in vitro and that OXT injected into the third ventricle modifies the activities of OXT cells in suckled lactating rats. Eventually neurosecretory cells can be excited by iontophoretically applied OXT. The aim of this study is to clarify the effects of OXT on SON neurons and to determine the sensitivity of OXT to these neurons using the rat hypothalamic slice preparations. Application of OXT to the bathing medium (3×10^{-7} M) made excitation in 13 (93%) of 14 cells which fired continuously and 26 (81%) of 32 cells which fired slowly and irregularly. By contrast, only 2 of 26 phasically firing neurons which were putative vasopressin neurons were excited and none of the SON cells tested were inhibited. The excitation was reversibly antagonized by a synthetic OXT analogue (d[Try(Me)^2]VDAVP). To investigate whether the responses were due to direct or indirect effect on the neurons, a low Ca^{2+} (0.5 mM) and high Mg^{2+} (9 mM) medium was used. Four neurons which had shown excitatory responses to OXT in control medium were still excited after blockade of synaptic transmission, suggesting that the SON neurons themselves were sensitive to OXT. When the actions of vasopressin (AVP) were compared with those of OXT, all 17 neurons tested which had shown clear excitatory responses to OXT showed a much weaker excitatory responses to AVP or no response at all at the same concentration. The results suggest that OXT exerts predominantly excitatory effects in the SON and that putative OXT cells are more likely to be affected than putative vasopressin cells.

186.10 **STUDIES OF THE TOXICITY OF DIISOPROPYLFLUOROPHOSPHATE: SPECIFIC CHOLINERGIC SUPPRESSION OF PROLACTIN, LUTEINIZING HORMONE AND THYROTROPIN SECRETION IN INTACT RATS.** H.G. Fein* and R.C. Smallridge* (SPON: F. Manning). Div. of Medicine, Walter Reed Army Institute of Research, Washington, D C 20307

Diisopropylfluorophosphate (DFP) is an anticholinesterase that has been employed as a model agent for studies of organophosphate poisoning. Recently, pituitary cells have been shown to have muscarinic cholinergic receptors. Therefore, we examined some hormonal effects of DFP in normal animals. DFP (or peanut oil vehicle) was administered subcutaneously to male Sprague-Dawley rats (250-350 gm). Animals were sacrificed by decapitation, trunk blood was collected, and sera were analyzed by radioimmunoassay. At 3 h after injection, DFP (0.6-2.4 mg/kg) caused a dose-dependent rise in corticosterone (cort). It also resulted in a dose-dependent reduction of prolactin (PRL) at 1.4 mg/kg and above (suppression of 64-80 % vs controls, $p<.05$) and of thyrotropin (TSH) at 1.8 mg/kg and above (56-87 % suppression, $p<.05$). Luteinizing hormone (LH) was increased 142 % at 0.6 mg/kg ($p<.01$), but was suppressed at 1.4 mg/kg and above (47-50 %, $p<.05$). The suppressive effects of DFP at 2.4 mg/kg were seen on TSH by 1 h after injection and persisted for 18 h; PRL was suppressed from 1 to 6 h and LH was suppressed at 3 and 6 h; cort was elevated from 1 to 9 h. Pituitary PRL content was increased 200 % ($p<.01$) within one half hour after administration of 2.4 mg/kg of DFP; this effect persisted for 18 h (240 % increase, $p<.01$). Pretreatment with intraperitoneal atropine at doses of up to 30 mg/kg one-half hour before DFP (2.4 mg/kg) had a dose-dependent effect of partially ameliorating the cholinergic symptoms caused by DFP, but did not affect the suppression at 3 h of PRL or LH or stimulation of cort. Atropine at 300 µg/kg had no effect on the suppression of TSH by DFP, but a reversal of TSH suppression occurred after 3 and 30 mg/kg of atropine. Thirty mg/kg of atropine did not block the suppression of PRL at 3 h by 1.8 mg/kg of DFP but did block the suppression of TSH and LH. To determine if the suppression of TSH by DFP might be due to the effects of increased cort levels, we administered DFP (2.4 mg/kg sc) to adrenalectomized rats. At 3 h, cort and PRL levels were unchanged from control but TSH was suppressed ($p<.01$). Conclusions: increased cholinergic tone induced by DFP markedly suppressed serum PRL, LH and TSH but raised intrapituitary PRL, suggesting DFP may act by inhibiting hormone release without having a primary effect on synthesis. Although increased cort levels may have partly mediated effects on PRL, the suppression of TSH appeared to be a direct effect of DFP, confirming other studies in our laboratory showing cholinergic suppression of secretion from cultured pituitary cells.

186.11 SEPTAL NEURONS WITH PROJECTIONS TO THE MEDIAN EMINENCE ARE INFLUENCED BY THE SUBFORNICAL ORGAN IN THE RAT. S.D. Donevan and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The subfornical organ (SFO), a small circumventricular structure, is believed to play an essential role in the control of pituitary hormone secretion. In addition, lesion of the SFO has been shown to disrupt the estrous cycle in the rat, suggesting a possible role in the control of gonadotropin secretion. As well as its efferent projections to the supraoptic and paraventricular nuclei, the SFO also sends axons to the medial septum and diagonal band of Broca (MS-DBB) and the preoptic area. These regions have been shown, using immunohistochemical techniques, to contain the majority of luteinizing hormone releasing hormone (LHRH) containing neurons in the rat brain, many of which project to the median eminence. Electrical and chemical stimulation in these regions leads to an increase in the release of LHRH into portal blood. The present study examines the effect of electrical stimulation in the SFO on the activity of putative LHRH containing MS-DBB neurons that project to the median eminence.

Male Sprague-Dawley rats (140-320 g) were anesthetized with urethane (1.4 g/kg), placed in a stereotaxic frame, and a concentric bipolar stimulating electrode was implanted in the region of the SFO. The medial basal hypothalamus was then exposed using a transpharyngeal approach and a bipolar stimulating electrode positioned in the median eminence. Extracellular single unit recordings were then obtained from MS-DBB neurons antidromically identified as projecting to the median eminence. The effects of electrical stimulation in the SFO on the activity of these identified MS-DBB neurons were then assessed using peristimulus histograms. The anatomical locations of the stimulating and recording electrodes were histologically verified following each experiment.

Recordings were obtained from a total of 447 neurons in the MS-DBB, 18 of which were antidromically identified as projecting to the median eminence (mean latency 18.6±1.3, SEM). Of those antidromically identified cells tested with SFO stimulation 44% responded with an increase in firing rate, 11% a decrease in firing rate, while the the remaining 44% were unaffected.

These studies describe a predominantly excitatory connection between the SFO and putative LHRH containing MS-DBB neurons projecting to the median eminence, and provide further evidence of a role for the SFO in regulating gonadotropin secretion.

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186.12 ANGIOTENSIN II ACTS AT THE SUBFORNICAL ORGAN TO INFLUENCE PARAVENTRICULAR NUCLEUS TUBEROINFUNDIBULAR NEURONS. A.V.Ferguson. Dept. Physiol. Queen's University, Kingston, Ont., Canada K7L 3N6.

The subfornical organ (SFO) is now well established as playing a major role in the control of posterior pituitary hormone secretion through it's efferent connections with the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei. The observations; 1) that SFO lesions abolish ACTH release in response to systemic AII (Keil and Thrasher Fed. Proc. 45:166 1986), and 2) that electrical stimulation in SFO causes the release of ACTH (Ferguson and Plotsky, unpublished observation) suggest a role for the SFO in the control of the secretion of this hormone from the anterior pituitary. Within the PVN are several groups of parvocellular neurons with axonal projections to the median eminence. One of these groups of neurons contain CRH. I have recently reported that electrical stimulation in SFO influences the excitability of these PVN neurons (Ferguson, A.V., Can. J. Physiol. Pharmacol., 1987 - in press). These studies were undertaken to further examine the role of SFO efferents in the control of PVN tuberoinfundibular neurons.

Urethane anaesthetised (1.4 g/kg) male Sprague-Dawley rats were used in all experiments. The medial-basal hypothalamus was then exposed through a transpharyngeal approach and extracellular single unit recordings were obtained from PVN neurons antidromically identified as projecting to the median eminence but not to the posterior pituitary. The effects systemic administration of AII (100-500 ng N=48) or adrenaline (100-500 ng N=20) on the activity of these neurons were examined. Of 48 identified cells tested 54% were specifically activated by AII, while 29% were similarly influenced by adrenaline and AII. In a second series of experiments in which greater than 50% of the SFO was destroyed by electrolytic lesion, only 7% of 30 identified tuberoinfundibular neurons tested were found to be specifically activated by AII.

These studies demonstrate that circulating AII influences the activity of PVN neurons projecting to the median eminence. They also suggest that such effects result from an action of this peptide at the SFO further supporting a role for this circumventricular structure in the control of anterior pituitary hormone secretion.

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186.13 **CHANGES IN CEREBRAL AND PITUITARY GLUCOSE UTILIZATION AFTER WATER DEPRIVATION AS DETERMINED BY AUTORADIOGRAPHIC MEASUREMENT OF (1-^{14}C)-GLUCOSE UPTAKE.** S. A. Oglesby, G. E. Duncan*, R. S. Greenwood, R. B. Meeker, J. N. Hayward, and W. E. Stumpf, Curriculum in Neurobiology and Departments of Neurology and Anatomy, University of North Carolina, Chapel Hill, NC 27514.

A high resolution autoradiographic technique was used to investigate effects of water deprivation on cerebral glucose utilization in conjunction with measurements of plasma vasopressin, osmolality, and hematocrit levels. Male Sprague Dawley rats were deprived of water (food ad libitum) for 48-52 hours and (1-^{14}C)-glucose was injected via chronic venous catheters implanted at least 5 days prior to water deprivation. Small venous blood samples (0.2 cc) were taken at the beginning and end of the deprivation period (blood replaced with equal volume of saline) and assayed for hematocrit and plasma osmolality. Rats were anesthetized with isosmotic Na pentobarbital 30 min after injection of the labelled glucose. Left ventricular blood was then taken for determination of plasma radioactivity, endogenous glucose and vasopressin (RIA) content followed by decapitation and removal of brain for mounting and freezing. Autoradiograms were prepared by thaw mounting 4 µm cryostat sections on to nuclear emulsion (NTB-3) coated slides. Radioactivity in selected brain regions was quantitated by automated silver grain counting with an Artek counter.

Water deprived rats showed an increase in plasma osmolality (+11 mosmol/kg), hematocrit (+8%), and vasopressin (+81 pg/ml) while body weight decreased by 17% compared to controls. In water deprived rats significantly higher silver grain densities, relative to controls, were found in the paraventricular nucleus, supraoptic nucleus (SON), anterior hypothalamic area, lateral hypothalamic area, periventricular nucleus, somatosensory cortex, and neural lobe of the pituitary. In contrast, no significant changes were seen in the subfornical organ, OVLT, or the region dorsolateral to the SON.

These results suggest that when rats are dehydrated by water deprivation, glucose utilization increases dramatically in somatosensory cortex as well as in several hypothalamic areas traditionally implicated in osmoregulation but with no concomitant changes in regions thought to be possible sources of osmoreception.

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186.14 EFFECTS OF NEONATAL GLUTAMATE TREATMENT ON GALANIN IMMUNOREACTIVITY IN THE RAT BRAIN. S.M. Gabriel, J.I. Koenig, U. McGarvey*, K.J. Swartz*, J.B. Martin and M.F. Beal. Department of Neurology, Massachusetts General Hospital, Boston MA 02114.

Galanin is a resently isolated gut peptide which has a wide distribution in the central nervous system and displays considerable heterogeneity across species. We have developed a radioimmunoassay (RIA) to galanin using antibodies generated in rabbits to synthetic porcine galanin conjugated to BSA. Radiolabeled porcine galanin was produced using the chloramine-T method and purified by HPLC. Studies with the synthetic porcine galanin fragments, galanin(1-10), galanin(7-16), and galanin(15-29) indicated that the galanin antibody is primarily directed towards the conformation of the midportion of the galanin molecule. There was no crossreactivity with a number of other peptides tested. The RIA detects 2 fmoles porcine galanin standard when performed under dysequilibrium conditions. However, under dysequilibrium conditions acid extracts of rat and primate brain displace ^{125}I-galanin binding poorly. In contrast, under equilibrium conditions, the assay has a sensitivity of 6 fmoles and acid extracts of rat, rabbit, human and baboon brain as well as rat pancreas displace ^{125}I-galanin binding in a parallel fashion. Sephadex G-50 chromatography and HPLC chromatography of rat brain acid extracts reveal one molecular form of galanin-like immunoreactivity. On HPLC, however, rat galanin-like immunoreactivity elutes earlier than either porcine standard or porcine brain extracts. This may indicate that the rat and porcine galanin molecules are not identical.

Since galanin neurons in the arcuate nucleus may be important in regulating neuroendocrine function, galanin-like immunoreactivity was measured in brain regions of adult male rats treated with monosodium glutamate during the neonatal period. Galanin-like immunoreactivity in control littermates were 124±11 ng/mg protein in the median eminence, 10.4±0.4 ng/mg in the medial basal hypothalamus, 11.2±0.4 ng/mg in the preoptic area, and 3.61±ng/mg in the septal region. Galanin-like immunoreactivity was reduced 57% in the median eminence, 15% in the medial basal, 22% in the preoptic region and 27% in the septal region. The concentrations of galanin-like immunoreactivity were unchanged in parietal cortex (control= 0.3±0.03 ng/mg). These studies suggest that glutamate-sensitive galanin neurons in the arcuate nucleus project throughout the medial basal forebrain of the rat. The presence of a galanin pathway from the arcuate nucleus to the median eminence indicates that this peptide may have a role in the regulation of anterior pituitary hormone release. Supported by DK07561(SMG), AG05134(MFB), and AM26252(JBM).

186.15 POSSIBLE ROLE OF HYPOTHALAMIC GROWTH HORMONE-REGULATING FACTORS IN THE SUPPRESSION OF GROWTH HORMONE DURING MATERNAL DEPRIVATION OF YOUNG RATS. L.M. Juárez* and L.A. Meserve* (SPON: R. Kantner). Dept. of Biol. Sci., Bowling Green State Univ., Bowling Green, OH 43403-0212.

The clinical syndrome of psychosocial dwarfism has been directly linked to a modification of growth hormone-mediated developmental mechanisms. In maternal deprivation, the animal model of this syndrome, short-term separation of young from the mother has been shown to directly suppress the release of growth hormone by mechanisms unrelated to pituitary-adrenal stress response. The purpose of the present study was to examine possible signals which trigger the suppression of GH during maternal deprivation of rats by quantifying hypothalamic content of growth hormone release-inhibiting hormone (GHRIH) and growth hormone-releasing hormone (GHRH). Circulating levels of GH and somatomedin C were used as peripheral correlates of hypothalamic peptide effects. Ten day old rat pups were maternally deprived in an incubator for 1 or 6 hours; controls remained with the mother during the deprivation period. At the end of the time period, pups were decapitated, trunk blood was collected and centrifuged, and the serum was frozen. Hypothalami were rapidly excised and homogenized in 2N acetic acid. Peptides were extracted by boiling the homogenates for 5 minutes. Homogenates were centrifuged, then the supernatents were flash frozen with liquid nitrogen and lyophilized. Serum samples and reconstituted hypothalamic extracts were subsequently subjected to specific radioimmunoassay for GH and somatomedin C, and for GHRIH and GHRH, respectively. Analysis of hypothalamic peptide content revealed maternal deprivation to elevate GHRIH levels significantly when values for the 1 and 6 hour deprivation were combined. Hypothalamic GHRH content was not significantly modified. Peripheral levels of GH and somatomedin C were compatible with the interpretation that elevated hypothalamic GHRIH represented an increase in its synthesis and secretion in maternally deprived pups. These results suggest an influence of GHRIH in maternal deprivation. Further studies are required to fully characterize the modifications at higher centers, and at the hypothalamic level, which result in suppression of GH by this condition.

186.16 **rGRF(1-29)NH$_2$-INDUCED GROWTH HORMONE SECRETION IN GENETICALLY AND DIETARY OBESE RATS.** P. Gaudreau, G. Renier*, M. Houde-Nadeau* and P. Brazeau*. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center and Dept. of Nutrition, University of Montreal, Montreal, Canada, H2L 4M1.

Growth hormone (GH) plays an important role in metabolic processes, especially in the stimulation of lipolysis. In genetically obese rats (Heiman et al., Am. J. Physiol., 249, E380-E384, 1985) and in dietary obese humans (Davies, R.R. et al., Clin. Endocrinol., 23, 521-525, 1985) a reduced GH secretion has been observed. In a search for a better understanding of this phenomenon, we have studied the rat growth hormone releasing factor (1-29) amide (rGRF)-induced GH response in adult male Zucker rats, dietary obese Sprague-Dawley rats and in their lean littermates. All the rats were evaluated under sodium pentobarbital anesthesia (40 mg/kg b.w., i.p.). Thirty min after anesthetic administration, rGRF was injected i.v. at a dose of 0.4 ug/kg b.w. and 4.0 ug/kg b.w. in Zucker rats or at a dose of 0.8 ug/kg b.w. and 4.0 ug/kg b.w. in dietary obese rats. Blood samples were collected every 4 min from the jugular vein, starting 4 min before, ending 20 min after rGRF injection. The sera were assayed for rGH by radioimmunoassay. In genetically obese rats (453 ± 12 g) administration of 0.4 ug/kg b.w. rGRF resulted in a rGH rise to 159 ± 16 ng/ml at 4 min post-injection while administration of 4.0 ug/kg b.w. resulted in a rGH rise to 718 ± 134 ng/ml at 8 min post-injection. The same doses increased rGH to 767 ± 85 ng/ml ($p < 0.02$) and to 3553 ± 526 ng/ml ($p < 0.05$) respectively, in lean littermates (345 ± 14 g). In dietary obese rats (551 ± 31 g), administration of 0.8 ug/kg b.w. rGRF resulted in a rGH rise to 776 ± 259 ng/ml at 4 min post-injection while administration of 4.0 ug/kg b.w. resulted in a rGH rise to 1249 ± 275 ng/ml at 8 min post-injection. The same doses increased rGH to 1057 ± 468(NS) ng/ml and to 2314 ± 389 ng/ml ($p < 0.1$) respectively, in lean littermates (458 ± 5 g). In vitro preliminary experiments using perifused anterior pituitary cells of these animals indicate a decrease in somatotroph sensitivity to rGRF. Determination of pituitary rGH content and characterization of pituitary rGRF receptors will be needed to further elucidate physiological deficiencies involved at the pituitary level in obese animal models.

187.1 CATECHOLAMINE AND NEUROPEPTIDE RATIOS IN TISSUE AND DURING RELEASE FROM THE DOG ADRENAL. S.L. Chritton*, M.K. Dousa*, C. Grabau*, D.R. Roddy*, T.L. Yaksh* and G.M. Tyce. Depts. of Physiology and Neurosurgery, Mayo Clinic and Foundation, Rochester, MN 55905.

The adrenal medulla contains epinephrine (E), norepinephrine (NE), dopamine (DA), [Met]enkephalin-like immunoreactive material (ME-IRM), and neuropeptide Y-like immunoreactive material (NPY-IRM). This study compares the ratios of these autocoids in unstimulated adrenal tissues and in perfusates of isolated adrenals before, during, and after high and low levels of nicotinic stimulation. One of a pair of dog adrenals (n=5 in each group) was retrogradely perfused at 37°C with oxygenated modified Krebs-Ringer solution. After 65 min of perfusion, medium containing $5x10^{-5}$M or $3x10^{-6}$M 1,1-dimethyl-4-phenylpiperazinium (DMPP) was introduced into the perfusion cannula for 2 min, followed by 48 min of perfusion without DMPP. Measurement of E, NE, and DA in extracts of unperfused and perfused adrenals and in perfusates was by HPLC with electrochemical detection; ME-IRM (antibody #N382) and NPY-IRM (antibody #221) were determined by radioimmunoassay. Comparison of perfused and unperfused adrenals showed no measurable depletion of autocoid stores; indeed, DA tissue content was increased following stimulation in each pair studied. The results are summarized in the table:

	Tissue Content (μg/g±S.E.)	Efflux in Perfusate (ng/min±S.E.) Basal	Low DMPP	High DMPP
EPI	1329±418	641±132	1053±305	2930±396
NE	281±61	69±17	143±60	640±111
DA	7.2±1.2	4.8±1.3	8.5±2.5	37.4±7.8
ME-IRM	2.22±0.36	1.32±0.23	2.01±0.88	8.51±2.48
NPY-IRM	0.049±0.012	0.025±0.003	0.089±0.071	0.190±0.058

The ratio of E to NE was significantly lower in unstimulated tissues and high DMPP perfusates than in basal and low DMPP perfusates. Comparing NE to DA, the ratio in tissue stores was higher than in any perfusates. Similarly, the E to DA ratio was higher in tissue than in high DMPP-evoked release. The tissue ratio of ME-IRM to NE tended to be lower in tissues than in basal perfusates. The ratios of NPY-IRM to the other autocoids did not change significantly from tissue to perfusates. These results demonstrate that E, NE, and DA are not released in the same manner from the dog adrenal. During basal conditions, there is preferential efflux from tissue stores of E, DA, and ME-IRM relative to NE. During high DMPP-evoked stimulation, DA is preferentially released from tissue stores in proportion to E and NE. DA stores in tissues increased following stimulation with DMPP. Supported by grant NS 17858.

187.2 RELEASE OF CATECHOLAMINES, [MET]ENKEPHALIN AND NEUROPEPTIDE Y FROM EX SITU PERFUSED ADRENALS AFTER CHOLINERGIC RECEPTOR STIMULATION. M.K. Dousa*, S.L. Chritton*, D.L. Lucas*, C. Grabau*, T.L.Yaksh* and G.M. Tyce (SPON: C.L. Kornblith). Depts. of Neurosurgery and Physiology, Mayo Clinic and Foundation, Rochester, MN 55905.

Chromaffin cells in adrenal medulla contain catecholamines (CA) as well as different neuropeptides. The present study is focused on the concurrent release of free CA, [Met]enkephalin-like immunoreactive material (ME-IRM), and neuropeptide Y-like immunoreactive material (NPY-IRM) from adrenals after repeated stimulation by carbamylcholine (carbachol). The experiments were carried out on dog adrenals (n=5) which were surgically removed and retrogradely perfused at 37°C by oxygenated modified Krebs-Ringer solution. After 60 min of adjustment period, four stimulations (S_1, S_2, S_3, and S_4) by carbachol ($3x10^{-5}$M) were introduced, each stimulation lasting 2 min with 40-min periods between subsequent stimulations. Samples from perfused adrenals were collected before, during, and after each stimulation by carbachol. In each perfusate sample, norepinephrine (NE), epinephrine (E), and dopamine (DA) were determined by HPLC with electrochemical detection, while ME-IRM and NPY-IRM were measured by specific radioimmunoassays. Total evoked releases from 10-min samples collected during and after S_1 expressed as means ± S.E. in pmol/min/adrenal gland were: NE, 3,095 ± 300; E, 14,028 ± 1,596; DA, 188 ± 22; ME-IRM, 12.75 ± 2.87; and NPY-IRM, 0.036 ± 0.014. Net evoked releases (R_1, R_2, R_3, and R_4) were obtained by subtracting the basal releases at given times from total evoked releases. R_1, R_2, R_3, and R_4, when expressed as average-fold increases of baseline, were surprisingly stable for each measured substance, i.e., after each carbachol stimulation, R_1, R_2, R_3, and R_4 were for NE, 4.6-fold; for E, 1.7-fold; for DA, 3-fold; and for ME-IRM, 4-fold higher than their respective baselines. NPY-IRM basal release was very low, in some samples below detection level; however, during stimulation the release significantly increased. R_2, R_3, and R_4 expressed as percents of R_1 were for both NE and E on average 80%, 68%, and 50%, respectively. However, DA was secreted during repeated stimulations at more stable rates: R_2, 95%; R_3, 82%; and R_4, 67% of R_1. The composition of adrenal perfusate during basal release was stable during perfusion, averaging 10% NE, 89% E, and 1% DA of the total pool of CA. During stimulations the percentages changed: NE and DA percentages increased to averages of 18.3% and 1.2%, respectively, with E averaging 80.5% of total CA. These results indicate that net release of all autocoids is reduced during repeated stimulations by carbachol but that DA release is reduced less than the others. During stimulation, NE and DA are preferentially released over E. The release of ME-IRM by carbachol paralleled release of NE more closely than release of E.
Supported by Diabetes Training Grant 5 T32 AM07352

187.3 MONOCLONAL ANTIBODIES AGAINST UBIQUITIN INHIBIT HIGH AFFINITY CHOLINE TRANSPORT IN RAT BRAIN SYNAPTOSOMES. M.-T.T. Nguyen*, H.T. Smith*, V. Fried*, C. West*, and E.M. Meyer (SPON: W. Millard). Dept. of Pharmacology and Dept. of Anatomy and Cell Biology, Univ. of Florida, Gainesville; and Dept. of Biochemistry, St. Jude Children's Hospital, Memphis, TN.

Since it was previously shown that polyclonal antibodies against ubiquitin bind to rat cerebral cortical synaptosomes with an affinity similar to that with which they block high affinity choline transporters in that preparation, we investigated whether monoclonal antibodies against this peptide similarly affect transport activity. Two mouse hybridoma antibody preparations, termed 4-2D8 BA9 (an IgG1) and 1-2H11 AH5 (an IgM) were found to be specific for ubiquitin. The former antibody preparation inhibited acetylcholine synthesis in rat cerebral cortical synaptosomes by up to 85 + 10% (+ S.E.M.) and total choline uptake (1 uM choline) by up to 68 + 8%. The IgM, in contrast, inhibited these cholinergic markers by only 58 + 7% and 38 + 4%, respectively. Another monoclonal antibody specific for Band 3 anion transporters (an IgG 1 also) with no cro-s-reactivity for ubiquitin was also found to inhibit these transport processes, but by 40 + 6% and 31 + 6%, respectively. None of these antibodies significantly affected low affinity choline uptake. Since culture supernatants were used for these studies, it is possible that some of the observed inhibition was due to other factors in the medium. Alternatively, 4-2D8 BA9 is more efficacious a transport-inhibitor than the other 2 antibodies due to its recognition of a specific epitope of ubiquitin exposed along the extracellular surface of the plasma membrane. Western blot analyses of synaptosome plasma membranes with these 3 antibodies revealed only 1 significant band in the one experiment performed to date. This was at an apparent MW of 25,000-30,000, and it was observed only with 1-2H11 AH5. This band was observed consistently in similar studies using the polyclonal antibody against ubiquitin, as reported earlier.

The present data continue to support the hypothesis that ubiquitinylation of a few extracellular plasma membrane proteins, such as lymphocyte homing receptors and neuron-specific transporters may be involved in cellular recognition processes. The precise function of this ubiquitinylation of the neuronal choline transporter remains unclear, however.

187.4 PARTIAL PURIFICATION OF DIHYDROTETRABENAZINE BINDING ACTIVITY FROM BOVINE *CORPUS STRIATUM*. Joseph A. Near and Michael S. Vincent. Medical Sciences Program and Department of Pharmacology, Indiana University School of Medicine, Bloomington, Indiana 47405.

Accumulation of catecholamines by synaptic vesicles is accomplished by a reserpine sensitive transport system energized by an ATPase-generated proton electrochemical gradient. Two classes of inhibitors block catecholamine uptake and effect depletion through interaction with the vesicle tranporter complex. The first class includes reserpine and other competitive inhibitors of transport, while the second class comprises the benzoquinolizines tetrabenazine and dihydrotetrabenazine that are not competitive with substrate. Recent evidence suggests that the transporter in both peripheral and central catecholamine storage granules contains distinct binding sites for each class localized on separate subunits. Solubilization of a subunit from the transport complex of bovine striatum retaining dihydrotetrabenazine binding activity was sought in an attempt to characterize binding and purify the subunit.

Solubilization was accomplished following the general methods previously described for adrenal chromaffin granules (D. Scherman and J.-P. Henry, *Biochem.* 22:2805, 1983). Cholate (10%) solubilized 50 to 60% of the tritiated dihydrotetrabenazine binding activity from crude bovine striatal synaptosomes. Nonlinear least squares curve fitting analysis indicated a single class of binding sites (Kd = 10nm) and inhibition studies showed sensitivities similar to those in purified synaptic vesicles (tetrabenazine > reserpine > serotonin > dopamine > norepinephrine). The solubilized binding activity exhibited a half life at 4 degrees in excess of five days. Further purification was accomplished by adsorption to fluphenazine-linked sepharose (H. Charbonneau and J. Cormier, *Biochem. Biophys. Res. Commun.* 90:1039, 1979) and elution with 10% cholate. This resulted in a 50-75 fold enrichment over starting material with an overall yield of approximately 25% of the original particulate binding activity. Assuming a 1:1 stoichiometry between dihydrotetrabenazine binding sites and 70 kilodalton subunit a further 100-fold purification will be required to achieve homogeneity. These results indicate that the tetrabenazine binding subunit can be purified in an active form which may be useful in reconstitution studies and antibody elicitation.

187.5 NEUROPEPTIDE Y STIMULATES CHOLINERGIC RECEPTOR MEDIATED SECRETION FROM THE PERFUSED BOVINE ADRENAL GLAND. T.D. Hexum, C. Cherdchu* and L.R. Russett*. Dept. of Pharmacol., Univ. Neb. Med. Ctr. Omaha, NE 68105.

Neuropeptide Y (NPY) has recently been shown to be co-stored with norepinephrine and to participate in various autonomic functions. This peptide is present in the bovine adrenal medulla and released in response to nicotinic receptor stimulation along with catecholamines and pro-enkephalin-derived peptides (Hexum et al., 1986 Soc. Neurosci. Abs., 12, 997). Since bovine adrenomedullary membranes contain binding sites for N-[Propionyl-^{3}H]-NPY (Higuchi, et al., 1987, Fed. Proc., 46, 1448) and NPY is present in the splanchnic nerve (Hexum et a., 1986, Soc. Neurosci. Abs., 12, 997) we proposed that NPY might have a role in the secretion of catecholamines and the pro-enkephalin-derived peptides. We studied its effects using the retrogradely perfused bovine adrenal gland in the presence of various nicotinic receptor agonists. NPY alone had no effect on the secretion of either catecholamines or pro-enkephalin-derived peptides. However, the infusion of 1 x 10^{-8} M NPY in the presence of 5 x 10^{-5} M 1,1-dimethyl-4-phenylpiperazinium (DMPP) resulted in an increase of 50% or more ($p < 0.05$) in the release of norepinephrine, epinephrine and enkephalin-like peptides compared to the release in the presence of DMPP alone. The effect was dose dependent up to 1 x 10^{-7} M NPY. NPY (1 x 10^{-8} M) had a similar effect when either 5 x 10^{-5} M acetylcholine or 5 x 10^{-5} M nicotine were present in the infusing medium.

It has been reported that NPY inhibits the nicotine induced release of norepinephrine and epinephrine from cultured bovine chromaffin cells (Higuchi, et al., 1987, Fed. Proc., 46, 1448). The results described above prompted us to confirm this data using cultured bovine chromaffin cells. We observed that after the introduction of 1 x 10^{-8} M NPY into the culture medium along with 5 x 10^{-5} M DMPP, chromaffin cells responded with a decreased release of norepinephrine, epinephrine and pro-enkephalin-derived peptides ($p < 0.05$) when compared to the release with DMPP alone. This is the first reported instance where the adrenal medulla responds to the administration of regulator substances such as peptides in a manner distinct from that seen with isolated chromaffin cells.

These results strongly implicate a role for NPY in the regulation of adrenomedullary secretion in the bovine adrenal gland and suggest that the immediate environment of the chromaffin cells is important in this effect. (Supported by the American Heart Assoc., Inc., with funds contributed from the Nebraska Affiliate).

187.6 EFFECT OF 3-ACETYLPYRIDINE ON SEROTONIN UPTAKE AND RELEASE FROM RAT CEREBELLAR SLICES. C. Beas-Zárate*, A. Morales-Villagrán*, G. Tapia-Arizmendi* and A. Feria-Velasco. Facultad de Ciencias, Universidad de Guadalajara and Unidad de Investigación Biomédica de Occidente, I.M.S.S. Guadalajara, Jalisco. MEXICO.

Several studies have shown that cerebellum receives indoleaminergic nerve fibers[1,2], mostly influencing the Purkinje cell discharges[3]. Furthermore, recent data from our laboratories[4,5] have demonstrated endogenous release of serotonin (5-HT) and Na^+-dependent uptake and Ca^{++}-dependent release of exogenous 5-HT from tissue slices, homogenates and synaptosomal fraction of rat cerebellar molecular layer. Although several studies oriented to know the neurotransmitter produced by climbing fibers have been done, ruling out some of the classical transmitter substances (GABA, glutamate, acetylcholine), at present, the neurotransmitter released from climbing fibers remains unknown. The aim of this work was to measure the 5-HT uptake and release from rat cerebellar slices, 18 days after intraperitoneal injection of 3-acetylpyridine (3-AP)(65 mg/Kg) to adult rats. A histological study of medulla oblongata in these animals showed complete destruction of neurons in the inferior olivary nuclei.

A significant reduction of 5-HT uptake (60%) and its V_{max} (60%) was seen in the cerebellar preparations obtained from rats injected with 3-AP. Also, the Ca^{++}-dependent release of 5-HT from those preparations was found to be similar to basal values, in spite of a depolarizing stimulus with high-K^+ in the medium (53 mM).

Results suggest that the 5-HT could play a role as neurotransmitter produced by some climbing fibers, aside from some evidences reported in the literature documenting the role of aspartate as neurotransmitter in other group of climbing fibers[6].

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187.7 THE EFFECTS OF pH ON RESERPINE BINDING TO CHROMAFFIN GRANULE MEMBRANES. J.D. Deupree and W.F. Herblin. Dept. of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68105 and Medical Products Dept., E.I. duPont de Nemours and Company, Wilmington, DE 19898.

The carrier molecule used to transport catecholamines into synaptic vesicles and chromaffin granules can be studied using [^{3}H]reserpine, but this binding is strongly affected by pH. The binding of increasing concentrations of reserpine to chromaffin granule ghosts was measured in PIPES buffer at pH 6.61, 7.04, and 7.35 and in HEPES buffer at pH 6.8, 7.32, and 7.86. The binding data were analyzed using the MLAB mathematical modeling program. Binding of [^{3}H]reserpine increased with pH, indicating that the unionized form of the ligand was binding to the membranes with the highest affinity. When Kd and Bmax values were calculated based on the concentration of unbound, unionized reserpine, much of the variability was eliminated. There was still a non-random trend toward the over-estimation of binding at low pH which could not be eliminated by either varying the pKa of reserpine or assuming that the ionized form of reserpine could also bind to the site. Extending the model to include an ionizable group on the reserpine binding site eliminated the non-random residuals. The fit to this model suggests that the unionized form of reserpine binds to the unionized site with a Kd of 2.89 $\pm$ 0.1 nM, and the binding site can ionize with a pKa around 6.6. The results are consistent with the concept that catecholamines are transported into the granules in the neutral form. The results suggest that the catecholamine binding site may be deprotonated when exposed to the outside of the granules in the more alkaline environment and protonated when exposed to the more acidic interior of the granules. This would favor the binding of catecholamines on the outside of the granules and the release of catecholamines from the carrier on the inside of the granules. These results should be of interest to other scientists working with ligands which can ionize around the pH of the normal assay such as morphine or naloxone. (Funded by NINCDS #NS15187)

187.8 AN IN VIVO STUDY OF DOPAMINE RELEASE IN STRIATUM: THE EFFECT OF AMINO ACIDS. M.J. During*, I.N. Acworth*, R.J. Wurtman. Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Intracerebral dialysis was used to monitor extracellular levels of dopamine and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum of chloralose/urethane anaesthetized rats. Levels of these compounds were determined after intraperitoneal (i.p.) administration of the amino acids tyrosine, phenylalanine, valine and alanine. Tyrosine was given i.p. at doses of 50 and 100mg/kg, all other amino acids were at 200mg/kg.

Probes were also placed in the nucleus accumbens and dopamine release was followed after a dose of tyrosine (200mg/kg). Control rats received normal physiological saline. Rats were maintained at a temperature of 37°C and kept at a light level of stable anaesthesia by administering additional doses of chloralose/urethane as required. After an initial period of injury release (60-90 mins post probe implantation), stable concentrations of dopamine were obtained. Treatments were administered after a minimum of three stable 15 min collections. Dialysates were collected in 0.5M perchloric acid (flow rate 1.5ul/min) and were directly injected on to a reverse-phase, isocratic HPLC system with an ESA Coulochem 5100 detector. The basal dopamine concentrations in dialysates were similar in all groups, the mean concentration being 4.62±0.3nM, as the in vitro probe callibration gave recovery of 16±1%, the basal dialysates therefore reflected an extracellular dopamine level of 29±3nM. Dopamine release following treatment was determined for every animal by recording the percentage change from its stable baseline. Rats receiving normal saline showed no change in dopamine release during the 120 min following its administration. In contrast tyrosine at both 50 and 100mg/kg, transiently increased dopamine release by 28.2±3.8% and 28.5±3.5%, peaking after 90 minutes and 75 minutes (for the 50 and 100mg/kg doses respectively.) In the nucleus accumbens tyrosine (200mg/kg) increased dopamine release by 220±28%. A dose of phenylalanine (200mg/kg) that, in rats, elevates plasma tyrosine also increased striatal dopamine release by 59±11% peaking at 75 mins. Valine or alanine failed to affect striatal dopamine release. None of the treatments affected DOPAC or HVA levels, in all of the treatment groups. These data affirm that tyrosine administration can enhance brain dopamine release, and indicate that, under normal conditions, this effect is soon terminated, probably by trans-or presynaptic feedback mechanisms.

These studies were supported from a grant from the Brain Sciences and Metabolism Charitable Trust.

187.9 UPTAKE OF GLUTAMIC ACID INTO SYNAPTOSOMES AND BULK-PREPARED ASTROCYTES. J.M. TUCHEK*, D.D. Johnson and R.I. Wilcox*, Dept. of Pharmacology, College of Medicine, Univ. of Saskatchewan, Saskatoon, Sask. S7N OWO.

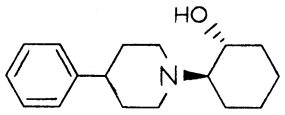
Although it is well established that both nerve endings and astrocytic cells have uptake mechanisms for glutamic acid, the reported transport constants (K_T) for glutamate vary anywhere from approximately 2 μM to 30 μM and more (see Schousboe, A. (1981) Int. Rev. Neurobiol. 22, 1-45). In view of studies reporting that the concentration of glutamic acid in brain extracellular fluid and cerebrospinal fluid is less than 10 μM (Hamberger et. al. (1983) in "Glutamine, Glutamate and GABA in the Central Nervous System" pg. 473-492, Alan R. Liss, Inc.), transport constants for glutamate above 10 μM would seem to have little physiological relevance. Furthermore glutamate receptor binding studies tend to suggest that the K_D of the glutamate post-synaptic receptor is approximately 1 μM or less. Thus, if reuptake is the main mechanism responsible for terminating the response in glutaminergic synapses then the K_T for glutamate would be expected to be a correspondingly low micromolar concentration.

We have examined the transport of glutamic acid into synaptosomes freshly isolated from rat and avian cerebella and into bulk-prepared astrocytes from rat brain. The uptake of [^{3}H] Glu (47,7 Ci/mmol, NEN) into synaptosomes (50-150 μg Lowry protein) or astrocytes (approx. 60 μg Lowry protein) was studied at 30°C in an incubation mixture that also contained 136 mM NaCl, 5 mM KCL, 1.3 mM $CaCl_2$, 1.3 mM $MgCl_2$ 10 mM glucose and 10 mM Tris/HCl pH 7.4 buffer in a total volume of 500 μl. One minute after the addition of protein, a 400 μl aliquot was taken and filtered on Whatman GF/C filters and washed 2x with 5.0 ml 0.9% NaCl, 5 mM Tris/HCl buffer pH 7.4. The radioactivity on the filter paper was analyzed using standard liquid scintillation counting methods. Eadie-Hofstee analysis of the data indicated that both avian and rat cerebellar synaptosomal transport systems had two affinities for glutamate. The average K_m for the high affinity site was approximately 2.5 μM and 7 μM for the low affinity site in both species of animals. The glutamate transport mechanism in the bulk-prepared astrocytes also displayed two affinity sites for glutamate with K_{Ts} of 4.5 and 11 μM. Synaptosomes prepared from avian cerebral hemispheres, a tissue containing relatively low amounts of glutaminergic nerve endings in comparison to the cerebellum, contained a single affinity transport system for glutamate (K_T= 7-8 μM). The results indicate that glutaminergic nerve endings have a two-affinity transport system for glutamate with K_{Ts} at physiologically relevant glutamate concentrations. Synaptosomes from avian cerebral hemispheres also transport glutamate but with a single, low affinity K_T (7-8 μM). Supported by the MRC.

187.10 THE VESAMICOL (AH5183) RECEPTOR IN VP_1 CHOLINERGIC SYNAPTIC VESICLES: PARTIAL PURIFICATION. Ben A. Bahr and Stanley M. Parsons. Department of Chemistry, University of California, Santa Barbara, California 93106.

The compound 1-trans-2-(4-phenylpiperidino)-cyclohexanol (1-vesamicol; 1-AH5183) inhibits the uptake and storage of [^{3}H]acetylcholine (ACh) by purified Torpedo VP_1 synaptic vesicles (SV). 1-[^{3}H]Vesamicol binds enantioselectively to a saturable receptor present on VP_1 vesicles which bind 300-400 pmoles of the ligand per mg vesicle protein, or 6-8 ligand sites/SV. Cholic acid (5%, w/v) successfully solubilizes nearly all of the vesamicol receptor (VM-R) present in a 0.8 mg/ml SV suspension. Dilution of the cholic acid (0.5-1.0%, w/v) and the addition of phosphatidylcholine (1 mg/ml) and glycerol (25%, w/v) were necessary to stabilize the solubilized VM-R. IC_{50} values for vesamicol analogs ranging over three orders of magnitude in potencies, measuring either inhibition of [^{3}H]ACh uptake in SVs or inhibition of 1-[^{3}H]vesamicol binding to solubilized VM-R, are nearly unaltered by the solubilization procedure. Therefore, the membrane-bound and detergent-solubilized forms of the VM-R are pharmacologically indistinguishable.

After silica-based DEAE ion-exchange chromatography, the binding specific activity of solubilized VM-R increased 6-10 fold. Chromatography of this material on an agarose-wheat germ lectin column achieved an additional 3-fold increase in binding specific activity, to 5500-6500 pmol/mg protein. This partially purified receptor chromatographed on an HPLC gel permeation column with an apparent particle molecular mass (including bound detergent and phospholipid) of approximately 300,000 daltons, in a final yield of about 30%. The protein subunit structure is currently being studied and will be discussed.
(Supported by NIH grant NS15047 and the Muscular Dystrophy Association.)



(VESAMICOL)

187.11 TAURINE RELEASE FROM ASTROGLIAL CELLS IS NOT DEPENDENT ON INTRACELLULAR Ca^{++}. D.L. Martin[1], J.A. Connor[2], and W. Shain[1], [1]Laboratory of Neurotox. & Nerv. Sys. Disorders, Wadsworth Center for Labs and Research, Albany, NY 12201 and [2]AT&T Bell Laboratories, Murray Hill, NJ 07974.

Beta-adrenergic receptor-stimulated taurine release from astroglia is not inhibited when Cd^{++}, Co^{++} or Mn^{++} are substituted for Ca^{++} or by the Ca^{++} channel blockers nifedipine, diltiazem, or verapamil (Martin et al., Neurosci. Abst. 12:107, 1986). The role of intracellular Ca^{++} on release was evaluated using parallel experiments designed to measure taurine release and intracellular Ca^{++} content. Taurine release was measured by loading cells with tracer quantities of ^{3}H-taurine and monitoring release of radiolabelled taurine. $[Ca^{++}]_i$ was determined by loading cells with the Ca^{++}-sensitive dye fura-2 and measuring the fluorescence ratio (340/380 nm) by digital imaging. Analyses of taurine release and intracellular Ca^{++} were made using three different experimental media to manipulate intracellular Ca^{++} -- control (1.2 mM Ca^{++}), low Ca^{++} (0 Ca^{++}, 10 uM EGTA), high Ca^{++} (100 uM Ca^{++}, 100 uM ionomycin). Cells were stimulated with 100 nM isoproterenol (IPR) under control conditions in all experiments. IPR stimulation in control medium resulted in no apparent increase in $[Ca^{++}]_i$. When the medium was changed to produce low $[Ca^{++}]_i$, $[Ca^{++}]_i$ decreased from highly variable levels to 30-60 nM within 3 min. This lower $[Ca^{++}]_i$ was maintained for as long as cells were observed (20 min). No change in $[Ca^{++}]_i$ was observed in response to IPR. When cells were returned to control medium, there was a transient increase in $[Ca^{++}]_i$. This was variable with peak $[Ca^{++}]_i$ = 300-350 nM, and returned to control levels within 3-4 min. In parallel taurine release experiments, IPR responses were obtained in control conditions and after 30 min in low Ca^{++}. Taurine release was the same under both conditions. In addition there was no taurine release when the low Ca^{++} medium was replaced with control medium. This latter observation suggests that elevation of $[Ca^{++}]_i$ alone will not stimulate release. When ionomycin was added to produce high $[Ca^{++}]_i$, the fura-2 signal rapidly became saturated indicating that $[Ca^{++}]_i$ was > 1 uM. In taurine release experiments ionomycin did not stimulate release. Thus, even very high $[Ca^{++}]_i$ did not stimulate release. These results indicate that beta-adrenergic receptor-stimulated taurine release from astroglial cells can take place without measurable changes in $[Ca^{++}]_i$. In addition, they demonstrate that elevation of $[Ca^{++}]_i$ does not elicit release suggesting that Ca^{++} does not function as a second messenger for, or regulator of taurine release. Clearly, taurine release from astroglia is distinctly different from neuronal vesicular release. Supported in part by NS21219, AA07155 and USAF OSR F49620-85-C-0009.

187.12 REGULATION OF NOREPINEPHRINE UPTAKE BY DIVALENT CATIONS AND ATP IN PC12 CELLS. J.E. Chaffee, Y.H. Ehrlich, and E.D. Hendley. Depts. of Physiology & Biophysics, Psychiatry and Biochemistry, Univ. of Vermont, Burlington, VT 05405.

The potential regulation of norepinephrine uptake by extracellular ATP and divalent cations was examined in PC12 cells, a model of noradrenergic neurons, derived from a rat adrenal medullary pheochromocytoma (Greene and Tischler, PNAS 73: 2424, 1976). The uptake of ^{3}H-(-)-norepinephrine (^{3}H-NE, 1 μCi/ml, 0.4 μM NE) in PC12 cells was determined at 37°C for 3 min in a HEPES-buffered Krebs-Ringer solution (KRH in which $[Ca^{2+}]$ and $[Mg^{2+}]$ were 1.2 mM). Following incubation, the cells were collected by vacuum filtration and the amount of ^{3}H-NE incorporated was determined by liquid scintillation counting. Specific, transport-mediated uptake, referred to as uptake 1, was defined by the addition of 1 μM nisoxetine. Omission of calcium and magnesium ions from the incubation medium reduced uptake 1 by approximately 75%. Replacement of either ion alone, in concentrations ranging from 0.1 mM to 1.2 mM, restored uptake to control levels in a concentration-dependent manner. Extracellular ATP (100 nM) was able to stimulate uptake 1 above control levels by approximately 60%, but only if calcium ions were present in the medium. Previous experiments, in which PC12 cells were incubated with extracellular γ-^{32}P-ATP followed by SDS-PAGE and autoradiography, demonstrated a NE-dependent phosphorylation of surface membrane proteins. These results describe for the first time that NE uptake in PC12 cells requires divalent cations, as has also been demonstrated for NE uptake in brain synaptosomal preparations. These results are consistent with our hypothesis that NE uptake is regulated by a membrane protein kinase that utilizes extracellular ATP, NE, and millimolar concentrations of divalent cations. (Supported by NSF: R11-8610679 and USAFORSR: 84-0331).

187.13 PHOSPORYLATION DEPENDENT AND INDEPENDENT TRANSMITTER RELEASE MECHANISMS IN PC-12 CELLS. H.J.G. Matthies*, H.C. Palfrey*, and R.J. Miller. (SPON: W. Woolverton). Dept. of Pharmacological and Physiological Sciences, U. of Chicago, Chicago,IL 60637

It is well established that an increase in intracellular [Ca^{2+}] can trigger release of neurotransmitters. However the intracellular locus of action of Ca^{2+}has not been identified. As secretion from permeabilized cells seems to be dependent on MgATP, it has been suggested that a Ca^{2+}dependent phosphorylation reaction plays a central role in this process. We have studied the role of different kinases in the release of catecholamines from PC-12 pheochromocytoma cells.

In wild type PC-12 cells (PC-12WT), secretion of catecholamines could be evoked by depolarization or by ionomycin, a Ca ionophore. Evoked release was enhanced by phorbol esters (PE) or by agents that increased cAMP such as 2-chloroadenosine (2CA). As demonstated previously PE was no longer effective in PC-12WT in which protein kinase C (PKC) had been eliminated by down-regulation. In a mutant PC-12 cell line that lack functional cAMP dependent protein kinase (PKA) (A126-1B2, supplied by Dr J.A. Wagner, Dana-Farber Cancer Institute), 2CA was no longer effective but PE still enhanced release. Following chronic PE treatment, release from A126-1B2 cells was no longer enhanced by either 2CA or PE. In A126-1B2 cells permeabilized by digitonin, PE but not cAMP enhanced release induced by Ca^{2+}. Following down-regulation of PKC neither cAMP nor PE enhanced release from permeabilized cells.

Calmodulin(CaM) has been proposed to be the Ca^{2+} receptor involved in transmitter release. In permeabilized, down-regulated A126-1B2 cells several inhibitors of CaM including calmidizolium, mastoparan, and 48/80 failed to block Ca^{2+} induced release even at concentrations snown by others to inhibit CaM dependent phosphorylation. Furthermore release could be produced by Ba^{2+} which does not activate CaM but not by Mn^{2+}which does. Neomycin which does inhibit phospholipase C amongst other actions was able to block secretion from down-regulated permeabilized A126-1B2 cells.

We conclude that Ca induced secretion of catecholamines from PC-12 cells does not depend on the activity of PKC, PKA, or a Ca/CaM dependent Kinase. The locus of action of Ca and MgATP in secretion does not appear to involve protein kinase-mediated events, but kinases may regulate the process.

187.14 MOLECULAR WEIGHT AND PURIFICATION OF THE Ca^{2+}/Mg^{2+} ATPASE OF CHOLINERGIC SYNAPTIC VESICLES. Susan K. Yamagata* and Stanley M. Parsons. (SPON: Steven K. Fisher). Dept. of Chemistry., Univ. Calif. Santa Barbara, Santa Barbara, CA 93106.

Synaptic vesicles from cholinergic nerve terminals are known to contain a membrane associated Ca^{2+}/Mn^{2+} dependent ATPase. This ATPase is thought to set up a proton gradient across the vesicle membrane from which the energy stored in the gradient can in turn be used to drive the uptake of acetylcholine into vesicles. Here, we describe the molecular weight determination and isolation of Ca^{2+}/Mg^{2+} ATPase from the electric organ of Torpedo californica.

The ATPase can be successfully solubilized in octaethylene-glycoldodecyl ether ($C_{12}E_8$) and stabilized with phosphatidylserine, glycerol, dithiothreitol and protease inhibitors. Furthermore, phosphatidylserine was found to activate the ATPase. To determine it's molecular weight the solubilized enzyme was subjected to size exclusion chromatography and sedimentation velocity centrifugation on glycerol gradients made up in H_2O and D_2O. This gave a Stoke's radius of 78 $\pm$ 1 Å for the detergent solubilized complex and a sedimentation velocity coefficient of 6.8 $\pm$ 0.2 $S_{20,w}$. This data was used to deduce a partial specific volume of 0.81 + 0.01 cm^3/g, f/f_0 = 1.75 $\pm$ 0.02 and a protein molecular weight of 222,000 $\pm$ 10,000 (n=3).

Purification of the detergent solubilized and stabilized Ca^{2+}/Mg^{2+} ATPase was achieved using band sedimentation velocity centrifugation on glycerol density gradients followed by sequential chromatography on hydroxylapatite, wheat germ lectin affinity and gel filtration columns. The final yield was approximately 6% of the total starting activity. When examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis a triplet set of polypeptides was observed which varied in their relative intensities from preparation to preparation. The molecular weights of these polypeptides were 93,000, 91,000 and 85,000 M_r. Also observed was a diffuse polypeptide centered about 61,000 M_r. These subunit molecular weights together with the total molecular weight of the enzyme suggest that this ATPase may be of the phosphoryl-enzyme intermediate class of ATPases.

187.15 ULTRASTRUCTURE OF EXOCYTOSIS IN RAT ADRENAL MEDULLA. S.W. Carmichael, J.C. Brooks, R.K. Malhotra, T.D. Wakade, and A.R. Wakade, Dept. of Anatomy, Mayo Clinic, Rochester, MN 55905, Dept. Basic Sci., Marquette Univ., Milwaukee, WI 53233, and Dept. Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY 11210.

Using a method employing tannic acid, we previously demonstrated exocytosis in cultured adrenal chromaffin cells (Am.J.Anat. 178:85-89, 1987). The current study utilized a modification of this technique in an attempt to demonstrate exocytosis in an intact adrenal medulla. The adrenal glands of rats were retrogradely perfused as described previously (J.Physiol. 313:463-480, 1981). During the perfusion, the organs were either unstimulated (control), electrically stimulated (10 Hz) in the presence or absence of calcium ions), stimulated with excess potassium ions, stimulated with vasoactive intestinal polypeptide, or the tonicity of the perfusion medium was varied in a manner that has been shown to cause explosive secretion of catecholamines from the adrenal medulla (J. Neurosci. 6:2625-2634, 1986). These treatments were followed by perfusion with a solution containing tannic acid followed in turn by a buffered solution of glutaraldehyde. Blocks of medulla were excised and prepared for electron microscopy by routine methods. Figures interpreted to represent exocytosis were observed. The figures were essentially identical to those seen in cultured cells. Exocytotic figures were only rarely seen in unstimulated specimens and were not observed in specimens perfused with calcium-free solution. In the other specimens, exocytotic figures were frequently observed along the plasma membrane in a subendothelial position. In addition, figures were frequently observed in positions that appeared to be on the lateral aspect of the cell. Evidence was obtained to suggest that adrenal chromaffin cells may not only secrete their vesicular contents directly into the subendothelial space, but also from the sides of the cell where the secretory products then proceed toward a vessel, either along a narrow intercellular cleft or through an intercellular cannaliculus. Furthermore, our evidence suggests that the secretory products of chromaffin cells may be carried across endothelial cells to enter vessels. This was most strongly suggested in specimens stimulated with potassium. Figures were also obtained that suggested membrane retrieval occurs over all aspects of the plasma membrane.

187.16 DIFFERENTIAL SENSITIVITY TO ETHANOL OF CALCIUM-DEPENDENT DOPAMINE RELEASE FROM PC12. S.B. Oldham*, T. Ritchie and E.P. Noble*. Alcohol Research Center, Neuropsychiatric Institute, UCLA, Los Angeles, CA 90024.

PC12 cells were prelabeled with (^3H) dopamine and the effects of K^+ and bradykinin were studied on dopamine release. Increasing concentrations of K^+ (4.8 mM to 70 mM) and bradykinin (10^{-8}M to 10^{-5}M) dose-dependently enhanced dopamine release. The K^+- and bradykinin-induced releases were dependent on the presence of extracellular Ca^{2+} and were significantly inhibited by 1 mM Co^{2+} or Ni^{2+}. BAYK 8644 (10^{-7}M), a stimulator of the voltage-dependent Ca^{2+} channel, and Verapamil (10^{-5}M), an inhibitor of this channel, respectively enhanced or decreased K^+-induced dopamine release, whereas these substances were without effects on bradykinin-stimulated dopamine release. Exposure of PC12 cells for 6 days to 200 mM ethanol resulted in a significant increase in dopamine release when stimulated with K^+ concentrations greater than 50 mM (mean increase of 128 $\pm$ 9% of untreated cells, $p < 0.0005$); however, the EC50 for K^+ remained unchanged (38 $\pm$ 1 mM). When bradykinin-induced dopamine release was measured in cells exposed to ethanol, neither the maximal secretory response nor the EC50 for this secretagogue was altered. This study shows that the effect of bradykinin on dopamine release is not exerted through voltage-dependent Ca^{2+} channels. Moreover while the present data support previous studies showing enhanced voltage-dependent Ca^{2+} channel activity as a consequence of chronic alcohol treatment (Messing, R.O. et al. PNAS 83:6213, 1986), such treatment does not affect other Ca^{2+} channels in PC12 cells involved in exocytotic release. This selective effect on Ca^{2+} channels by ethanol may explain in part the changes in the CNS during ethanol dependence. (This study was supported by The Seaver Institute.)

187.17 CHOLINE UPTAKE AND INCORPORATION INTO PHOSPHOLIPIDS BY NEUROBLASTOMA CELLS: EFFECT OF HEMICHOLINIUM-3 (HC-3) AND 4-METHYLPIPERIDINE DERIVATIVES OF HC-3. K.Y. Sheff, M.A. Yorek, J.P. Long*, and J.G. Cannon*. Depts. of Internal Medicine and Pharmacology, College of Medicine and College of Pharmacy, University of Iowa, Iowa City, IA 52242.

N41A3 Neuroblastoma cells rapidly take up [methyl-^{3}H] choline and incorporate it into phosphatidylcholine. Choline uptake by neuroblastoma cells occurs by high- and low-affinity uptake systems. K'm and V'max for high- and low-affinity uptake are 6.08 and 679.81 uM and 64.11 and 781.57 pmol/min/mg protein, respectively. The high affinity choline uptake system is sodium dependent.

After a 6 h period about 30% of the choline taken up is incorporated into phospholipid. Greater than 90% of the incorporation is into phosphatidylcholine and a small fraction into lysophosphatidylcholine and sphinogomyelin. Hemicholinium-3 (HC-3) and its 4-methylpiperidine derivatives, A-4 and A-5, all decrease high-affinity choline uptake and choline incorporation into phospholipid. The degree of inhibition of choline uptake and incorporation into phospholipid differs among the 3 compounds examined. When added to the incubations, the tertiary amine A-4 was more effective than A-5, quaternary amine derivative, which was more effective than HC-3 in decreasing choline uptake and its incorporation into phospholipid. There was no significant difference in the potencies of the d, l or meso isomeric forms of A-4 or A-5 on choline uptake from their respective racemic mixture. The extent of inhibition of choline metabolism by these compounds was also concentration-dependent. A-4, at a concentration of 50 uM and after a 6 h incubation, decreased 5 uM choline uptake by 42% and incorporation of choline into phospholipid by 25%. Following a 6 h incubation, A-5 and HC-3 at 50 uM decreased choline uptake by 24% and 14%, respectively, and choline incorporation into phospholipid by 16% and 4% respectively, from 5 uM choline. The ID_{50} for choline uptake by A-4, A-5 and HC-3 was determined to be 28.3, 46.8 and 118.0 uM, respectively. Incubation of neuroblastoma cells with 50 uM A-4, A-5 or HC-3 for a minimum of 24 h causes a decrease in intracellular choline levels. Control cells contain 10.73 nmol/mg protein of choline. Culturing cells in the presence of 50 uM A-4, A-5 or HC-3 for a minimum of 24 h decreases intracellular choline levels by 60, 34 or 26%, respectively. These studies show that two HC-3 derivatives, A-4 and A-5, are more active than HC-3 in decreasing choline metabolism and intracellular choline levels in a cultured neural cell line.

187PO CALCIUM CHANNEL ANTAGONISTS INHIBIT DOPAMINE RELEASE IN VIVO MEASURED BY TRANS-STRIATAL DIALYSIS METHOD. E. Carboni*, M. Morelli and G. Di Chiara. Inst.of Exp. Pharmacology and Toxicology, University of Cagliari, Viale A.Diaz, 182, 09100 Cagliari, Italy.

Calcium ions are essential for the release of neurotransmitter. The study of calcium uptake in CNS has been related to the role that this ion can play in release processes. Although the action of calcium channel antagonists on neurons and CNS preparations has been widely investigated, only recently, it has been provided evidence showing that calcium channel antagonists inhibit voltage dependent calcium uptake in synaptosomes and in neurones. However the effect of dihydropyridines on synaptosomes is debated; it might imply that this preparation may not be suitable for this purpose. We have studied the effect of Ca-antagonists on the release of dopamine in two different models either in vivo or in vitro. We have measured the action of the dihydropyridine (+)PN-200-110 on the release of dopamine estimated by the trans-striatal dialysis method. The drug was applied directly to the striatum by including it in the Ringer used for dialysis. (+)PN-200-110 rapidly reduced in a dose related manner the release of dopamine by about 10, 30 and 50% from basal values when added at concentrations of 0.1, 1 and 10 uM which correspond to about 15 fold lower concentrations in the extracellular space. Dopamine returned to basal level when the drug was withdrawn from the Ringer. The effect of various calcium channel antagonists was studied also in striatal synaptoneurosomes depolarized by increasing conc. of KCl (from 15 to 60 mM). Dopamine was separed by rapid filtration through Whatman GF/C filters and measured in the filtrate by reverse phase-HPLC. DA-release was depolarization and calcium dependent. In this preparation dihydropyridines failed to inhibit dopamine release at concentrations up to 10 uM. Our results indicate that in agreement with previous results (Carboni, E., Wojcik, W. J. and Costa, E., Neuropharmacol., 24: 1123, 1985) dihydropyridines may play a role in the control of calcium uptake and neurotransmitter release although in preparations "in vitro" this effect for still unknown reasons is difficult to evidentiate.

CORTEX: MOTOR CORTEX II

188.1 A PATHWAY MEDIATING SOMAESTHETIC PROJECTIONS TO THE MOTOR CORTEX IN THE CAT. Y. Padel and L. Relova*. Equipe "Mécanismes Sensori-Moteurs", Lab. Neurosciences Fonctionnelles, CNRS, 31, chemin J. Aiguier, Marseille, France.

It has been known since 1939 that the pyramidal cells of the motor cortex can be made to discharge at extremely high frequency with sensory stimulation of the body (Adrian, E.D. & Moruzzi, G., J. Physiol. (Lond.) 97:153, 1939). In view of their short latencies these somaesthetic responses seemed unlikely to take paucisynaptic pathways, such as the trancerebellar pathway via the ventrolateral nucleus of the thalamus to area 4, or the system comprising the dorsal columns, the medial lemniscus and the ventrolateral nucleus of the thalamus, with the sensory cortex acting as a relay. It was therefore assumed that there existed a "direct" projection to the motor cortex from some thalamic region receiving short-latency spinal afferents (Albe-Fessard, D. & Rougeul, A., Electroencephal. Clin. Neurophysiol., 10:131, 1958). Many attempts have been made to identify this sensory pathway to area 4, but the results obtained so far have been highly controversial. Various nuclei have been suggested as candidate thalamic relay sites, and the very existence of a direct thalamocortical link arising in a thalamic region receiving spinal afferents has been questioned up till recently (Greenam, T.J. & Strick, P.L., Brain Res., 362:384, 1986).

In a preliminary series of experiments on cats, it was therefore attempted to establish whether it was possible to record short-latency somaesthetic responses from neurones belonging to area 4. It was confirmed that these cells had axons projecting into the spinal cord by checking that they responded antidromically to stimulation of the contralateral dorsolateral cord where the corticospinal bundle was situated. In these experiments, motor cortical area 4 was completely cut off from all its "classical" afferents: the corticocortical connections were abolished by lesion of the transcallosum pathway and an incision was performed between the sensory cortex and the motor cortex. The transcerebellar circuits towards area 4 were eliminated by transsecting the ascending brachium conjunctivum, and the dorsal spinal somaesthetic pathways, i.e. the dorsal columns and spinocervicothalamic bundles, were transsected within the cervical cord.

In these preparations, neurones in cortical area 4 at the origin of the corticospinal pathway responded consistently to peripheral activation. Electric shocks applied to the limbs were found to elicit spike bursts with very short latencies as well as complex excitation/inhibition sequences.

The pathway along which these sensory messages are conveyed to the motor cortex was found to be located in the ventral and ventrolateral spinal cord. The thalamic region acting as a relay was delimited using a combination of spinal lesion, intracortical microstimulation, and field potential mapping techniques.

188.2 FUNCTIONAL ROLE OF THE SENSORY CORTEX IN LEARNING OF FOOD TAKING MOVEMENT IN CATS. T. Sakamoto*, K. Arissian*, H. Asanuma (SPON: L.L. Porter). The Rockefeller University, New York, N.Y., 10021.

It has been shown repeatedly that removal of the sensory cortex produced little, if any, motor deficits. We have recently demonstrated that association input from the sensory cortex (area 2) produced long lasting exitability changes (LTP) in motor cortical neurons (Brain Research, 1987) suggesting that the association input is related to motor learning. To examine whether this input actually participates in the learning process, we trained cats with or without the sensory cortex intact.

A total of 3 cats was used. After unilateral ablation of the sensory cortex (areas 2, 2 pri and 5) by suction, cats were trained to pick up a small piece of food (cat food) from a beaker rotating at a given speed. The beaker was placed at some distance (2 inches) from the cage, so that the cat had to bring the food back to the cage across a space. Therefore, cats had to learn a special motor skill which consisted of spatially and temporally organized flexion and supination of the forepaw to pick up a piece of food. The learning process (pick up movement) was examined by counting the success rate at a fixed rotating speed (0.4 rev/sec). Both forelimbs, ipsilateral (normal) and contralateral (operated) to the lesioned cortex were trained equally and the performance of the normal limb was used as a control.

In all cats (n=3), the learning process for the operated limb was slower than that for the normal limb. The normal limb reached a stable state of performance (80% success rate) within 2 to 4 weeks, whereas the operated limb necessitated 3 to 8 weeks. There was a statistically significant difference in success rate between the two limbs during the initial 2 weeks ($P < 0.05$). In each cat, the success rate for the operated limb was lower than that of the normal limb. However, there was no significant difference in performance between the two limbs after completion the training.

To evaluate the effects of sensory cortex ablation on the learned motor skill, the sensory cortex for the normal limb was removed after completion of the training (n=2). Following recovery from the operation, the success rate returned to the preoperative level within three days for one cat and a week for the other. This recovery period was significantly shorter than the initial learning period for the operated limbs (3 weeks and 5 weeks). The results strongly suggested that the sensory cortex played a role in learning a specific motor pattern but that the sensory cortex was not essential in maintaining a learned motor skill.

(Supported by the NIH Grant NS 10705).

188.3 THE EFFECTS OF SOMATOSENSORY CORTICAL LESIONS ON THE PARAMETERS OF MASTICATORY MOVEMENTS OF THE RABBIT. S.Enomoto*, G.Schwartz, and J.P.Lund. Département de stomatologie et Centre de recherche en sciences neurologiques, Univ. de Montréal, Canada.

It has been known for many years that ablation of the sensorimotor cortex disrupts the ability to take in, manipulate and prepare food for swallowing. Furthermore, the results of studies using electrical stimulation and microelectrodes suggest that cortical neurons participate in the control of the parameters of the masticatory cycle. However, if food is manually placed between the molar teeth, animals seem to chew it in a rather normal way (Bremer, F., Arch. Int. Physiol., 21: 308, 1923). In the belief that cortical lesions should cause identifiable deficits in this type of movement, we have begun a longitudinal study in rabbits.

The animals were anesthetized with sodium pentobarbital (30 mg/kg, supplemented with ketamine hydrochloride) and chronic Emg electrodes were implanted into the major jaw muscles. A holder for a small light bulb was attached to the midline of the mandible and the rabbit was placed in a frame. Two plugs were attached to the skull so that the head could be replaced in the same position day after day. Movement of the light bulb was recorded in three directions with photodiode transducers: before recovery from the anesthetic the system was calibrated. Over the next few days the rabbits were fed rabbit chow, raisins and pieces of carrot of standard dimensions, while jaw movements and Emg activity were recorded on tape. The animals were then re-anesthetized and the masticatory area of the sensorimotor cortex was ablated. Feeding was repeated daily for up to two months. Data was replayed from tape and analysed with computer assistance.

The major effects of cortical lesions are as follows: 1) The number of cycles needed to transport the food to the molar teeth (preparatory cycles) and to grind it up (reduction cycles) increases; 2) The mean cycle time of preparatory cycles is increased, but there is little change in the length of the reduction cycles; 3) The vertical amplitude of both the reduction and preparatory cycles falls, and there are decreases in velocity in both the opening and closing directions; 5) The number of distinct phases per cycle increases; 6) These effects diminish with time with one exception - the velocity of the Slow Closing phase (in which the food is crushed between the teeth) never returns to normal values. The results support the hypothesis that the sensorimotor cortex participates in the generation of the pattern of the masticatory cycle. It may be particularly important in controlling the grinding force during the reduction cycles, since the fall in velocity during Slow Closure never recovers. Supported by the Canadian MRC.

188.4 MODULATION OF SOMATOSENSORY INPUT TO SENSORY CORTEX DURING ISOTONIC AND ISOMETRIC LIMB MOVEMENTS IN THE MONKEY. W. Jiang*, Y, Lamarre, M-T. Parent*, S. Marchand* and C. E. Chapman. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada.

It has previously been shown that somatosensory transmission in the lemniscal system is diminished prior to and during voluntary movement. Both central and peripheral feedback probably contribute to this decrease, but their relative importance has not yet been determined. The purpose of this study was to evaluate the contribution of peripheral feedback to the modulation by modifying the feedback associated with the movement. The effects of systematically changing either the type of movement (isometric vs. isotonic) or the direction of movement (flexion vs. extension) were studied.

A monkey, macaca mulatta, was conditioned to perform rapid isotonic and isometric flexions and extensions of its elbow in response to the presentation of, respectively, a tone or a light cue. EMG recordings were taken from selected arm and shoulder muscles by means of chronically implanted electrodes. Recordings were made from the contralateral primary somatosensory cortical representation of the arm. Intracortical evoked potentials were elicited by a brief airpuff (20 ms) directed within the receptive field of the recording site. The airpuff was applied at different delays following the cue to move, concentrating on the initial part of the movement. Control (no movement) records were regulately alternated with the movement trials. The overall analysis (25 experiments) showed a significant decrease of the evoked potentials during both isotonic and isometric movements and during both flexion and extension. While the results of individual experiments showed some differences with the type and direction of the movement, no consistent differences were seen. For the isotonic condition, the response was decreased to 47 ±12% of the control during flexion and 38±12% during extension. For the isometric condition, the response showed a similar decrease, to 39 ±11% for flexion and 46 ±12% for extension. The absence of any difference in the decrease during these different types and directions of movement suggests central feedback plays a large role in this modulation. Supported by the Canadian MRC and the FRSQ.

188.5 SENSORY TO MOTOR TRANSFORMATION WITHIN AREA 5 OF THE POSTERIOR PARIETAL CORTEX IN THE MONKEY. J. Seal* and J. Requin. CNRS Lab. de Neurosciences Fonctionnelles, 13402 Marseille Cedex 9, France.

Using the technique of single unit recording in behaving monkeys, numerous authors have described changes in neuronal activity of the posterior parietal cortex which precede the onset of movement. However, the functional interpretation of these changes is not clear, some authors explaining them as being the result of sensory mechanisms and others as representing a form of motor command. In the present study, we have examined whether the early changes in area 5 can be explained in terms of sensory or motor mechanisms. A rhesus monkey was rewarded when it performed correctly in three experimental conditions: (1) execution of a forelimb movement on presentation of an auditory cue, (2) withholding the movement after presentation of the cue and (3) self-paced forelimb movement. Single neuron activity was recorded in condition 1 and only those neurons which modified their activity prior to movement were subsequently studied in the other two conditions. For each neuron studied, a series of trials were recorded in one condition before changing to another condition.

Of the 254 task-related neurons recorded in condition 1, 64 neurons presented changes in activity prior to movement. These neurons could be divided into three groups according to whether or not they responded in the other two conditions. The "sensory" type neurons responded whenever the cue was present (conditions 1 and 2), the "motor" type neurons whenever the movement was present (conditions 1 and 3) and the "sensori-motor" type neurons responded in all three conditions. Examination of the peri-event histograms showed that the neuronal response of the "sensori-motor" type neurons in condition 1 appeared to have two components, the first related to the cue and the second related to the movement. Statistical analysis of the discharge of this group of neurons showed that the onset of the neuronal response was related to the auditory cue rather than the onset of movement. From an analysis of the timing of neuronal responses, we found that the order of change in activity after the stimulus was, first, the "sensory" type then the "sensori-motor" type and finally the "motor" type neurons.

We have interpreted these results as indicating the presence in area 5 of a central nervous mechanism for sensory to motor transformation by which the neuronal response to a sensory cue becomes transformed into a motor output. The initial change in activity of the "sensori-motor" type neurons is induced by the "sensory" type neurons and this change may represent the sensory component of the neuronal response. The second, motor, component may occur when, under the influence of facilitatory effects, the activity of the "sensori-motor" type neurons reaches the threshold level necessary for the activation of the "motor" type neurons which governs the eventual execution of movement. External influences from structures involved in, for example, the recognition of the stimulus as being the signal for movement or motivation may act on the excitability of the "sensori-motor" type neurons so as to regulate whether or not the movement occurs. Recent experiments have shown that somaesthetic input may be a modulating influence.

188.6 TECHNIQUES FOR ANALYZING ADAPTIVE CHANGE IN COMPLEX NEURAL SYSTEMS. D. Birt , S. Aou, and C.D. Woody, Mental Retardation Res. Ctr., Brain Res. Inst., Depts. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024.

Both neurophysiologic experiments (Woody, Ann. Rev. Psychol., 1986) and theoretical analyses of artificial neural networks (Hopfield and Tank, Science, 1986) indicate that adaptive changes which support learned behavior may arise from relatively simple changes in large numbers of widely distributed elements. Mapping/labelling mechanisms permit the individual elements to participate in a variety of different adaptations. Empirical studies show that the correlation between behavioral change and any randomly chosen neural unit is small and that meaningful analysis of the network requires methods for identifying the ensemble of elements which participate in a particular conditioned behavioral response.

We have developed and tested methods which facilitate the analysis of neural networks underlying the short latency conditioned eyeblink produced by pairing click (CS) with a tap to the glabella (US). Intracellular recordings from neurons of the pericruciate cortex of awake cats were obtained during associative pairings of CS and US and during appropriate control conditions. The impulse behavior of each cell was described using sets of categorizations based on such measures as the cell's spontaneous activity, the onset latency and magnitude of its response to the CS and a different, neutral auditory stimulus (DS), the magnitude of response at latency periods similar to those of major components of the conditioned behavioral response, and the relationship of spike activity with spontaneously occurring myographic activity. Other computerized procedures permitted measurement of such basic cellular properties as excitability, input resistance, parameters of action potentials (rise time, duration, amplitude, afterhyperpolarization), and magnitude and time course of evoked post synaptic potentials. A relational data base program was then used to select subsets of units whose spike activity was most closely correlated with the experimental condition and with behavioral change. In addition, the trial to trial variations in behavioral response and in neural response were correlated to further define those units whose activity was most closely related to the behavioral change. These subsets of units were then used to test specific hypotheses about conditioning related changes in basic cellular properties.

Results of these analyses show that adaptive changes occur preferentially in subsets of units that reflect specific functional outputs of the network, such as the ability to discriminate stimuli and the ability to control appropriate motor responses. (Supported by NICHHD and AFOSR).

188.7 **APPLICATION OF MULTI-CHANNEL TOPOGRAPHIC EEG MAPPING IN ASSESSMENT OF BRAIN FUNCTION IN BEHAVING RATS.** **R.G. Bickford, G. Buzsaki, B. Allen* and F.G. Gage. Dept. of Neurosciences, School of Medicine, Univ. of Calif., La Jolla, CA 92093.**

Topographic maps of spectral EEG and EP have gained popularity in the clinical diagnostic field. These rat experiments aim at: 1) clarifying the neurophysiologic mechanisms underlying the toposcopic display by means of lesion studies in the animal, and 2) using maps as evidence of electrical rhythm restitution following fetal cell grafting experiments.

Methods. Using stainless steel screws embedded into the skull and head mounted cathode followers, artifact-free EEG and EPs have been obtained in rats during wheel running, drinking etc. The 16 channel data is input to the Minicears (LSI 11/23) computer which generates topographic maps. Color maps of the same data are generated on the Zenith 100 computer and color printer system in three perspectives (Allen). In this study we show examples 1.) of how wave patterns with similar frequency and amplitude characteristics can be dissociated based on different spatial distributions and 2.) effect of subcortical lesions on neocortical EEG.

1. Two types of hippocampal rhythmic slow wave activity (RSA) exist in the rat: one is sensitive to muscarinergic blockers, and the second is non-cholinergic (Vanderwolf and Robinson, 1981). Topographic maps derived from the skull revealed that the two types of RSA are produced by different dipoles, i.e. by different sets of synapses.

2. Unilateral lesion of the nucleus basalis increased the power of neocortical delta activity unilaterally (Fig.), but decreased high voltage spindles in the same hemisphere.

On-line color video displays of the EEG and animal behaviour will be presented. **Supported by grant from J.D. Douglas Foundation and Alzheimer grant No. PHS-AGO 5131-03.**

Before Surgery **After Surgery**

188.8 INTERRUPTION OF VOLUNTARY MOVEMENT BY AN ELECTRICAL OR MAGNETIC CORTICAL STIMULUS IN MAN. J.C. Rothwell, B.L. Day, P.D. Thompson and C.D. Marsden. (SPON: E.A. Jackson). Dept. Neurology Institute of Psychiatry, De Crespigny Park, London SE5 8AF, U.K.

There are now two methods available for stimulating the brain through the intact scalp: high voltage electrical stimulation devised by Merton and Morton (Nature, 285: 227, 1980), and magnetic stimulation pioneered by Barker et al (Lancet, i: 1106-1107, 1985). Both methods have been used successfully to activate areas of cortex and produce elemental sensations or movements. Here we show that such stimuli are capable of interrupting, in addition to mimicking, normal brain function.

Six normal subjects were asked to react to an auditory signal given 1s after a visual warning by flexing their wrist through 20-30° as rapidly as possible. 25% of trials were controls in which no tone followed the warning. Surface EMG recordings from wrist flexor and extensor muscles revealed that the movement was accompanied by a normal bi/triphasic pattern of activity. In about a third of trials, at random, an electrical or magnetic stimulus was given over the contralateral central area of cortex at different intervals after the reaction signal. The intensity of stimulation was above the threshold needed to produce a response in relaxed muscles.

The most striking effect was seen if the stimulus was given so that the muscle response to the cortical shock occurred before the expected time of onset of movement. In this case, the entire movement was delayed, without disrupting the form of the EMG bursts, for periods of up to 200ms depending upon the intensity of stimulation. If the stimulus came later, in the middle of the first burst of agonist EMG activity, only the antagonist (and second agonist) burst was delayed. The delay could not be explained entirely by inhibition of spinal cord mechanisms after the shock since an H-reflex or a second electrical stimulus to the cortex could produce activity in this interval. Neither was the delay caused by interference with perception of the tone, or by changing the intention of the subject to respond. If subjects were required to react bilaterally, and the stimulus was given only to one side of the head, then the movement was delayed only in the arm contralateral to the stimulus.

We conclude that such stimuli can delay the execution of voluntary motor commands, probably by an action at the level of the cerebral cortex.

188.9 FOCAL CEREBRAL STIMULATION IN MAN WITH THE MAGNETIC COIL. V.E. Amassian, R.Q. Cracco and P.J. Maccabee*. Depts. of Physiology and Neurology, SUNY Health Sci. Ctr. at Brooklyn, New York 11203.

The magnetic coil when centered at the vertex has been reported to stimulate arm muscles at least 2 ms later than when transcranial electrical stimulation is used, suggesting that only 'I' waves are evoked in corticospinal neurons (Day et al, 1986, J. Physiol., 378, 36P). Theoretically, with tangential orientation, a substantial fraction of the magnetic flux is coupled transcranially. The resulting electrical field is also tangentially oriented, i.e., optimally oriented for stimulating tangential intrinsic cortical fiber systems and corticocortical afferents. When the plane of the coil is angulated towards the vertical, the fraction of the magnetic flux penetrating the cerebrum is reduced but the orientation of the induced electric field should approximate that of corticospinal neurons in motor cortex and their trajectory in white matter, thus resulting in earlier, 'D' discharge. Using the Cadwell magnetic coil on ourselves, we tested this prediction by comparing the latency of activation of contralateral arm muscles to transcranial electrical and magnetic coil stimulation. The previously described focal anodic configuration was used to avoid distress with electrical stimulation (Amassian, V.E. and Cracco, R.Q., 1987, Neurosurgery, 20: 148-155). The minimum latencies of EMG responses in forearm flexors (e.g., 13.3 ms) and extensors (e.g., 14.8 ms) to near-maximal stimulation with the magnetic coil situated several cm lateral to the midline and optimally oriented towards the vertical, matched those obtained with focal anodic stimulation, also during voluntary contraction. Thus, given the appropriate coil orientation, direct activation of corticospinal neurons is attainable with the magnetic coil. With sub-optimal magnetic coil stimuli, latency jumps as brief as 1.2-1.9 ms were detected in the EMG, possibly reflecting the D-I or inter I periodicity in corticospinal discharge.

Movements evoked by threshold stimulation with the magnetic coil were studied by gluing fluorescent spots onto the tips of the digits, illuminating the spots with UV light and storing the XY coordinates at 60 Hz of each spot with a videcon-computer system. By appropriately angulating the magnetic coil over lateral motor cortex, movements were evoked predominantly of a single contralateral digit. Either flexion or extension could occur. Slight changes in angulation or in placement resulted in movement predominantly of another digit. Such localized responses imply focal cerebral activation had occurred. Other stimuli resulted in movements of many digits. To resolve whether these reflected movements at more proximal joints, a fluorescent spot mounted on a light rod was attached to the hand. Movements occurring at the wrist and digits in any given axis could then be differentiated from those occurring only at the digits.

188.10 LOCALIZATION OF ELECTRICAL BRAIN ACTIVITY IN THE SOMATOSENSORY CORTEX VIA BIOMAGNETIC TOPOGRAPHIC BRAIN MAPPING. L. Narici*§, I. Modena*§, G.L. Romani, P. Rossini*°. Istituto di Elettronica dello Stato Solido - CNR - via Cineto Romano 42, 00156 Roma, ITALY.

Neuromagnetic investigation performed during the last few years has repeatedly shown an impressive capability to perform a three-dimensional source localization on the basis of the measured spatial distribution of magnetic field over the scalp. The ability of the data analysis to calculate location and strength of a bioelectric source depends, of course, on the geometry of the source itself. Here we present a further application of this method to Somatosensory Evoked Field study. We, in fact, have been able to follow, with a time resolution up to a millisecond, the evolution of the position, direction and strength of the equivalent source for the electrical activity evoked in the brain areas adjacent to the central sulcus during the stimulation of the contralateral median nerve at wrist. These results show a reasonable constant behaviour over the subjects we studied. Strength and direction of the equivalent sources seem to behave often as these were undergoing rotations. This would suggest consecutive activations of different areas of the cortex were the curvature of the cortex itself is stronger (for example along the post central wall of the central sulcus down to the floor of the rolandic fissure) or for successive activation of different areas (for example motor/somatosensory). Experiments have been carried out in different conditions to gather more information about the interested areas. In certain cases it has been possible to show that the direction of the current flow is perpendicular to the direction of "motion" of the source. This would provide a hint for an activity following a perpendicular path to the rolandic fissure and moving down/up-ward along the fissure itself.

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188.11 NEW VIBRATION EVOKED POTENTIAL METHOD: COMPARISON WITH CEREBRAL BLOOD FLOW MEASUREMENTS IN HUMANS. AZ Snyder*, JS Perlmutter*, PT Fox, ME Raichle (SPON: S Eliasson) Washington Univ Sch of Med, St. Louis, MO 63110.

A new vibration evoked potential (EP) technique was developed in order to provide a means of comparing electrophysiological and regional cerebral blood flow (rCBF) responses to stimuli of the same somatosensory modality. Vibrotactile stimulation is much more effective than direct electrical shocks delivered to peripheral nerves in activating rCBF. Accordingly, the new EP method is based on steady state amplitude modulation of electromechanically generated vibration. The time dependent potential, v(t), sent to the electromechanical vibrator may be expressed as $v(t) = A(1 + \cos(2\pi f_{mod} t))\cos(2\pi f_{vib} t)$, where A is mean amplitude, and f_{mod} and f_{vib} are the amplitude modulation and carrier frequencies, respectively. In pilot studies involving neurologically normal human volunteer subjects, in which f_{mod} was systematically varied between 2 and 43 hz with f_{vib} held constant at 128 hz, it was determined that, as regards topographic distribution, the responses evoked by vibration of the palm and finger pads of one hand are similar to short latency (30 - 40 msec range) somatosensory EPs obtained by direct median nerve shocks.

A clinical application of the new technique is illustrated in the following case presentation. The patient was a 67 year old dextral woman with a 45 year history of unilateral action dystonia which was responsive to Sinemet. EPs and rCBF increments were measured in response to vibrotactile stimulation of each hand individually before and about 30 min after treatment with Sinemet. rCBF responses were determined by $H_2{}^{15}O$ and positron emission tomography (PET) as previously described (PNAS 83:1140, 1986). Vibration of the uninvolved (left) hand induced normal rCBF and electrical responses in the contralateral hemisphere before and after Sinemet. In the pre-Sinemet condition, vibration of the involved (right) hand elicited an abnormally low rCBF response in the contralateral hemisphere (patient: 12% vs. 8 normals: 26 + 5%). The pre-treatment right hand vibration EPs exhibited an abnormal topographic shift towards the temporal regions of the scalp and response dynamics which were asymmetric with respect to stimulated hand. EPs and rCBF responses were normal and symmetric after Sinemet.

188.12 SPATIOTEMPORAL VOLTAGE MAPPING OF MEDIAN NERVE SOMATOSENSORY EVOKED POTENTIALS BEFORE AND AFTER DIGITAL FILTERING. C. G. Widmer. Dental Research Center, Emory University, Atlanta, GA 30322.

Median nerve somatosensory evoked potential recordings consist of both fast (high frequency) and slow (low frequency) components which have been attributed to subcortical and cortical structures in the afferent pathway. Median nerve high frequency evoked potentials include the P14, N17, N20, P22 and P27 components while the low frequency potentials include N18, N30 and P45 (Desmedt, et al., EEG clin. Neurophysiol., 68:1-19, 1987). Topographic mapping studies have been helpful in establishing the spatial distribution of these potentials, although some of the high frequency components (N17, N20, P22) are superimposed on a lower frequency negativity (N18) and are more difficult to observe in a topographic map. The purpose of this study was to selectively remove the slow or fast components by digital filtering and to create a topographic map of the remaining high or low frequency potentials.

In ten subjects, median nerve evoked potentials were simultaneously recorded from 12 cephalic sites located over the left hemisphere including FZ, PZ, CZ, FP1, F3, C3, P3, O1, F7, T5, T3 and A1 (10-20 International System) with reference to the neck. Data were collected using a bandpass of 1 to 1000 Hz, sampled at 4000 Hz and averaged for 512 stimuli. The stimulus of the right median nerve consisted of a 0.2 ms pulse at a stimulus frequency of 2.2 stimuli/second. Analysis time was 64 ms post-stimulus. Digital filtering consisted of two bandpasses: (1) 1 to 62 Hz to identify the low frequency components and (2) 62 to 1000 Hz to identify the high frequency components. Digital filtering of the recordings was obtained by calculating the Fast Fourier Transform, windowing the data and then calculating the inverse Fast Fourier Transform to reconstruct the filtered SEP.

Spatiotemporal voltage maps of the low pass filtered recordings demonstrated that the highest amplitude of the N18 negativity was widespread over the parietal region, the N30 potential was greatest over the frontal region and the P45 potential was located overlying the central sulcus. Mapping of high pass filtered recordings showed that the P14 and N17 potentials were widespread over the frontal, central and parietal regions with smaller amplitude over the occipital area, the N20 potential was more localized at the mid-parietal recording site P3, the P22 potential was located over the central sulcus and the P27 potential was maximal in the parietal region. These topographic maps were similar to those previously reported in the literature. Therefore, digital filtering can allow mapping of high frequency potentials without phase distortion and without the high-amplitude influence of slow wave potentials.

188.13 HUMAN MOTOR CORTEX IS FACILITATED PRIOR TO ONSET OF VOLUNTARY MOVEMENT. A Starr, M Caramia*, F Zarola* and P Rossini*. Lab. of Clinical Neurophysiology, II University of Rome, Rome, Italy 00173

Human motor cortex can be activated by the passage of a single brief electrical current through the scalp overlying the motor area causing a movement of contralateral muscles. The time-course of facilitation of motor evoked potentials (MEPs) to transcranial electrical stimulation delivered at varying intervals near the onset of a voluntary ballistic movement were studied in four normal subjects. MEPs were recorded from the left thenar muscles to unifocal anodal stimulation of the right scalp overlying the hand motor area delivered every 8 to 10 seconds. The level of current used was adjusted to be subthreshold for evoking an MEP while at rest but effective for evoking an MEP if the subject simultaneously contracted the thenar muscle. A click, randomly associated with the scalp stimulus was the signal for the subject to make a brief thumb press on a piston at short latency. The time interval between the electrical scalp stimulus and the click was adjusted so that the former occurred approximately between 150 msec before and 150 msec after the onset of the voluntary movement signaled by the EMG in the thenar muscles. The results showed that MEPs were not detected when the scalp was stimulated 80 msec or more prior to onset of voluntary movement. MEPs then appeared with increasing probability as the time interval before movement shortened reaching 100% with an interval of 40 msec or less. The amplitudes of MEPs in the 80-40 msec period preceding movement onset were small (>20% of maximum) and achieved maximum values 20 msec after movement onset.

188.14 NEUROCHEMICAL DIVERSITY IN THE HUMAN TEMPORAL NEOCORTEX K. Thapar*,N.C. de Lanerolle, J.P. Partington*, D.D. Spencer. Section of Neurosurgery, Yale University School of Medicine, New Haven CT 06510

Little is known of the neurochemical organization of the human neocortex . In the present study lateral temporal neocortex (LTN) removed during surgery for intractable partial complex epilepsy from 25 patients was used for the immunohistochemical localization of aminoacid (γ-aminobutyric acid-GABA, glutamate-GLU) and neuropeptide (somatostatin-SS, cholecystokinin-CCK, vasoactive intestinal polypeptide-VIP, neuropeptide Y-NPY, enkephalin-ENK, neurotensin-NT) containing neurons. Depth electrode studies prior to surgery had established the presence of seizure foci in the hippocampus but their absence in the LTN. Surgical access to the hippocampal seizure focus necessitated removal of this LTN tissue. Immersion fixed tissue was sectioned and immunostained by the PAP or Avidin Biotin complex techniques with primary antisera against the respective neurochemicals.

An abundance of neurons were immunoreactive for GLU. Most were pyramidal cells but a small number were interstitial neurons in the white matter. GABA cells were present in all cortical layers. However, their laminar density and cellular appearance were not uniform: a moderately high density was found in layer I, an even higher density in layers II - III, and a relatively lower density in the remaining cortical layers. At least three populations of SS neurons were recognized: a population of small bipolar neurons in layers II - III; large multipolar nonpyramidal cells in layers V - VI; and some interstitial neurons in the white matter . SS beaded fibres were present in all cortical layers, but were dense and coursing horizontally in layer I. NPY neurons were present throughout layers II - VI, but predominant in layers V - VI. Additional NPY neurons were present in the white matter. All NPY cells were large multipolar nonpyramidal cells with a varied and extensive dendritic pattern. A dense netwc k of NPY fibres coursed through the cortical layers, many extending vertically to the cortical surface. Horizontal NPY-fibres predominated layer I. A population of NPY neurons may co-localize with SS (Vincent et al.,1982). VIP neurons were small, fusiform and bipolar and uniformly present in all layers except layer I. Isolated, beaded VIP fibres were seen in the upper cortical layers. CCK neurons were present in all cortical layers: most numerous in layers II - III and most sparse in layer VI. Although most cells were small fusiform bipolar neurons, larger pyramidal shaped neurons were noted in layers II - IV. Some of the layer I CCK neurons were horizontally oriented. A loose network of CCK- fibres was present in layers I - II with addition fibres scattered in other layers. While no ENK or NT somata were localized within the LTN, scattered isolated fibres were infrequently present in all cortical layers. These results, while demonstrating a neurochemical diversity within the organization of the human LTN, show in particular: (1) The clear existance of GLU within pyramidal cells; and the absence in such cells of any other substance tested. (2)Neuropeptides are mainly within populations of intrinsic neurons which may modulate pyramidal cell activity. (3) Whereas some of these nurochemicals may be co-localized within the same neuron (eg. SS, NPY) independent populations of chemically distinct neurons can be inferred from variations in the proportions of their respective populations. (4) The white matter intrinsic neurons whose function is unclear, contain one or more of the substances GLU, SOM, NPY. These studies establish the basis for future examination of possible cortical reorganization in LTN seizure foci.

189.1 INTRACELLULAR INJECTION OF LUCIFER YELLOW INTO CORTICAL NEURONS IN LIGHTLY FIXED SECTIONS AND ITS APPLICATION TO HUMAN AUTOPSY MATERIAL. G. Einstein and D. Fitzpatrick. Department of Anatomy, Duke University, Durham, NC 27710.

Recently Masland and his colleagues have developed a technique for making intracellular injections into fixed retinal wholemounts (Tauchi, M. and R.H. Masland, Proc. Roy. Soc. Lond. B. 223:101-119, 1984; Sandell, J.H. and R.H. Masland, J. Neurosci. 6:3331-3347, 1986). We have adapted this method to study the intrinsic and projection neurons in the cerebral cortex of a variety of specimens, including those from human autopsy.

Tissue samples of cat and tree shrew visual cortex and human hippocampal cortex were fixed (either by perfusion or by immersion) with a mixture of 2.5% paraformaldehyde and 0.2% gluteraldehyde in 0.1M phosphate buffer (pH 7.4). Slices, 300-400 um in thickness were cut on an Oxford vibratome and collected in cold phosphate buffer. In order to visualize neurons under the fluorescent microscope slices were stained with 4',6-diamidino-2-phenylindole (DAPI). Under visual guidance, selected neurons were impaled by pipettes filled with a solution of 10% Lucifer Yellow CH in distilled water (final impedance of 120-175 megohms). Filling of the neurons was achieved by pulsing a negative current of about 4 nA (frequency, 2 Hz) until the cell and its dendrites fluoresced bright yellow. In some cases we used an antibody to Lucifer Yellow to achieve a stable, electron-dense reaction product (Taghert, P.H. et al.,Dev. Biol. 94: 391-399, 1982).

The dendrites of injected neurons in the visual cortex appeared to be well filled to their distal portions revealing differences in spine morphology and varicosities. In rapid autopsy material from patients with Alzheimer's Dementia, neurons in the hippocampus displayed a variety of morphological abnormalities including bulbous swellings, spines coated with filamentous material, and beaded processes. Equally good results were obtained with tissue fixed by either perfusion or immersion and neurons could be well-filled up to 48 hours after fixation. This technique allows one to obtain a high yield of stained neurons with exquisite morphological detail and may be particularly valuable for the study of diseased neurons from the human brain. We thank Dr. Donald Schmechel for providing us with rapid autopsy material and Dr. Corey Goodman for the antiserum to Lucifer Yellow. Supported by NEI grants EY06661, EY05546, and the Sloan Foundation.

189.2 COMBINATION OF FLUORO-GOLD AND RHODAMINE BEAD TRACT-TRACING WITH IN SITU HYBRIDIZATION HISTOCHEMISTRY. B.M. Chronwall, J.R. Dubin*, R.G. Krause II, M.E. Lewis, and J.S. Schwaber. Experimental Therapeutics Branch, NINCDS, National Institutes of Health, Bethesda, MD and Med. Prod. Dept., Experimental Station, E.I. DuPont Co., Wilmington, DE

In situ hybridization histochemistry has become an invaluable tool for examining the effects of physiological and pharmacological manipulations on specific mRNA at the single cell level in brain. Since treatment effects are likely to be related to the connectivity of the affected neurons, it would be desirable to have a rapid and simple method to determine the projection area of individual hybridzed neurons. The continued development of fluorescent retrograde axonal transport tracers has resulted in the introduction of a new generation of dyes, i.e. fluoro-gold and rhodamine beads, which are now the retrograde tracers of choice because of their ease of use, bright fluorescence, resistance to fading or leakage, and apparent lack of effect on cellular metabolism. We therefore sought to determine whether these tracers could be used in conjunction with our standard in situ hybridization procedures, which are carried out using formaldehyde-fixed brain sections with synthetic oligonucleotide probes labeled on the 3' end with multiple [^{35}S] or [^{125}I]-labeled nucleotides. Rats were anesthetized and either tracer was administered into the nucleus tractus solitarius by pressure injections via glass pipettes in volumes of 50-75 nl. After survival of at least one week, the animals were anesthetized and then perfused with 4% formaldehyde in PBS. The brains were cryoprotected by overnight immersion in 30% sucrose in PBS and frozen for cryostat sectioning.

The sections were hybridized with labeled somatostatin oligonucleotide (39mer), washed in descending concentrations of SSC, and emulsion dipped according to standard methodology. Following variable exposure times (5-30 days), the slides were then developed, fixed, and washed in water. Sections containing rhodamine beads were air dried and mounted in Entellan, while sections containing fluoro-gold were dehydrated through ascending concentrations of ethanol and xylenes and then mounted in Krystallon prior to coverslipping. The tract-tracing and in situ hybridization labels were viewed nearly simultaneously (with transient blockade of the subcondenser light path) using epifluorescence for the dyes and darkfield transmission microscopy for the autoradiographic grains. Many dye-filled and hybridization-positive cells were detected in the paraventricular nucleus of hypothalamus and central nucleus of amygdala, but only a small subset of neurons in either nucleus were actually double labeled. This method has proved to be straightforward and now provides investigators with the means to easily study specific mRNA in relation to the projections of given neurons within complexly organized brain nuclei.

189.3 VISUALIZATION OF RETROGRADELY-TRANSPORTED FLUORO-GOLD IN LIGHT AND ELECTRON MICROSCOPIC PREPARATIONS. L. Schmued,* K. Kyriakidis,* J.H. Fallon and C.E. Ribak. Dept. of Anatomy and Neurobiology, Univ. of Calif. Irvine, CA 92717.

The advantages of axonally transported Fluoro-Gold as a retrograde fluorescent marker are numerous and have been described previously (Schmued and Fallon, 1986). The present study was conducted to determine whether Fluoro-Gold is visible in either semi-thin sections for light microscopy or thin sections for electron microscopy. Rats were pressure injected with 0.1 ul of 2.5% Fluoro-Gold into either the striatum or the spinal cord. After survial times, ranging from 3-20 days, they were perfused with 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, and blocks of tissue from the substantia nigra or red nucleus, paraventricular nucleus and cerebral cortex were obtained. Sections were cut at 200 um on a vibratome and analyzed on slides with the fluorescent microscope using ultraviolet excitation to determine the extent of retrograde labeling. Sections that contained labeled cells were embedded in plastic for electron microscopy. Semi-thin sections of unosmicated tissue displayed high resolution fluorescent labeling of somata and dendrites. In contrast, osmicated tissue had the fluorescence quenched but numerous dense punctate structures were observed in the perikaryal cytoplasm of labeled neurons in toluidine blue stained sections. The unosmicated tissue did not display these granules and this finding suggested that the granules are composed of membranes since osmium is lipophillic and membranes are primarily composed of lipid. It is interesting to note that neurons that did not send axons to the injection sites were not labeled. Adjacent thin sections examined with the electron microscope displayed numerous electron-dense lipofuscin-like organelles in the perikaryal cytoplasm. The electron density of these organelles was greater than that of ordinary lysosomes. Another distinctive organelle that was observed in these preparations included relatively dense concentric lamellated bodies of various sizes that were found in the somal and dendritic cytoplasm. The presence of swollen multivesicular bodies in somata was also found in these labeled somata. Control sections did not display these organelles. Also, small neurons in the red nucleus that lack a spinal cord projection did not display these organelles whereas the large projection neurons displayed them. Therefore, the electron-dense lipofuscin granules, lamellated bodies and swollen multivesicular bodies suggest that these organelles are sites of Fluoro-Gold localized in the somata and dendrites of retrogradely-labeled neurons. It is not known whether it is the Fluoro-Gold itself, or its physiologic effect on membrances which provides the electron dense staining. This study extends the previously described advantages of Fluoro-Gold tract tracing to the electron microscopic level. One anticipated advantage of this new application at the ultrastructural level is its combination with other electron microscopic labels, such as horseradish peroxidase. (This work was supported by NIH Grants NS 15669 and NS 15321).

189.4 RETROGRADE TRANSPORT OF THE LECTIN PHASEOLUS VULGARIS LEUCOAGGLUTININ BY RAT SPINAL MOTONEURONS. C.L. Lee, D.J. McFarland, and J.R. Wolpaw. Wadsworth Labs, NYS Dept Hlth, Albany, NY 12201.

The lectin Phaseolus vulgaris leucoagglutinin (PHA-L) has proved to be an excellent CNS anterograde tracer, giving Golgi-like labelling. We are exploring its use as a CNS retrograde tracer (McFarland & Lee, this vol.), and, in this study, as a retrograde tracer in the peripheral nervous system.

Rats (200-300 g) were anesthetized and a right C5-T2 dorsal root rhizotomy was performed aseptically. PHA-L (5-10 ul of a 5% solution) was injected into triceps brachii on both sides or just on the right side. After 2,3,4,5,6 or 10-day survival, rats were sacrificed by anesthetic overdose and perfused with phosphate-buffered saline and 4% paraformaldehyde. The C5-T2 spinal cord was cryoprotected and cut by cryostat into 30-um sections. Sections were reacted for PHA-L using goat PHA-L antiserum and the biotin-avidin (ABC) method.

No label was found in rats that survived 2 days. Labelled motoneurons were seen on the injected side(s) in all rats that survived 3 days or more. In rats injected bilaterally, labelling was comparable on the normal and rhizotomized sides. Motoneuron labelling was granular and appeared to be associated with the cell membrane and nuclear envelope. In some cells labelling extended into primary dendrites, but Golgi-like labelling was not seen. Primary afferent labelling was not apparent.

These results indicate that PHA-L undergoes retrograde transport from muscle in spinal motoneurons, since motoneuron labelling was comparable on normal and rhizotomized sides. Furthermore, the lack of primary afferent labelling makes anterograde transsynaptic labelling very unlikely. This demonstration of retrograde transport in the peripheral nervous system increases the possible uses of PHA-L as an anatomical tracer.

(Supported by NIH 22189 and by United Cerebral Palsy)

189.5 BIDIRECTIONAL AXONAL TRANSPORT OF PHASEOLUS VULGARIS LEUCOAGGLUTININ AFTER INJECTION INTO RAT HIPPOCAMPUS. D.J. McFarland and C.L. Lee. Wadsworth Labs, NYS Dept Hlth, Albany, NY 12201.

Phaseolus vulgaris leucoagglutinin (PHA-L) is an excellent anterograde tracer in the central nervous system (CNS). We have shown retrograde transport of this lectin in the lower motoneuron (Lee, McFarland, and Wolpaw this vol.) and are seeking evidence for CNS retrograde transport. The hippocampus was chosen as the site of injection due to its well-known circuitry and orderly cyto-architecture.

Male rats (250-300 g) were anesthetized and placed in a stereotactic frame. PHA-L (0.05-0.10 ul of a 5% solution) was injected into the dentate gyrus on one side over at least 5 min. Two or three days later, rats were deeply anesthetized and perfused with buffered saline and 4% paraformaldehyde. After overnight immersion in the same fixative and cryoprotection, 30 um sections were cut by cryostat. PHA-L was detected by goat antiserum to PHA-L in conjunction with the avidin-biotin method. At both 2 and 3 days after injection, anterograde labelling was seen in the mossy fiber system of the dentate granule cells, in the Schaffer collaterals of CA4 pyramidal cells, and in the commissural/ipsilateral associational system. Golgi-like filling of axons and collaterals was present. In addition, granular labelling of soma membranes and some principal dendrites was seen in dentate interneurons bilaterally and in regio inferior interneurons on the contralateral side. This granular neuronal labelling was probably the result of retrograde transport because it occurred early and was not in close proximity to extensive anterograde labelling.

These results strongly suggest that PHA-L undergoes bidirectional axonal transport in the commissural/associational system of the rat hippocampus.

189.6 CHOLERA TOXIN CONJUGATED WITH HORSERADISH PEROXIDASE: TIME-DEPENDENT VISUALIZATION OF ANTEROGRADE AND RETROGRADE TRANSPORTED REACTION PRODUCT. M. Kalia, M.E. Hudson* and J.J. Viola. Dept. Pharm., Thomas Jefferson Univ., Philadelphia, PA 19107.

Horseradish peroxidase (HRP) conjugated to cholera toxin (CT) has been shown to be a valuable tracer for the study of anterograde (Trojanowski et al, '81) and retrograde (Trojanowski, '82; Wan et al, '82a) transport in central or peripheral neurons. Recent studies have utilized this material almost exclusively for demonstrating details of the dendritic morphology of retrogradely labeled cells (Wan et al, '82b; Shapiro and Miselis, '83; Shapiro and Miselis, '85). Studies of efferent pathways have used wheat germ agglutinin conjugated to HRP as a preferred substance for anterograde tracing. In this study, we examined the time-dependent labeling of cholera toxin-horseradish peroxidase (CTHRP) in a combined system using both anterograde and retrograde transport. The present study examines the usefulness and time-dependency of CTHRP as an anterograde tracer and compares it with new and existing data on retrograde transport. CTHRP conjugates were made with glutaraldehyde activation according to the method of Avrameas and Ternynck ('71) as described by Gonatas et al ('79) and Trojanowski et al ('82). Type VI HRP was obtained from Sigma Chemical Co. and CT from Schwarz/Mann. Ten µg of 0.1% solution of CTHRP was injected into the extensor digitorum longus (EDL) muscle of a rat in which all other nerves had been sectioned to avoid surreptitious uptake by diffusion of the label to the fascial compartments of the muscle. Survival times from 1 to 21 days were allowed prior to perfusion transcardially with 1% paraformaldehyde, 1.25% glutaraldehyde in phosphate buffer solution, followed by 5% sucrose in phosphate buffer at 4°C. The tetramethyl benzidine method for HRP histochemistry was used to demonstrate HRP reaction product in serial, horizontal, frozen sections of the lumbar cord. Sections were examined without counterstain under bright and dark field illumination. The number of retrogradely labeled neurons and anterogradely labeled bouton terminals was counted in every section and quantitative time-related differences were determined using morphometric analysis (Bioquant Systems). The following striking similarities/differences between retrogradely and transganglionically (anterograde) transported CTHRP were observed: a) survival time required for initial appearance of retrograde transport was one day and for transganglionic transport, three days; b) period required to reach peak labeling from the onset of labeling was 48 hours for both retrograde and transganglionic transport; c) duration of peak responsiveness was six days for retrograde transport and one day for transganglionic transport; d) total duration during which labeling could be detected was over 19 days for retrograde transport and 9 days for transganglionic transport. These results demonstrate that CTHRP is detectable in both retrogradely and transganglionically transported forms and that the optimal visualization of each of these transported forms is different, making it possible to select different survival times in order to optimize visualization for the pathway in a system under investigation. This study is the first demonstration of significant amounts of transganglionically transported CTHRP and provides evidence that CTHRP is far superior to WGAHRP and Pha-L (Phaseolus vulgaris-leucoagglutinin) as a transganglionic label. In addition, the discrepancy observed between the optimal duration and latency of labeling in the two instances (retrogradely transported and transganglionically transported) will permit investigations of systems in which one or the other type of transport is being investigated. By selection of the optimal duration of survival, it is possible to study CTHRP transported either in the retrograde or anterograde direction. Quantitative data from this study indicates that since optimal visualization of retrogradely and transganglionically transported CTHRP does not occur simultaneously, experiments involving selected survival periods can be used to analyze motor and sensory systems independently. (Supported by USPHS Grants HL30991, HL31997, and HL33632 to MK.)

189.7 SUICIDE TRANSPORT ACTIVITY OF VISCUMIN (MISTLETOE TOXIN), *NAJA NIGRICOLLIS* CYTOTOXIN AND THE HYBRID CYTOTOXIN, RICIN B-CHAIN DISULFIDE COUPLED TO THE RIBOSOME INACTIVATING PROTEIN FROM BARLEY. M. Ovadia*, F. Stirpe*, T.N. Oeltmann* and R.G. Wiley (SPON: J.T. Wall). Oncology and Neurology Depts., Vanderbilt University and VAMC, Nashville, TN 37232.

Suicide transport, anatomically selective destruction of neurons by axonally transported cytotoxins, is a useful experimental strategy. A number of agents are known to be effective, particularly the toxic lectins such as ricin; however, none are ideal. In our ongoing attempts to identify new and potentially more useful agents, we sought to determine the suicide transport activity of the toxic lectin from mistletoe, viscumin (V), the purified cytotoxin from the venom of *Naja nigricollis* (**NnCyTox**) and the hybrid cytotoxin composed of the ribosome inactivating protein (RIP) from barley disulfide coupled to ricin B-chain (**RTB-SS-BarRIP**). All three agents are potent cytotoxins in vitro. The toxins were dissolved in saline and pressure microinjected unilaterallly into either the vagus or sciatic nerves or caudate nucleus of 32 anesthetized adult, male Sprague-Dawley rats. Toxin doses were 0.2-1.8 ug of V, 2-20 ug of **NnCyTox** and 0.18-6.5 ug of **RTB-SS-BarRIP**. After survival times of 2-17 days, rats were reanesthetized and perfused transcardially with FAGLU fixative followed by sucrose. Cryostat sections of appropriate ganglia, brain and spinal cord were stained with cresyl violet to assess neurotoxicity. Sections containing the substantia nigra were processed for catecholamine histofluorescence. V was reliably neuronotoxic to vagal sensory and motor neurons and sciatic dorsal root ganglion neurons and less consistently to substantia nigra, pars compacta neurons but not sciatic motor neurons. Both **NnCyTox** and **RTB-SS-BarRIP** were neuronotoxic to vagal sensory neurons only; either of these later agents could be used to produce a selective vagal sensory dennervation. V generally produced incomplete lesions at maximally tolerable doses suggesting limited usefulness as an experimental tool. Ricin, modeccin and volkensin remain the most generally useful, nonspecific suicide transport agents known. (This work supported by Veterans Administration Medical Research Service.)

189.8 TOPOGRAPHY OF NUCLEUS BASALIS CHOLINERGIC NEURONS PROJECTING TO DEFINED CORTICAL AREAS IN THE RAT. M. Piotte, R. Egizii*, G.E. Lucier and A.C. Cuello. Depts. of Pharmacology and Therapeutics, McGill Univ., Montreal, Quebec, H3G 1Y6 and of Medical Physiology, Faculty of Medicine, Univ. of Calgary, Calgary, Alberta, P2N 4N1.

It has now been well established that the Nucleus Basalis of Meynert (NBM) sends numerous cholinergic fibers throughout the neocortex. At present, however, there is relatively little information in the rat concerning the exact limits of the NBM and its precise cortical projection sites. This lack of information stems mainly from the difficulty in restraining spreading of the retrograde labeling substances, such as horseradish peroxidase (HRP), to well defined cortical areas. This problem, however, can be overcome by the administration of HRP in a slow release gel. The cortical projections of NBM cholinergic neurons were thus studied combining HRP retrograde labeling and choline acetyltransferase (ChAT) immunocytochemical staining. A small piece (<1 mm in diameter) of a polyacrilamyde slow release gel containing 15% HRP was carefully inserted beneath the surface of each of the following cortical areas (Zilles,K., The Cortex of the Rat, A Stereotaxic Atlas, 1985) in adult male Wistar rats (270-320g): frontal, parietal, temporal and occipital cortices. Forty-eight hours later the animals were perfused and their brains removed. Serial 40-50 um thick sections of the NBM were cut on a sledge, reacted for HRP, washed in phosphate buffer, and processed for ChAT immunocytochemistry by means of the peroxidase-antiperoxidase method of Sternberger (Immunocytochemistry, 2nd ed., 1979). NBM neurons were examined under a light microscope attached to a projector and traced onto paper. Three types of neurons were identified: those which were exclusively ChAT immunoreactive, those which were exclusively HRP positive and double-labeled neurons which contained both reaction products. Although the majority of cells were only ChAT immunoreactive, the proportion of the different types of labeled neurons varied along the rostro-caudal extent of the NBM as well as with the site of HRP implant. Results are discussed in view of the implications that differently located cortical lesions have on cholinergic neurons in various portions of the NBM.

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189.9 ALZHEIMER'S PLAQUES AND TANGLES: A CONTROLLED AND ENHANCED SILVER STAINING METHOD. S.K. Campbell*, R.C. Switzer, III, and T.L. Martin* (SPON: E. O'Connor), University of Tennessee, Department of Pathology and Medical Biology, Knoxville, TN 37920.

The hallmark neuropathological features of Alzheimer's disease are the neuritic plaques and neurons with neurofibrillary tangles (nft). Traditionally, these features have best been histologically visualized by one of a number of protocols utilizing silver. Because of a lack of control in certain steps in many of these protocols, the silver methods are notorious for being capricious. In particular, erratic staining and other problems are common in methods that use a silver-diammine solution (silver nitrate and ammonium hydroxide). Furthermore, the step involving reduction (final development of silver impregnated structures) typically is not easily controlled.

We have combined the silver-pyridine-carbonate incubation step of Hicks (J Lab Clin Med 31:1375, 1946) with the physical development procedure of Gallyas (Acta Neuropathol Berl. 16:39, 1970) to achieve control of the staining reaction. This method works equally well on paraffin or freeze-cut sections. In addition to easily and consistently achieving a high quality of staining of plaques and tangles, the contrast was greatly improved due to little or no staining of myelin. By adjusting the time in the developer, the density of staining can be controlled so that features of interest to a given investigator could be accentuated. We also found that the staining of glia and background neuropil could be reduced by increasing the amount of pyridine in the silver-pyridine-carbonate solution, thus allowing fuller development of the staining of plaques, especially minute ones. This yields spectacular sections that have dark plaques against a clear background, which makes these sections readily amenable for computer image analysis. Neurons with nft's become more orange with increasing pyridine concentrations. With such enhancement, we have found that plaques occur in caudate and putamen more frequently than our other stains had led us to believe. This method allows a wide range of control of silver staining and a means of regulating the amount of staining of normal tissue elements. A synopsis of the procedures is as follows. From water, transfer sections through: 1) 0.6%NH_4OH-5'; 2) H_2O x 2-1' ea; 3) AgPC-40'; 4) 1% citric acid-3'; 5) 0.5% acetic acid-3'; 6) Phys. developer 1.5-3' (3-7' for paraffin); 7) 0.5% acetic acid-3'; 8) H_2O-3'; 9) Mount, air dry; 10) dehydrate, clear, coverslip. AgPC: add to 60ml 1% $AgNO_3$, 2.7ml pyridine, then 45ml 1% K_2CO_3 (anhyd.); phys. developer: add to 50ml 5% Na_2CO_3, 45ml (40ml for paraffin) of [Sol'n B=0.2% NH_4NO_3, 1% tungstosilicic acid (MCB #TX1618)<, 5ml (10 ml for paraffin) of Sol'n C [Sol'n C= Sol;n B with 1.8% formaldehyde (0.7ml of 37% stock/100 ml of B)]. Supported by ADRDA and Robert H. Cole Fdn.

189.10 NEURONAL HETEROGENEITY AND INCREASE OF FUCHSINORRHAGIC MOTONEURONS IN MURINE MUSCULAR DYSTROPHY. M.A. Khan* (SPON:R.Harvey). Health Sciences., NRCAE, NSW 2480, Australia.

The non-enzymatic histochemical technique Haematoxylin-Basic fuchsin-Picric acid (HBFP) is widely used in the histopathological investigation of the extent of infarction in myocardium. The positive crimson red staining that results due to basic fuchsin binding is termed "Fuchsinorrhagia". The HBFP-negative components of the tissue stain blue or yellow. Application of HBFP technique to rat liver sections revealed two populations of hepatocyte nuclei; some were blue and some were red (Khan,M.A., Histochem.J.,10, 641, 1978; Khan,M.A. & Eberhardt,N.H., Acta. Histochem., 65, 146, 1979). The questions studied in this investigation, based on the HBFP stain, were: Does a heterogeneity exist in the motoneuronal and Purkinje cell populations of mammals? What are the tinctorial characteristics of Purkinje neurons during ontogeny? Whether or not motoneurons of the dystrophic mouse show any variation of the histochemical reaction in comparison with the controls?

Neonatal and adult rats (1,2,3,4,6,8,12 and 26 week old) and 4 4-month old homozygous normal and 7 dystrophic mice of Bar Harbor 129/ReJ-dydy strain were used in this study. They were anaesthetised with ether; their cerebellum and thoracic spinal cord were quickly excised and fixed for 24 hours in Carnoys fixative. Paraffin embedded tissue sections were processed according to the HBFP technique (Khan, M.A. & Eberhardt, N.H. Acta Histochem., 65, 146, 1979).

A marked heterogeneity between Purkinje neurons became evident in rats of all ages, except for the one week old; two varieties were easily identifiable on the basis of presence and absence of fuchsinorrhagia. In HBFP-positive neurons, a gradation of fuchsinorrhagic staining, ranging from low to high intensity was present in both nuclei and cytoplasm. A total of 4838 Purkinje neurons were examined and percentage of HBFP-positive fuchsinorrhagic population showed an age dependent linear increase from the second week postnatal onwards. A similar heterogeneity was evident among motoneurons of both normal and dystrophic mice. The majority of motoneurons in normal mice were HBFP-negative and only about 6% were fuchsinorrhagic. The spinal cords of dystrophic mice revealed about 150% increase in the fuchsinorrhagic motoneurons. In dystrophic mice, neither the size of HBFP-positive motoneurons nor the size of their nuclei/nucleoli differed greatly, either amongst themselves, or in comparison with those of the normal mice.

Recent immunochemical studies have substantiated the existence of Purkinje neuronal heterogeneity. Most of the basic histone proteins are soluble and hence removed during fixation. The fuchsinorrhagic neurons, be they Purkinje or motoneurons, may be due to a greater proportion of phosphate groups, fat and carbohydrate components. Nonhistone nuclear proteins which are acidic, heterogeneous, tissue and species specific may participate in fuchsinorrhagia. However, fuchsinorrhagia may not correlate with neuronal maturation since cerebellar maturation is complete by 8 weeks while the highest percentage of fuchsinorrhagic Purkinje cells occurs at 6 months.

189.11 A NEW METHOD FOR ANTEROGRADE AXONAL LABELING IN THE CENTRAL NERVOUS SYSTEM USING A HORSERADISH PEROXIDASE-PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L) CONJUGATE. D.A. Pandya (1), J.B. Rubin (2), R.E. Fine (3) and P.B. Cipolloni (4). Depts. of Anatomy (1,4), Biochem. (3) and Neurol. (4), Boston Univ. Sch. of Med., Boston, MA, 02118 and ENR VA Hosp., Bedford, MA. 01730 (1,2,3,4).

Specific anterograde axonal marking in the CNS of higher animals for quantification at the ultrastructural level has proven to be difficult. All available techniques have at least one major shortcoming. For instance, the use of horseradish peroxidase(HRP)-wheat germ agglutinin conjugate as an anterograde axonal marker is complicated by concurrent retrograde transport of the compound. Further, PHA-L, which can be used successfully for solely anterograde axonal labeling, requires the use of secondary methods for visualization of labeled axons; ie. introduction of HRP to the lectin through an avidin-biotin system (Gerfen and Sawchenko, Brain Res. 290: 219-238, 1984). However, this technique does not routinely allow for optimum tissue fixation necessary for ultrastructural examination. If measures are taken to ensure good tissue fixation, complete visualization of the transported marker is not easily obtained. In order to obviate the need for subsequent attachment of HRP to the previously transported PHA-L, we have conjugated HRP to PHA-L with the bidirectional cross-linker sulfo-disuccinimidyl suberate forming a compound that is transported anterogradely when injected iontophoretically and can be visulized with the usual HRP-DAB reaction.

In developing methods for the use of this conjugate we focused on two fundamental goals: 1) efficient anterograde marking; 2) limited retrograde filling. As PHA-L has already been shown to be transported predominately in the anterograde direction when iontophoretically injected, the second of these goals involved limiting the conjugate's entry into axon terminals via routes commonly taken by the HRP moiety of the compound; namely, fluid-phase endocytosis and, possibly, receptor-mediated uptake. The fluid-phase uptake of the compound was limited by injecting low concentrations of the conjugate and the receptor-mediated uptake was inhibited by co-injecting an excess of denatured-HRP.

The conjugate was evaluated as an anterograde-specific axonal marker with iontophoretic injections into auditory, visual, somatosensory and motor cortices of the rat. In all cases axonal filling occurred in expected projection sites. The extensive anterograde marking we have observed occurs in the virtual absence of retrograde labeling of neurons.

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190.1 ACTIVITY-DEPENDENT FLUORESCENT LABELING OF NEURONS BY TRAPPED REDISTRIBUTION. L. M. Okun* (SPON: D. Yoshikami). Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

I am exploring a new approach designed to mark living neurons with a persistent fluorescent label as the consequence of a transient period of depolarization. The approach depends on fluorescent dyes which are membrane permeant, but intracellularly trappable, anions. The underlying assumptions, combining elements from two earlier methodologies, are (1) that the permeant dye ions will be passively "redistributed" to establish a quasi-equilibrium intracellular concentration that depends on the membrane potential (Vm) during the labeling period (cf. Sims, et al. Biochem. 13: 3315, 1974) and (2) that an enzymatic conversion of intracellular dye to impermeant form (Rotman & Papermaster PNAS 55: 134, 1966; Tsien Nature 290: 527, 1981) will, if concentration limited, trap for later inspection an image (time integral) of that Vm-dependent redistribution. [Strictly, this applies only if trapping is slow relative to the redistribution process (permeation). If, at the other extreme, trapping is much more rapid, all entering dye might be captured, preserving an image of unidirectional, inward flux - a "trapped flux" measure, also expected to be Vm-dependent but generally less sensitively so than "trapped redistribution." At either extreme, or in likely intermediate cases, neurons depolarized during labeling should trap more of the (anionic) dye.]

Promising candidates as dye species with the properties required for this application are certain acyloxyalkyl esters of carboxylated polymethine oxonols. I have prepared a selected member of this class, coba-2,1*(3)/PM, the pivaloyloxymethyl ester of a carboxyalkyl-derivatized barbituric acid trimethine oxonol, and have found that it produces a bright, differential, and persistent (hrs. at room temperature) labeling of cultured chick-embryo dorsal root ganglion neurons depolarized in its presence for a few minutes by isotonic KCl or by veratridine, or of cultured sympathetic neurons depolarized by carbachol. The label is uniformly distributed within cells, is highly resistant to photobleaching, and is not obviously toxic (though it does sensitize cells to photodamage). There is little labeling of dead cells, debris, or flattened nonneuronal cells so that labeled neurons stand out brightly against faint backgrounds in culture.

It remains to be seen whether the approach can be extended to labeling of neurons depolarized in intact tissues, e.g. by a train of action potentials. Several strategies might be employed to augment the approach, if necessary, for this purpose, including use of drugs to enhance or prolong depolarizations, inhibit hyperpolarizations, and control extracellular enzymes, increase of the charge (valence) of the permeant dye to steepen the redistribution's dependence on Vm, and normalizations against second, analogous dyes to correct for labeling variations from factors other than Vm. If the extension to tissue labeling does prove workable, the approach would provide a means for identifying neurons in subsequently dissociated cell samples on the basis of their activation by specific inputs in situ, and would thus complement the recently introduced use of retrograde-transport fluorescence labeling to identify dissociated neurons according to their targets of synaptic output in situ (McPheeters & Okun Soc. Neurosci. Abstr. 6: 733, 1980). In addition, the principles underlying design of the dyes should be applicable to construction of labels bearing other signals, e.g. ones involving positron emission or NMR, susceptible to noninvasive detection. Synthesis of variants suited to fixation for histology should also be possible.

Preliminary results suggest that relatively slowly trapped, weakly ionic dyes, such as CF/PM (carboxyfluorescein pivaloyloxymethyl ester), might provide a related class of "trapped redistribution" labels for transiently altered pH gradients across membranes.

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190.2 **IN VIVO VOLTAGE MAP OF THE HIPPOCAMPUS IN CONTROL AND SEIZING RATS USING VOLTAGE SENSITIVE DYE.** R. M. Dasheiff. Dept. of Neurology and Psychiatry, Univ. of Pittsburgh Epilepsy Center, and Veterans Administration Hosp.,Pgh. PA 15213

Monitoring the electrical activity of the brain is a necessary requisite to understanding brain function. This is especially true for epilepsy research. A new technique has been developed to map changes in membrane potential throughout the brain of awake animals. Voltage sensitive dye (VSD) DiO-C(2)-5 is injected into the carotid artery while the EEG is recorded from electrodes in the hippocampus. The brain is stained by the dye, and the differential accumulation of dye reports the averaged membrane potential during the 20 sec injection. The brain is immediately removed and frozen to preserve this voltage map. Cryostat cut sections are thaw mounted onto slides and coverslipped with permount. A computer fluorescence microscope scans each brain slice and produces a pseudocolor, digital image on a TV monitor. Each pixel represents a 10 micron diameter spot.

Eight anatomical regions within the hippocampus were analyzed: stratum oriens (SO), CA1 pyramidal cells (CA1), stratum radiation (SR), lacosum moleculare (LM), molecular layer of the upper and lower blade of the dentate gyrus (MLU) (MLL), granule cells in the upper and lower blade (DGU) (DGL). Each region is identified using the dark field image produced by the scanning computer microscope. The area is then re-directed to the fluorescence image that was scanned separately but in register with the dark field image. The absolute concentration of VSD is calculated for each area from specially constructed dye standards. Voltage differences are calculated from changes in dye concentration using the results of in vitro hippocampal slice experiments (J.Neurosci.Methods 13:199-212, 1985).

Four sets of rats, grouped CON, KA, BIC, were used and each group was yoked throughout the experiment from operation to scanning. Seizures were produced by injecting 12mg/kg kainic acid (KA), i.p., or 0.25 mg/kg bicuculline (BIC) intrajugularly. Controls (CON) received saline.

	SO	CA1	SR	LM	MLU	DGU	DGL	MLL
CON	-60± 2	-57± 4	-60± 3	-50± 4	-55± 3	-55± 3	-54± 8	-50± 5
KA	-62± 7	-61± 9	-63± 7	-57± 9	-60± 7	-60± 7	-62± 7	-58±22
BIC	-50±15	-44±22	-50±18	-36±29	-50±16	-51±15	-43± 7	-39±21

Values in the table are millivolts $\pm\sigma$. The variation in voltage among the 8 regions in CON are statistically significant, $P<.05$ using ANOVA $F(7,24) = 3.4$. Comparing all groups in CON vs. KA, $P<.005$, paired t test, $df=31$; CON vs. BIC $P<.005$.

The application of VSD as a histological method has produced a voltage map. KA seizures produced a uniform depolarization throughout the hippocampus whereas BIC seizures produced a hyperpolarization. The large σ in the seizing rats is apparently due to biological variability since the technique produced tight data in controls. Future studies will provide a 3 dimensional map of the entire brain to visualize the voltage changes in seizure pathways.

190.3 QUANTITATIVE HISTOFLUORESCENCE OF HIPPOCAMPAL MOSSY FIBER ZINC. C.Y. Montano*, E.J. Kasarskis and D.D. Savage. (SPON: G. Ballam). Dept. Pharmacology, Univ. New Mexico School of Medicine, Albuquerque, New Mexico, 87131 and Dept. Neurology, Univ. Kentucky Medical Center., Lexington, Kentucky, 40536.

6-Methoxy-8-p-toluenesulfonamide (TSQ) has been shown to be a useful fluorescent probe for histochemically detectable zinc in brain. However, utilization of zinc:TSQ fluorescence as a quantitative tool required further characterization of the histofluorescence and development of zinc standards.

Spectrofluorometric experiments were conducted to optimize reaction conditions and determine metal cation specificity. One micromolar zinc was added to 0.003% TSQ (in barbital-acetate buffer, pH 10.2). The excitation and emission peaks for zinc:TSQ fluorescence were found to be 362 and 488 nm respectively. Maximum fluorescence occurred between pH 10 and 11. Zinc:TSQ fluorescence was linear over a range of 100 nM to 10 micromolar zinc. Zinc yielded the highest fluorescence among 16 metal cations tested. Strontium was the only other cation to show appreciable fluorescence. The presence of magnesium or calcium in concentrations up to 10 mM did not affect the fluorescence of 1 micromolar zinc.

Zinc:TSQ histofluorescence was examined using an image analysis system at 32.5X magnification. Zinc standards were made by mixing known amounts of zinc with Immuno-Bed embedding medium. Eight micron sections of the polymerized blocks were cut and mounted onto microscope slides. Eighty microliters of TSQ solution was applied to sections, coverslipped and examined one minute later. Fluorescence, expressed as grey levels, was plotted against the log zinc/100 microns^2. Zinc:TSQ fluorescence was linear over a range 1.5 to 105 femtograms zinc/100 microns^2.

Zinc:TSQ fluorescence in brain tissue was characterized using eight micron horizontal sections of rat ventral hippocampal formation (HPF). Fluorescence was measured in the s. lucidum and adjacent s. radiatum of HPF CA3. Specific mossy fiber zinc fluorescence was defined as the difference in fluorescence between the two regions. Mossy fiber fluorescence increased linearly up to 10 micron thick sections. The fluorescence decay constant in tissue was 107 seconds. Mossy fiber fluorescence varied linearly with TSQ concentrations from 0.5 to 30 mM TSQ. Using four sections each from ten male Sprague-Dawley rats, ventral HPF mossy fiber zinc averaged 25.3 +/- 1.6 femtograms zinc/100 microns^2.

This quantitative histofluorescence technique can be used for the study of hippocampal mossy fiber zinc in a variety of nutritional deficiency, teratological and other pathological states. (Supported by the M.B.R.S. program and NIH-AA06548.)

190.4 **A Novel Method for the Relative Quantitation of Lipofuscin Using a Computer Image Analyses System.** C. Jengeleski M.F. Casanova *, V.E. Koliatsos. Clinical Brain Disorders Branch, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032

Lipofuscin granules normally accumulate in certain populations of large neurons within the central nervous system. Although these granules may balloon cells during aging without loss of function, an overabundant accumulation of lipofuscin may be a sign of neuronal degeneration. Thus, the presence of lipofuscin in small neurons, astrocytes, endothelial cells and free in the neuropil is called pigmentary atrophy and has been causally incriminated in Alzheimer's disease, amyotrophic lateral sclerosis, schizophrenia, Huntington's disease, lipofuscinosis and many other diseases. Present day microspectrophotometric techniques for the quantitation of lipofuscin measure absorbance of aggregate fields and rely on the autofluorescence of lipofuscin. We hereby describe a method for the relative quantitation of lipofuscin which preserves the topographical relation of the cells measured, is not limited by artifacts accrued to fluorescence, has excellent inter-rater reliability and is capable of automated processing. Tissue was formalin-fixed paraffin embedded, cut at 20 um thickness and stained for lipofuscin using a modification of the Gabe's method (Bull Mic appl Paris 3:153, 1953). Shrinkage considerations were avoided by quantification of lipofuscin on sections delimited by natural anatomical boundaries and calculating for area, not volume. A LOATS computer image analysis system interfaced to a Nikon microscope digitized microscopic slide sections and displayed them on a monitor. Software routines allowed calibration of illumination, objectives and areas. Transmission values of lipofuscin staining were isolated using a boundary function and contrast adjusted to all gray levels (color steps) of the displayed image. Re-digitization of the orginal slide with the new coloration scheme gave all pixels having an optical density range similar to lipofuscin. The overall pattern of the image was identical to its corresponding lipofuscin autofluorescence. Multiplication of the resolution by the number of pixels gave the area of the slide covered with lipofuscin. The mean optical density and its variation determined the amount of lipofuscin stained per cell, while both the full range of gray levels and their three dimensional representation gave an idea as to its texture. Area measurements were within the 95% confidence limit as determined by repeated regional computations of a microscale standard visualized at the same magnification as the digitized lipofuscin slides. The method described is not limited to lipofuscin but with the use of special stains it can be applied to the quantitation of most cell components including neuromelanin, iron, calcium and chromatin.

190.5 PHOTOCATALYZED REPLACEMENT OF FLUORESCENT MARKERS WITH A DIAMINOBENZIDINE REACTION PRODUCT. J. H. Sandell and R. H. Masland. Massachusetts General Hospital, Boston, MA 02114.

In 1982 Maranto reported that the fluorescence of lucifer yellow in injected neurons could be converted to an electron-dense product by irradiating the labeled cell with intense blue light in the presence of diaminobenzidine (DAB) (Science, 217: 953-955). Sandell and Masland subsequently showed that the fluorescence of a physiologically accumulated molecule, 5,7 dihydroxytryptamine, could be photoconverted in the same way, yielding a Golgi-like stain of the whole population of retinal indoleamine-accumulating cells. Here we describe an extension of these methods to a wider series of fluorescent molecules.

Our photooxidation procedure has been described (J. Neurosci., 6: 3331-3347, 1986). In most cases a piece of fixed rabbit retina containing fluorescent cells was placed on a slide under a drop of DAB solution (1.5 mg/ml in 0.1 M tris buffer, pH 8.2). The retina was irradiated under a fluorescence microscope, at wavelengths that excite the fluorescence of the molecule which with the particular tissue had been labeled.

The fluorescent molecules that caused photooxidation of DAB included 4,6, diamidino-2-phenylindole (DAPI), lucifer yellow CH, fluorescein, acridine orange, propidium iodide, ethidium bromide, and rhodamine. Their wavelengths range across most of the visible spectrum. Histochemically-induced fluorescence could also cause the photooxidation; examples were the aldehyde-induced fluorescence of 5,7 or 5,6 dihydroxytryptamine or norepinephrine. Immunofluorescent labeling (FITC or rhodamine) also caused photooxidation of DAB. Although most of our experiments were on retinal tissue, the reaction occurred in brain sections and cultured astrocytes as well.

The mechanism of the reaction is incompletely understood. As suggested by Maranto, it is probably an oxidation initiated by the generation of free radicals following excitation of the fluorescent molecule. It is blocked by antioxidants and when oxygen is prevented from reaching the tissue. A successful reaction requires bright fluorescence and strong irradiation.

There is variability in how well different fluorescent molecules react. Our results, however, indicate that a wide range of molecules can be photoconverted in this way. The reacted material is permanent and can be further processed to improve its optical quality. Because the reaction is monitored visually, it can be stopped when the density of reaction product is optimal. The method is compatible with electron microscopy. A limitation is the small size ($\sim$ 1 mm^2) of the field that can be irradiated with adequate intensity through the microscope.

190.6 STAINING OF NEURONS AND THEIR PROCESSES WITH CARBOCYANINE DYES IN FIXED TISSUE. P.Godement*, J.Vanselow*, S.Thanos and F.Bonhoeffer*. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen.

The fluorescent carbocyanine dyes, diI (1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate) and diO (3,3'dioctadecyloxacarbocyanine perchlorate) have been shown to be able to provide staining of neurons in vivo and in vitro (Honig and Hume,'85). Here we report that these dyes can also label neuronal and glial cell processes in post mortem brain tissue that has been fixed in aldehyde fixatives, and compare this labeling with that obtained in living tissue. Staining in fixed tissue was done on mouse and chick embryos fixed in 4% buffered paraformaldehyde solution. The dyes were applied to the retina, optic nerve, or retinal fiber layer. The brains were then stored for various periods of time (a few days to several weeks) in buffer at room temperature or at 4°C. Observations showed that i) fine orthograde labeling of axons, including their growth cones and terminal arbors, occurs upon storage of the tissue for periods ranging between a few days to several weeks, depending on the distance the dyes have to travel from the site of application. Staining in mouse embryos aged E13-E18 shows optic axons in the chiasm region (E13), approaching their targets (E14-E16), and, at later stages, ramifying within their targets, representing a diffusion length of 10-12 millimeters; ii) retrograde labeling of ganglion cells, including their dendrites and growing tips, can also be achieved with application of the dyes to the optic nerve or retina in mouse and chick embryos; iii) a comparison of retrograde labeling of ganglion cells in fixed chick retinas with that obtained either in chick embryo retinas kept in vitro, or in rat retinas following application of the dyes in vivo, shows that while in fixed tissue the dye seems to be contained in the outer membrane of cells, outlining their finest processes, in living tissue the dyes are mostly present in the form of intracellular granules which can also provide accurate silhouettes of dendritic processes. Thus, two mechanisms, lateral diffusion of the dyes along the membranes of fixed and living neurons, and their intracellular incorporation and axonal transport in vivo, provide two different ways of obtaining detailed staining of neuronal processes with these dyes in fixed and in living tissue.

The property of diI and diO to stain processes in fixed tissue, and the observation that processes can be stained over long distances with this method, should prove useful for embryological studies, especially of mammalian embryos in which in vivo staining is difficult, and also for neurobiological studies in which in vivo experiments are not possible.

190.7 CHARACTERIZATION OF LOBSTER ABDOMINAL POSTURAL INTERNEURONS AND EXTENSOR AND FLEXOR MOTOR NEURONS USING THE INTRACELLULAR DYE 5,6-CARBOXYFLUORESCEIN. G.M. Bablanian and C.H. Page. Rutgers University, Bureau of Biological Research, Piscataway, N.J. 08855.

We have explored the possible use of the dye 5,6-carboxyfluorescein (Eastman Kodak) as an alternative to the Lucifer Yellow staining method in the lobster, Homarus americanus. This fluorescent dye has been used in the mammalian central nervous system to examine dendritic branching in the superior cervical ganglion (Purves, D. and Hadley, R.D., Nature, 315: 404-406, 1985) and for quantifying dye-coupling in the hippocampus (Rao, G. et al., J. Neurosci. Methods, 16: 251-263, 1986).

Lobster abdominal extensor and flexor motor neurons and postural interneurons were filled with a 5% carboxyfluorescein-0.1 M potassium acetate solution using standard micropipettes (75-100 M ohms) backfilled with 4 M potassium acetate. Carboxyfluorescein was ejected from the electrode with 3-5 sec pulses of pressure (10-30 psi) until the cell was bright yellow (approximately 30 sec to 1 min). Following incubation for 1 to 48 hours at 10°C, to allow for diffusion of the dye, preparations were cleared in a solution of 5% n-propyl gallate in glycerol for two hours, and then viewed using standard epifluorescence.

Ninety percent of all fills attempted were successful. Motor neuron fills showed characteristic dendritic fields, similar to those observed in the best Lucifer Yellow fills, with axonal projections into the extensor and flexor roots (roots 2 and 3) extending for a distance of 6 mm and more. Fills of interneurons have shown similar results with axonal projection running through the hemiconnective for a distance of more than 6 mm. Compared to Lucifer Yellow, carboxyfluorescein-filled pipettes are less likely to clog in high potassium solutions, an advantage when working with marine organisms. Thus, the carboxyfluorescein method is an improvement in dye-filling technique for examining neuronal morphology in the lobster.

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190.8 DETECTION OF BIOCYTIN FOLLOWING ITS INTRACELLULAR INJECTION ALLOWS VERSATILITY IN THE LABELING OF RECORDED NEURONS. K. Horikawa and W.E. Armstrong, Dept. Anatomy and Neurobiology, Univ. Tenn., Memphis, The Health Science Center, Memphis, TN 38163.

Interest in the microscopic identification of neurons recorded intracellularly has instigated the use of several injectable markers. We explored the use of biocytin (MW=357), a biotin-lysine complex, attractive because of its low molecular weight, high solubility in water and affinity to avidin, the latter of which has been conjugated to several markers.

Solutions of 4-6% biocytin (Sigma) and 0.5M KCl were made in 0.05M Tris buffers of differing pH, influencing biocytin's net charge. Unbroken electrodes containing a glass microfilament were backfilled and had initial DC resistances of 30-100MΩ. Neurons impaled *in vitro* in hypothalamic explants and hippocampal slices were injected through an active bridge amplifier for 1-20min. Positive current injections consisted of depolarizing 1-2nA pulses (1Hz, 220msec). Negative currents were applied with 0.5nA hyperpolarizing pulses (1Hz, 220msec) on a continuous hyperpolarization of 0.5nA.

Fixed tissue (in 10% formalin) was sectioned at 40-60µm on a cryostat (after soaking in 10-30% sucrose) or Vibratome®. Rinsed sections were incubated 1hr in phospate buffered saline (PBS) containing 1% Triton X-100, then incubated with Avidin D conjugated to fluoroscein or rhodamine (1:200) or ABC solution for 1-2hr (reagents from Vector Labs). For fluorescence microscopy, rinsed sections were mounted in glycerol-PBS and examined with standard filter combinations. With the ABC technique, peroxidase activity was revealed using diaminobenzidine as the chromogen.

Labeling was best with positive currents in neutral or acidic pH (3.5), or negative currents with a pH of 9.0. However, with acidic buffer, electrodes quickly became very resistive. Neurons were recovered after 80% of the injections, with both fluorescence and ABC techniques. Labeling was detected after short injections (1-2min) with good impalements, but with poor impalements was weak even after longer injections. With good impalements, longer injections produced detailed labeling resembling that obtained with Lucifer Yellow or horseradish peroxidase.

Intracellular injection of biocytin is useful in that: 1) it is easily injected from electrodes with small tips but of relatively low resistance; and 2) injection of a single compound gives the experimenter the option of producing fluorescent or opaque images.

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190.9 GANGLION CELL STAINING IN THE RAT RETINA BY ANTIBODIES TO MICROTUBULE-ASSOCIATED PROTEIN (MAP) 1. L.J. McKerracher*, R.B. Vallee* and A.J. Aguayo. (SPON: D. Lawrence) Neurosci. Unit Montreal Gen. Hosp., McGill Univ., Mtl., Que., Canada H3G 1A4 and Worcester Foundation Expt. Biol., Shrewsbury, MA 01545.

We have used antibodies raised against high molecular weight bovine brain MAPS (Bloom, G.S., Schoenfeld, T.A., and Vallee, R.B., 1984, J. Cell Biol. 98:320-330) to stain adult Sprague-Dawley rat retinas. Three different monoclonal anti-MAP 1A antibodies exhibited identical staining. In radial cryostat or polyethylene glycol (PEG) embedded sections MAP 1A immunoreactivity is mainly confined to the fiber layer, ganglion cell layer, and the dendritic arborizations in the inner plexiform layer. There is some immunoreactivity in the innermost region of the outer plexiform layer. In the ganglion cell layer the largest cells are most intensely stained, suggesting that the smaller displaced amacrine cells may not be recognized by this antibody.

To determine if MAP 1A immunoreactivity in the ganglion cell layer is restricted to ganglion cells we have used several different approaches: a) in vivo retrograde labelling from axon terminals in the superior colliculus to identify retinal ganglion cell bodies labels nerve cells stained with anti-MAP 1A antibodies. b) extensive retrograde degeneration of retinal ganglion cells after axotomy in the optic nerve appears to be associated with a marked loss of immunoreactivity in the retina. c) double labelling of PEG sections with the nuclear stain DAPI and with anti-MAP 1A antibodies demonstrates that not all of the cells in the ganglion cell layer are antibody-labelled, suggesting that displaced amacrine cells may not immunoreact with anti-MAP 1A antibodies.

In contrast to anti-MAP 1A labelling, two different monoclonal antibodies to a related protein, MAP 1B (Bloom, G.S., Luca, F.C., and Vallee, R.B., 1985, Proc. Natl. Acad. Sci. 82:5404-5408), stain all of the different layers of the retina. Thus, high molecular weight MAPS are present throughout the retina yet MAP 1A appears to predominate in the ganglion cell somata and their axonal and dendritic processes. Although MAP 1A has a widespread distribution in many regions of the nervous system (Bloom et al. 1984, op. cit.) anti-MAP 1A antibodies should be valuable as a ganglion cell marker in the rat retina.

190.10 PHOTODEGENERATION OF NEURONS, N. Franceschini[X], S. Picaud[X] and H.J. Wunderer[X] (SPON: N. Ropert). C.N.R.S., Lab. de Neurobiologie, 31, Ch. J. Aiguier, 13402 Marseilles, France.

The fly retina is a crystalline matrix in which small neurons can be studied in vivo in their natural environment. Using epi-fluorescence microscopy, single photoreceptor neurons can be visualized in the intact animal if one simply neutralizes refraction at the corneal surface with a drop of water (Franceschini, N. et al., Science, 213, 1264, 1981). We report here that neurons can be induced to degenerate if they are irradiated in the presence of a dye added to the extracellular fluid.

Sulforhodamine 101, a green absorbing dye, was applied to the extracellular space of the retina of the fly musca domestica and a selected patch of receptor neurons was irradiated for 30 minutes with green light (546nm Hg-line) under a conventional epifluorescence microscope. Two kinds of phenomena were observed depending on the survival time after the irradiation:

1) at zero survival time, irradiated neurons displayed a dark, electron dense cytoplasm that allowed them to be easily recovered, traced and scrutinized both with the light- and electron microscope. The dark axonal staining was fine-grained and did not obscure presynaptic specializations;

2) after several days survival time, the photo-exposed neurons started to be phagocytosed by the surrounding pigment cells in much the same way as degenerated neurons are phagocytosed by glial cells in vertebrates. Several pieces of evidence suggest that the "photodegeneration" we have observed is actually based on "photosensitization" --a deleterious effect that is readily known to alter excitable membranes, whether the dye is applied intra- or extracellularly (Oxford, G.S. et al., J. Membr.Biol., 36, 159, 1977).

In conclusion preselected neurons can be induced to degenerate on command in the nervous system of a living and adult animal. "Photodegeneration" can be achieved with single cell resolution and does not require any mechanical contact with the neurons. The simplicity, precision and adaptability of the procedure offer interesting prospects for both anatomical and physiological studies of neural networks.

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190.11 IN SITU FIXATION OF RAT SPINAL CORD USING MICROWAVE RADIATION. D.J. Gower, C. Hollman and M. Tytell. Section on Neurosurgery, Department of Surgery, and Department of Anatomy, Wake Forest University Medical Center, Winston-Salem NC 27103.

The spinal cord is resistant to fixation by simple immersion due to its muscular, bony, and dural investitures. Removal of the cord before fixation may structurally alter the tissue. Furthermore, during the time required for removal, early postmortem degenerative changes may occur, interfering with the evaluation of spinal cord injury. We have examined the use of in situ microwave-facilitated fixation of the spinal cord to improve the quality of tissue preservation and to reduce the occurrence of early postmortem anoxia.

Adult male rats were anesthetized and decapitated, and several centimeters of spinal column with surrounding muscles were removed. In one group (n = 15) the spinal cord was carefully dissected out and fixed by immersion in Bouin's fluid for a period of at least 36 hours. The time necessary for dissection ranged from 10 to 20 minutes. In the second group (n = 22) each tissue block was placed in 100 ml of Bouin's solution and exposed to microwave energy in a 600 watt microwave oven (Amana Corp.) to raise it to temperatures ranging from 60° to 82° C for one to five minutes. The microwave-fixed spinal cords were then allowed to cool to room temperature, dissected out, and embedded in paraffin. Spinal cords from both groups were stained with hematoxylin and eosin and compared to tissue prepared by perfusion fixation. Tissue sections were also examined immunohistologically with antibody against heat shock protein-70 (HSP-70; gift of Dr. William Welch, Cold Spring Harbor Laboratory), a stress protein whose synthesis may be elevated in slowly dying, anoxic tissue.

In the immersion-fixed spinal cords, tissue preservation, particularly of white matter, was clearly inferior, and HSP-70 was present in small quantities, primarily in the white matter. In the spinal cords fixed with microwave energy, there was no detectable synthesis of HSP-70, and the morphology of neurons and glia in the gray matter and axons in the white matter was comparable to that obtained with perfusion fixation.

These results indicate that microwave-facilitated fixation at 60° C for 5 minutes is comparable to perfusion fixation in quality of tissue preservation and is far superior to dissection of the unfixed cord for immersion fixation. Microwave-facilitated fixation thus allows simple and very rapid processing of tissues.

190.12 DESIGN OF AN INVERTED "SLAM " FREEZER FOR ULTRASTRUCTURAL-MICROANALYTICAL STUDIES OF IN VITRO BRAIN SLICES. C.S. Wallace, R.E. Crang*, and W.T. Greenough. Neur. & Behav. Biol. Prog. and Depts. Plant Biol., Psychol., and Anat. Sci., and Center For Elec. Micr., Univ. of Illinois, Urbana-Champaign, IL 61801.

For certain electron microscopic procedures, such as ultrastructural x-ray microanalysis, in which the in situ location of cellular constituents is of interest, quick freezing is the method of choice for tissue preservation. In addition, quick freezing may provide a more accurate representation of in vivo morphology as compared to that obtained by conventional chemical fixation methodologies. Of the freezing methods available, the cold-metal block method, such as that refined by Heuser & Reese, has the advantage over other freezing methods of allowing uniform freezing over a large surface, which has proven particularly useful for examining both peripheral nerve-muscle preparations and CNS regions such as the hippocampus. However, because most currently available "slam" freezing techniques require tissue to be suspended in an inverted position prior to freezing, tissue for which electrical stimulation may be desired, such as hippocampal slices, cannot be frozen directly in the tissue chamber in which electrical stimulation occurs. We describe here an inverted slam type freezer that plunges a liquid nitrogen cooled polished copper freezing surface onto slices of tissue maintained in vitro. This design is intended to facilitate studies of the events that occur at short intervals following electrical stimulation by minimizing the handling of tissue during the period between incubation and freezing and by providing better access for stimulating and recording electrodes immediately prior to freezing. Studies are currently in progress to determine the best methods for achieving the shortest possible time interval between electrical stimulation and freezing of tissue.

191.1 RESPIRATORY SINUS ARRHYTHMIA IN NORMAL INFANTS AND INFANTS WHO SUBSEQUENTLY DIED OF SIDS. K.A. Kluge*, R.M. Harper, V.L. Schechtman, A.J. Wilson* and D.P. Southall* (SPON: E.G. Zimmermann). Dept. of Anatomy and Brain Research Inst., UCLA, Los Angeles, CA 90024; Dept. of Medical Physics, Univ. of Sheffield, U.K.; and Cardiothoracic Inst., Brompton Hospital, London.

Reduced heart rate variability has been found in infants who subsequently died of the sudden infant death syndrome (SIDS) (Schechtman et al., in preparation), as well as in siblings of SIDS victims (Harper et al., Sleep 5:28, 1982) and "near-miss" for SIDS infants (Leistner et al., J. Pediatr. 97:51, 1980). This study examines a component of heart rate variability, respiratory sinus arrhythmia, the respiratory-related modulation of heart rate, in control infants and in infants who subsequently became victims of SIDS, in an attempt to isolate group differences that might elucidate the mechanism of death.

Twenty-four hour recordings of ECG and respiratory movements were obtained from 6,914 full-term infants. Of these, 18 recordings of infants aged 2-65 days who subsequently died of SIDS and 52 recordings of age-matched controls were submitted to analysis. Median respiratory rate and interquartile range were used to classify each nighttime (7pm-7am) minute of data as waking (AW), quiet sleep (QS), rapid eye movement sleep (REM), or indeterminate state using an automated sleep state classification procedure (Harper et al., EEG Clin. Neurophysiol., in press). Heart rate and respiratory waveforms were sampled at 16 samples/s and input to a spectral analysis package that calculated autospectra for heart rate and respiratory waveform and their cross-spectra for each 1-min epoch. Two measures of respiratory sinus arrhythmia were computed: "extent of sinus arrhythmia" (XSA), the logarithm of the power of the heart rate autospectrum in the band containing the peak in the respiratory autospectrum, and "coherence of sinus arrhythmia" (CSA), the cross-spectral coherence between heart rate and respiratory rate in this same band. Median XSA and CSA values in each sleep state for infants who subsequently died of SIDS and control infants were then compared using t tests.

For both the younger infants (those recorded in the first month of life) and older infants (those recorded after the first month), XSA values for the SIDS victims were significantly lower than those of the control infants during REM sleep ($p < .05$ and $p < .025$, respectively) and AW ($p < .025$ and $p < .05$). CSA values of the SIDS victims and controls were not significantly different in any sleep state or age group. The state-related reduction in respiratory modulation of heart rate variation suggests that an interaction between forebrain and brainstem cardiorespiratory areas is inappropriately functioning in victims of SIDS.

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191.2 DIFFERENCES IN THREE TYPES OF HEART RATE VARIATION IN NORMAL INFANTS AND VICTIMS OF THE SUDDEN INFANT DEATH SYNDROME. V.L. Schechtman, R.M. Harper, K.A. Kluge*, A.J. Wilson* and D.P. Southall*. Brain Research Institute and Dept. of Anatomy, UCLA, Los Angeles, CA 90024; Dept. of Medical Physics, University of Sheffield, U.K., and the Cardiothoracic Institute, Brompton Hospital, London.

Infants at risk for the sudden infant death syndrome (SIDS) exhibit lower overall heart rate variability than do controls. The purpose of this study was to determine which types of heart rate variation are diminished in infants who subsequently succumbed to SIDS.

Twelve-hour portions (7pm to 7am) of 24-hour recordings of ECG and respiratory movements were obtained from 18 SIDS victims and 54 control infants 2 to 65 days of age. Respiratory rate and variability were used to classify each minute of data as quiet sleep (QS), rapid eye movement sleep (REM), waking (AW), or indeterminate state (Harper et al., EEG Clin. Neurophysiol., in press). A peak/trough algorithm (Schechtman et al., in preparation) was used to determine for each minute the median extent of heart rate variation in three period bands (.9-3 s, respiratory-related heart rate variation; 4-7.5 s, mid-frequency heart rate variation; and 12-30 s, low-frequency heart rate variation). The median extent of each type of variation was determined by sleep state for each recording.

Subjects were divided into two age groups (2-30 days and 40-65 days), and one-way ANOVAs were performed for each measure in each sleep state for each of the two groups. Because cardiovascular variables change rapidly over the first month of life, age was used as a covariate in analyses of the younger age group. Since overall heart rate variation has been shown to vary with base heart rate (Mazza et al., Pediatr. Res., 14:232, 1980), each analysis was repeated with heart rate as a covariate to determine the contribution of basal rate to reduction of each type of variation.

In both age groups, the magnitude of respiratory-related heart rate variation was lower in SIDS victims than in controls during REM and AW. In the younger infants, mid- and low-frequency variation were also reduced during REM and AW. When heart rate effects were covaried, the SIDS victims in both age groups continued to show reduced respiratory-related variation during both REM and AW, and SIDS victims under 1 mo continued to show reduced mid- and low-frequency heart rate variation during AW. These results indicate a reduction in heart rate variation over a wide range of frequencies in infants who later died of SIDS, and that these reductions in variability are not mediated by the same factors that control basal heart rate.

Supported by HD-3-2830 and HD-22695.

191.3 **DISSOCIATED CELL CULTURES OF GUINEA PIG PREVERTEBRAL GANGLIA: PHYSIOLOGICAL AND CYTOCHEMICAL CHARACTERISTICS.** S.G. Matsumoto*, D. L. Kreulen and R. Gruener. Depts. Physiol. and Pharmacol., Univ. Ariz., Coll. Med., Tucson, AZ 85724.

The neurons of the prevertebral sympathetic ganglia (celiac, inferior and superior mesenteric) were grown in dissociated cell culture for periods of up to 16 weeks. The neurons were grown in both mass cultures (approximately 1,000 neurons/cm^2) and in microcultures (one to several neurons confined to a collagen drop ca. 0.5 mm diameter). The neurons were cultured in MEM with 2.5% guinea pig serum, NGF, and antibiotics added. In some instances conditioned medium (CM) obtained from cultures of non-neuronal cells derived from the gastrointestinal tract was added.

Immunoreactivity (IR) for the peptides NPY, Somatostatin (SOM) and VIP was observed in the cultures derived from 30-45 day gestation guinea pigs. The percentage of the neurons displaying IR for the peptides was: NPY (60%), SOM (30%) and VIP (<1%). Peptide-IR was observed in the somata after as little as 4 days in-vitro. The relative number of neurons displaying a given peptide-IR did not change over time from 4 days to over 5 weeks although there was an increase in the number of intensely-staining neurons. Cells grown in CM for 14-21 days showed fewer NPY-IR neurons (30%) without a significant change in total cell number.

Using standard intracellular recording techniques we have observed no spontaneous synaptic activity under control culture conditions. The range of resting potentials (-60 to -80 mV) and the action potential amplitudes (60-90 mV) obtained were comparable to those observed in the intact ganglia in-vitro. Patch clamp recordings, in the cell- attached mode, have been used to examine single channel activity in the neurons. The recordings were obtained 1-5 days after plating. In control cells (i.e. pipets containing only extracellular recording medium) channel activity was rarely observed when the patch was maintained at a holding potential of -80 mV. A concentration-dependent increase in channel openings (inward current) was observed when arginine vasopressin (AVP) was present in the pipet (10-100 nM). In three cases, sequential recordings (3 patches with pipets containing 0, 10nM and 100 nM AVP) from the same neuron showed this dose-dependent effect of AVP. The currents reverse at approximately -20 mV. AVP is a suspected mediator of a slow excitatory synaptic potential in the ganglia. VIP at a concentration of up to 10 uM had no effect.

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191.4 NEUROPEPTIDE Y SYNTHESIS IN SUPERIOR CERVICAL GANGLION DISSOCIATED CELL CULTURE. K. Marek* and R. Mains. Dept. Neuroscience, Johns Hopkins University, Baltimore, MD. 21205.

Neuropeptide Y (NPY) content and synthetic rate have been measured in rat superior cervical ganglion (SCG) dissociated cell culture. Cultures were maintained in either medium containing 5% rat serum or in complete serum free medium (CSFM) for up to 28 days. NPY content was measured by gel filtration and radioimmunoassay. Synthetic rate was determined after incubation in medium containing [^{3}H]tyrosine followed by immunoprecipitation and SDS-PAGE. The synthetic rate of NPY in day 14 cultures was 0.002 fmole/neuron/h. Similar experiments using [^{35}S]methionine to label SCG neurons, followed by SDS-PAGE or gel filtration, showed that proNPY was processed into NPY and a roughly 30 amino acid C-terminal peptide. Tryptic peptide analyses were used to compare the labeled rat NPY to authentic human NPY, which have identical amino acid sequences. Intact C-terminal peptide was compared in several HPLC systems to authentic human C-terminal peptide. About 30% of all cultured SCG neurons were immunostained for both NPY and the C-terminal peptide of proNPY.

Norepinephrine (NE) content, plus NE, acetylcholine (ACh) and NPY synthetic rates have been measured simultaneously in the cultures. NE levels were measured by RP-HPLC with electrochemical detection and synthetic rates were determined following incubation with [^{3}H]tyrosine or [^{3}H]choline followed by RP-HPLC. The ratio of NE:NPY synthesis was 250:1 in day 14 cultures maintained in either rat serum or CSFM. A developmental profile of NPY in SCG cultures showed an increase in synthetic rate from 0.0002 fmole/neuron/h at day 3 to 0.004 fmole/neuron/h at day 18, similar to that previously shown for NE (Mains,R. and Patterson,P., J Cell Biol., 59:361, 1973). SCG cultures showed transmitter phenotype plasticity as demonstrated by the increase in ACh synthesis in cultures grown with cholinergic inducing factor. Experiments are underway to determine the relative effects of further alterations in culture medium on NPY, NE and ACh synthesis and secretion.

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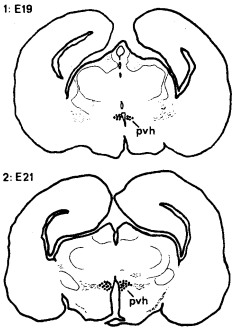
191.5 DEVELOPMENT OF THE SPINAL PROJECTION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS IN THE RAT. E.A.J.F. Lakke* and J.B. Hinderink* (SPON: P.G.M. Luiten). Lab. Anatomy & Embryology, University of Leiden, The Netherlands.

The paraventricular nucleus of the hypothalamus (PVH) provides a direct link between the hypothalamus and the preganglionic autonomic cell groups in brainstem and spinal cord. It influences the glucose homeostatic mechanism (Luiten et al., Progr. Neurobiol., 28:1-54, 1987), probably by modulating the activity of the adrenals by both neuroendocrine and autonomic pathways. Since glucose homeostasis is of utmost importance to the newborn animal, the neural circuitry necessary for its regulation has to develop before birth.

Timed pregnancy WAG rats were used, starting from the 16th day post-coitem (E16). The spontaneously breathing pregnant dams were operated under continuous flow O_2/NO_2 anesthesia. Injections with 5% WGA-HRP were made into the spinal cord of the fetuses through a small incision in the uterine wall. The fetuses were sacrificed after a 24 hrs. survival time and perfused transcardially with normal saline followed by a phosphate buffered (0.1 M, pH 7.2) solution containing 1.25% glutaraldehyde and 1% paraformaldehyde. The 40 µM serial freeze sections were processed for WGA-HRP histochemistry using TMB as the chromogen.

Though WGA-HRP was transported retrogradely from thoracic injection sites to the medullary raphe nuclei at E17 (injected at E16), no labeled cells were found in the PVH until E19 (injected at E18). These labeled cells are situated close to the ventricular wall (fig 1). At E21, i.e. just prenatally, the quantity of cells has increased, but they are still localized periventricular. The lateral descending fiber bundle of the PVH is labeled (fig 2). Postnatally the position of labeled cells shifts laterally, at P4 they have clearly separated from the ventricular wall.



The spinal projection of the PVH develops before birth, the first fibers arriving in the thoracic spinal cord between E18 and E19. This is early, since the PVH only becomes recognizable as a morphological entity at E17 (Hyyppä M., Z. Anat. Entwickl.-Gesch., 129:41-52, 1969). Neurons in the PVH start projecting their axons toward the spinal cord shortly after their birth from the ventricular matrix.

191.6 **GENDER DIFFERENCE IN A SUBPOPULATION OF SUPERIOR CERVICAL GANGLION NEURONS.** L.L. Wright and P.J. Calecas*. Boston University School of Medicine, Boston, MA 02118.

The number of superior cervical ganglion (SCG) neurons is equivalent in males and females at birth. However, by 15 days of age, males have significantly more neurons than females. This difference arises from differential neuron death and persists in the adult. The present study was undertaken to determine whether this difference exists uniformly throughout the ganglion, or only in a subpopulation of SCG neurons.

To study subpopulations of SCG neurons, one or both of the major postganglionic nerves was severed, thereby axotomizing and killing the neurons projecting through the severed trunk(s), and leaving for study those that project through the remaining nerve(s). Bilateral transection of the internal carotid nerve (ICN), external carotid nerve (ECN), or both nerves (ECN-ICN) was performed on male and female Sprague-Dawley rats within 36 hours after birth. All groups were compared with littermate sham-operated controls. At the age of surgery, there was no gender difference in number of SCG neurons (males have 30067, n=7, se=1768; females have 29264, n=5, se=2307). Animals were sacrificed on postnatal day 15 and their SCGs were removed, fixed and embedded. Neuronal nuclei were counted from thionin stained paraffin sections, and the total number of neurons per ganglion was calculated, correcting for sampling interval and split nuclei.

At 15 days of age, all three groups of sham-operated females had significantly fewer SCG neurons than their respective sham-operated male group, confirming our previous work. The number of neurons remaining in the SCG following neonatal transection of the ICN were not different in males and females, while the number remaining following transection of the ECNalone or in conjunction with the ICN was greater in males than in females. We conclude that the gender difference in numbers of SCG neurons lies in large part in those neurons projecting through the ICN. The number of neurons projecting out the ECN is equivalent in males and females.

NUMBERS OF SUPERIOR CERVICAL GANGLION NEURONS AT 15 DAYS OF AGE
(N per group, standard error of the mean)

	Males	Females
ECNX	12969 (11,1454)	9684 (12,1144)
littermate sham op.	29003 (12,983)	25634 (19,900)
ICNX	12226 (7,2040)	12516 (11,1411)
littermate sham op.	30034 (6,1412)	24395 (6,2694)
ECN-ICNX	3463 (16,517)	3882 (30,386)
littermate sham op.	32162 (4,1545)	27528 (6,1769)

This work has been supported in part by NIH grant NS21577 to LLW.

191.7 ONTOGENY OF ADRENERGIC FIBERS IN THORACIC SPINAL CORD IN RELATIONSHIP TO RETROGRADELY LABELED SYMPATHETIC PREGANGLIONIC NEURONS. H.Bernstein-Goral & M.C.Bohn. Department of Neurobiology and Behavior, State University of New York, Stony Brook, New York 11794

Epinephrine and its biosynthetic enzyme, phenylethanolamine N-methyltransferase (PNMT), are phenotypic markers for adrenergic neurons. PNMT is initially expressed in neuroblasts in the presumptive C1 region in the ventral lateral medulla oblongata on embryonic day 14 (E14; Bohn, et.al., Devel.Biol.,114:180,1986). In the adult rat, adrenergic bulbospinal axons from C1 neurons project to the intermediolateral cell column of thoracic spinal cord (Ross,et.al.,J.Comp.Neurol.,228:168,1984). To describe the development of this system, we have studied 1) the appearance and ontogeny of PNMT-immunoreactive (IR) fibers using an antiserum raised against rat PNMT, 2) epinephrine levels in thoracic spinal cord by HPLC, and 3) the relationship of adrenergic fibers to retrogradely labeled sympathetic preganglionic neurons (SPGN) at various postnatal ages.

PNMT-immunoreactive fibers are first observed in the embryonic lateral funiculus of spinal cord on E15. By E16, PNMT-IR fibers are located in thoracic spinal cord gray matter, exclusively associated with preganglionic nuclei including n.intermediolateralis principalis (IML) and funicularis (ILF), n.intercalatus (IC), and central autonomic nucleus (CAN). For two weeks after birth, there is a marked increase in the density of PNMT-IR fibers that declines at older ages. The changing fiber density is paralleled by epinephrine levels in thoracic spinal cord, which are 20 fold greater on postnatal day 10 compared to the adult. Neonatal adrenalectomy does not reduce spinal cord levels of epinephrine.

To study the developmental relationship of adrenergic fibers to identified SPGN, the adrenal medulla and superior cervical ganglion (SCG) were injected with the fluorescent retrograde tracer, true blue. Spinal cord sections containing true blue labeled preganglionic neurons were also processed for PNMT-IR. At postnatal ages 4,7,15,20,30,and 60 days, dense plexuses of PNMT-IR varicose axons enveloped every true blue labeled PGN soma and proximal dendrite, as well as many unlabeled neurons. In the IML and ILF, true blue labeled soma were silhouetted by PNMT-IR varicosities, while dense transverse bundles of PNMT-IR axons coursed between clusters of neurons in IC and CAN. Longitudinal PNMT-IR axons were observed along the ependymal cells of the central canal.

These results demonstrate that adrenergic axons are present in sympathetic nuclei of thoracic spinal cord during embryonic life. In the postnatal rat, sympathetic preganglionic neurons which project to SCG and adrenal medulla receive a rich plexus of PNMT-IR fibers suggesting that these neurons may be postsynaptic targets for adrenergic bulbospinal fibers. In the neonatal rat, these associations are particularly rich and epinephrine levels are high suggesting that epinephrine may play a role in spinal cord development and early autonomic function.

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191.8 NEURONS OF THE AVIAN CILIARY GANGLION CONTAIN SOMATOSTATIN. M .L. Epstein and J. L. Dahl. Depts. of Anatomy and Pharmacology, University of Wisconsin, Madison, WI 53706.

Immunocytochemical staining showed the presence of somatostatin-immunoreactivity (SOM-IR) in cell bodies of ganglia removed from newly-hatched chick and quail. Fibers showing co-localization of SOM-IR and choline-acetyltransferase immunoreactivity (ChAT-IR), a marker for cholinergic neurons, were found in the choroid layer of the eye. Immunostaining of the ciliary ganglion showed the presence of ChAT-IR in large ciliary neurons and a less intense ChAT-IR in the smaller choroidal neurons, which also contained SOM-IR. Somatostatin immunoreactivity in ganglia from hatched chicks eluted from high pressure liquid chromatography in the same positions as authentic SOM 14 and 28. We conclude that SOM is found in cholinergic choroidal neurons in the avian ciliary ganglion.

Supported by NIH DK32978.

191.9 ONTOGENY OF NEUROTRANSMITTERS/HISTOCHEMICAL MARKERS IN NEURONS OF THE PARACERVICAL GANGLION OF THE FEMALE RAT. K. Sullivan*, R.E. Papka and H.H. Traurig. Dept. of Anatomy and Neurobiol., Univ. of KY., Lexington, KY 40536.

The chemical neuroanatomy of the paracervical ganglia (PG), which provides much of the efferent autonomic innervation to the female reproductive tract, has been a focus of our research. Currently, we are interested in possible developmental interactions between steroid hormones and PG neurons and/or their chemical "signature". Prior to investigating such interactions, we undertook a baseline study of the developmental expression of histochemical markers exhibited by untreated neurons. Acetylcholinesterase(ACHE)$^+$ ("cholinergic"), tyrosine hydroxylase (TH)/dopamine beta hydroxylase(DBH)$^+$ ("noradrenergic"), neuropeptide tyrosine(NPY)$^+$, and vasoactive intestinal polypeptide(VIP)$^+$ markers are present in adult PG; thus, these were examined in the present study. Female rats were bred and litters killed at 0, 2, 4-5, 8-10, 15-16, 25-26 and 36 days postnatal. PG were removed and processed by standard procedures for immunofluorescence of neuropeptides and histochemical localization of ACHE. On day 0 the PG consisted of neuroblast-like cells, e.g. tightly packed, small, rounded or angular cells. ACHE staining revealed a compact PG in which neurons were difficult to discern; only a small amount of light-staining reaction product was present. However, DBH/TH$^+$ cells were evident at day 0 and were often clustered resembling SIF cells; thus, it was difficult to identify principal neurons. VIP$^+$ cells were numerous and evenly scattered among a large population of non-VIP$^+$ cells. A large population of cells weakly positive for NPY were present at day 0. Few ACHE$^+$ cells were evident at 4 days of age; however those present contained intensely stained pockets of reaction product. By day 4 and clearly by day 8 TH/DBH$^+$ principal neurons and SIF cells could be distinctly identified. With increasing age the number of intensely immunoreactive NPY$^+$ cells increased. No remarkable changes over time were evident with the VIP$^+$ neurons. By 26 and 36 days postnatal, the PG resembled that of the adult - the neurons were distinctly angular and multipolar, there was an increase in neuropil clearly separating the neurons; numerous ACHE$^+$, VIP$^+$ and NPY$^+$, but few TH/DBH$^+$ neurons were observed. It appears that cells with noradrenergic characteristics and VIP$^+$ cells are identifiable earliest in postnatal life relative to those with "cholinergic" markers or NPY. However by puberty the NPY$^+$ cells were most numerous followed by VIP$^+$, ACHE$^+$, and then noradrenergic. This study will serve as a baseline or library of information for future investigations of interactions of steroid hormones and ontogenetic expression of neurotransmitters/histochemical markers in PG neurons. (Supported by BRSG RR05374 and NIH Grant NS22526).

191.10 HORMONAL REGULATION OF NEUROTRANSMITTER SYNTHESIZING ENZYMES AND NEUROTRANSMITTERS IN AUTONOMIC GANGLIA. B.M. Schroeder* & R.W. Hamill. Depts. of Neurology & Medicine, Univ. of Rochester/Monroe Community Hospital, Rochester, NY 14603.

Gonadal steroids appear to influence a variety of morphological and neurochemical characteristics of peripheral sympathetic neurons. Previous studies by a number of investigators indicate that cell number, synapse number, transmitter enzyme activity and plasticity may all be influenced by hormonal factors. The present studies in the sympathetic hypogastric ganglion (HG) are an extension of previous investigations which indicate that testosterone (T) exerts regulatory effects on tyrosine hydroxylase (TH) activity, a marker of noradrenergic functional activity. The current experiments were designed to determine: 1. if T regulates neurotransmitter levels as well as TH in the HG and its major target the vas deferens (VD), and 2. if T regulates TH in the major pelvic ganglion (PG), an autonomic ganglion which is a mixed parasympathetic and sympathetic structure.

Adult Sprague-Dawley rats received either sham-operation or bilateral castration at approximately 60-70 days of age and were randomized to vehicle treated or T replacement treated groups. Animals were killed at various times after surgery and HG, PG, and VD were removed. TH activity was measured with a radiochemical assay and HPLC with EC detection measured transmitter molecules. Within one month, HG TH activity fell by about 85% (control:1661±209 pm/hr; castrates: 280±58 pm/hr: X ±S. E. M.). Within the same time frame HG NE levels decreased 30% and DA 44%. In the target VD there was a 65% decrease in TH activity but there was no change in NE levels (C=748±68.8; Cx=856±52.6 ng/-organ), however VD DA levels fell by approximately 50%. T treatment reversed these changes. Studies of the PG revealed that T regulates TH activity in a manner similar to the HG: TH activity fell by 80% within 4 weeks of castration and T treatment reversed these changes. Thus, gonadal steroids regulate transmitter molecules and enzymes within ganglia and the change in transmitter levels is delayed; also alterations occur initially within cell bodies before their axon terminals. Additionally DA appears to be altered before NE. Furthermore, since T regulates components of the PG (a mixed sympathetic and parasympathetic ganglia) as well as the HG, gonadal steroids exert a broad range of changes in various components of the peripheral autonomic nervous system.

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191.11 **NEUROTRANSMITTER METABOLISM IN PERIKARYA AND TERMINALS OF NEONATALLY DENERVATED OR HYPERINNERVATED SYMPATHETIC NEURONS.** Arnold J. Smolen and Patricia Beaston-Wimmer*. Dept. of Anatomy, The Medical College of Pa., Philadelphia, PA 19129.

The early postnatal period represents a time of rapid developmental changes in neurons in the superior cervical sympathetic ganglion (SCG) of the rat. In the present series of experiments, we examined the development of SCG neurons in two experimental conditions: one that results in permanent denervation of the sympathetic neurons and one that results in hyperinnervation of some of these neurons. Turnover of the neurotransmitter, norepinephrine (NE), was measured following synthesis blockade with alpha methyl paratyrosine, and was used as an index of sympathetic activity in neuronal perikarya and terminals.

In the first experiment, the cervical sympathetic trunk was resected at birth, resulting in a permanent deafferentation of SCG neurons. We observed that populations of neurons that project to different targets respond differently to neonatal deafferentation. NE content and turnover are normal in sympathetic terminals in the submandibular gland (SMG), while in the iris NE content is reduced by one-third, and there is no detectable turnover.

In the second experiment, we eliminated approximately half of the SCG neurons at birth by cutting their axons in the internal carotid nerve (ICN) unilaterally. The neurons that project through the external carotid nerve (ECN) survive this manipulation and become hyperinnervated by preganglionic axons. In this case, NE content and turnover in the SCG is only reduced by 25%, indicating that each surviving neuron, on average, contains more NE than normal and demonstrates increased turnover of transmitter. There is no detectable turnover of NE in the ipsilateral iris, which is completely denervated following ICN section. The pineal gland receives bilateral innervation from the SCG through the ICN, and therefore is partially denervated after neonatal section of one ICN. In this region, both levels and turnover of NE are normal, indicating that the intact innervation from the contralateral unoperated side is capable of compensating for the loss of the ipsilateral input. The SMG receives its sympathetic innervation through the ECN from neurons that survive section of the ICN. Increased activity of the terminals of these hyperinnervated neurons is expressed as an increased turnover of NE in the SMG, although the content of NE is unchanged from controls.

We conclude that, in general, transsynaptic influences are important in regulating neurotransmitter metabolism in developing sympathetic neurons. In some instances, however, development can proceed normally in the absence of transsynaptic influences.

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191.12 CATECHOLAMINE LEVELS AND TURNOVER IN THE SYMPATHETIC NERVOUS SYSTEM OF SPONTANEOUSLY HYPERTENSIVE RATS H. Trenchard, P. Beaston-Wimmer*, K.F. Greif and A.J. Smolen. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010 and Dept. of Anatomy, Medical College of Penna., Philadelphia, PA 19129.

The Spontaneously Hypertensive Rat (SHR) develops abnormalities in blood pressure regulation during the first two postnatal months which resemble human essential hypertension. It has been proposed that increased sympathetic nerve activity during the developmental period plays an important role in the etiology of the hypertension. As a measure of sympathetic activity, we assayed the content and turnover of norepinephrine (NE), the transmitter used by the sympathetic postganglionic neurons, in both SHR and in the related normotensive strain, the Wistar-Kyoto (WKY).

Levels of norepinephrine in the superior cervical ganglion (SCG) and its targets, the iris, pineal and submandibular gland(SMG) were compared in male SHR and WKY rats in the prehypertensive stage (6 weeks postnatal) and after hypertension is established (16 weeks). Catecholamines were extracted with perchloric acid and absorbed on alumina and quantified by use of HPLC with electrochemical detection. Turnover of catecholamines was determined by treatment for 3 hr with alpha-methylparatyrosine methyl ester to block new synthesis (Smolen et al, Dev. Brain Res. 23: 211, 1985).

NE levels in the SCG of 6 week prehypertensive SHR rats were twice that in age-matched WKY rats. Levels of NE were also elevated in the iris of 6 week SHR rats. In contrast, levels were significantly reduced in the pineal of SHR rats of the same age and equal in the SMG. At 16 weeks, when hypertension is established, NE levels in the SCG, iris and SMG of SHR rats were only slightly higher than in the normotensive WKY. Levels of NE in the pineal gland remained lower in SHR rats at 16 weeks.The continuous depression of NE levels in the pineal will require further investigation.

Turnover of catecholamines was elevated in the SCG and iris of 6 week SHR rats, and was reduced by more than ten-fold in the pineal. Turnover was not measurable in the SMG in either SHR or WKY rats at six weeks. At 16 weeks, turnover was slightly higher in the SCG and iris of SHR rats. However, turnover was significantly greater in the SMG of SHR rats, and remained reduced in the pineal. These results suggest that selective changes in turnover, dependent on the type of target, may occur. Since SCG innervation to the SMG is primarily to blood vessels, the increased turnover after the establishment of hypertension may reflect permanent changes in vascular innervation.

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191.13 SYMPATHETIC NEURONAL CONTROL OF CELL REPLICATION IN DEVELOPING RAT HEART AND KIDNEY: EFFECTS OF NEONATAL CENTRAL CATECHOLAMINERGIC LESIONS. S.F. Stasheff*, W.L. Whitmore*, F.J. Seidler*, K.L. Queen* and T.A. Slotkin (SPON: A.L. Sylvia). Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Recent studies suggest that norepinephrine released by sympathetic nerves serves to control the timing of cellular replication and differentiation in peripheral tissues. In the current study, this hypothesis was evaluated by central lesioning of neonatal rats with 6-hydroxydopamine (6-OHDA, 100 µg intracisternally on postnatal day 1 and 150 µg i.c. on day 6), a treatment known to attenuate sympathetic tone without physically destroying peripheral neurons. The degree and specificity of the lesion were verified by measurements which showed near-total depletion of CNS dopamine and norepinephrine content, with maintenance of peripheral norepinephrine stores. Adverse effects of 6-OHDA on development were noted in the heart but not the kidney. During the first two postnatal weeks, cardiac cell acquisition, as measured by DNA content, was slowed in the 6-OHDA group, and although some catch-up occurred by the end of the second week, the lesioned animals still displayed significant deficits in cell number into young adulthood. There was evidence for compensatory cellular hypertrophy, as protein/DNA (protein content per cell) was elevated during the period in which deficits of cell number were present. These results stand in contrast to the effects of large doses of catecholamines, which when administered acutely, shut down cardiac and renal DNA synthesis. Apparently, a low level of tonic sympathetic input serves to maintain cell acquisition during early development, whereas excessive tone terminates cell replication; indeed, the burst of sympathetic activity which occurs in the cardiac neuronal pathway during the third postnatal week is thought to provide the major signal for cessation of cell division. These data are consistent with a dual role of sympathetic activity in trophic control of peripheral tissue development: a low level of tonic input appears to be necessary for normal cardiac (but not renal) cell division to occur, whereas excessive input terminates mitosis in both tissues. The role of low-level tonic input is thus specific to some, but not all peripheral sympathetic pathways. (Supported by USPHS HD-09713 and EPA CR-813769).

191.14 NEURAL CONTROL OF MACROMOLECULE SYNTHESIS IN DEVELOPING RAT HEART, KIDNEY AND LUNG: RELATIONSHIP TO SYMPATHETIC NERVE DEVELOPMENT AND β-ADRENERGIC RECEPTORS. T.A. Slotkin, W.L. Whitmore*, L. Orband-Miller*, K.L. Queen* and K. Haim*. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Catecholamines derived from sympathetic neurons and/or the adrenal medulla have been hypothesized to play a role in setting the timing of cellular development. In the current study, we evaluated the relationships among the ontogeny of sympathetic projections to peripheral organs, the patterns of macromolecule synthesis in those organs, and the reactivity of synthetic processes to β-adrenergic stimulation by isoproterenol. In agreement with earlier results found in the cardiac-sympathetic axis, the major developmental rise in norepinephrine concentration and turnover occurred during the second to fourth postnatal weeks in renal and lung sympathetic pathways. Concurrently, the numbers of β-receptors, measured by iodopindolol binding, rose from the low levels characteristic of the neonate, to adult levels. Evaluations of sympathetic physiologic function (heart rate control, renin secretion) also indicate that the major phase of development occurs in this period. The developmental decline in DNA synthesis in heart, kidney and lung, also coincided with the maturation of sympathetic projections. Furthermore, direct stimulation of β-receptors by in vivo administration of isoproterenol was able to cause acute reductions in DNA synthesis in an age-dependent manner. In the heart, isoproterenol was first able to reduce DNA synthesis at 5 days of age and a maximal effect was seen at 9 days; this early phase was characterized by a rapid time constant of coupling of β-receptors to the DNA effect (maximal effect at 6 hr after isoproterenol). Reactivity was lessened by 12 days of age and thereafter displayed a longer time constant (maximal effect at 12 to 24 hr). Reactivity of DNA synthesis to isoproterenol challenge was slightly different in kidney and lung (detectable by 2 days of age), but bore similar developmental characteristics to the pattern in the heart (peak of reactivity at 9 days and a decline in reactivity and lengthening of the time constant after 16 days). No such relationship was evident for the basal ontogenetic pattern or effects of isoproterenol on RNA or protein synthesis. These results thus support the hypothesis that sympathetic input regulates cell replication patterns in developing peripheral organs, with a peak of reactivity corresponding to the point at which peripheral sympathetic function is first established (end of the first postnatal week to end of the third postnatal week). Because the coupling of β-receptors to DNA synthesis disappears with the maturation of full physiological function, sympathetic control of cell replication can thus occur only during a critical period in early development. The fact that β-receptors and their ability to modulate DNA synthesis appear prior to the completion of sympathetic neuronal development, suggests that teratologic changes in development of heart, lung and kidney, may be produced by premature exposure of the neonate to catecholaminergic drugs or by factors which accelerate sympathetic neuronal development.

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191.15 NEUROPLASTICITY OF SUBSTANCE P-LIKE NERVES IN PARTIALLY OBSTRUCTED RAT INTESTINE. T.H. Williams, G. Gabella, J. Jew, M.Q. Zhang and E. Jew. Dept. of Anatomy, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Over the past century several reports documented that, under the influence of partial obstruction, the gut wall exhibits remarkable hypertrophy occurring on the oral side of the stenosis site. Recently, the response in the intestinal musculature has been addressed both ultrastructurally and quantitatively in a series of studies (Gabella, G., Physiology of the Gastrointestinal Tract, pp. 335-381, 1987). The affected intestine is unchanged in length, but the thickness and circumference of the muscle coat are markedly increased. Previous studies have shown that hypertrophy of myenteric neurons and their sheath (Schwann) cells is substantial. The myenteric neuropil exhibits less growth than nerve cell bodies. In the present study we attempted to establish a histochemical profile for a representative neuropeptide in the myenteric plexus, and especially of neuroplastic changes occurring after introducing obstruction lesions. Many neuropeptide transmitters have been localized immunohistochemically in the myenteric plexus. Because of its functional importance and wide distribution in the GI system we selected substance P-like peptide as a relevant transmitter subsystem to investigate. Male Wistar rats, 3-4 months old, were anesthetized, and a short strip of acetate film 7 mm by 25 mm, preperforated with holes 21 mm apart, was positioned around the terminal loop of ileum. After 3-5 weeks, the animals were anesthetized and sacrificed; the distended portion of gut immediately proximal to the acetate strip was removed and opened lengthwise and placed in Zamboni's fixative solution. After an overnight wash in phosphate buffered saline, 35 µm thick tissue sections were cut with a freezing microtome and processed for substance P-like peptide immunohistochemistry using an avidin-biotin complex procedure. An analysis of changes in immunoreactive elements was based on i) comparison of sizes and staining characteristics of ganglia, and of connecting meshes of the plexus, in experimental versus control preparations; ii) determining sizes and labeling intensities of cell bodies in both groups; iii) evaluation of substance P-like fiber density, staining intensity and degeneration profiles between experimental and control preparations; and iv) changes in terminal axons, including fiber varicosities, staining intensities and numbers of substance P-positive processes in connecting meshes of the plexus. Interpretations were referenced to the measured elongation of the circular musculature. Changes in cell bodies, terminals, and nerve fibers including degeneration products are reported. (Supported by grants AM23986 and NS19578.)

191.16 CATECHOLAMINERGIC NEURONS IN DORSAL VAGAL MOTOR NUCLEUS PROJECTING TO THE UPPER ALIMENTARY TRACT OF THE RAT. D. Vyas*, D. Bieger and D. Hopkins (SPON: D. McKay), Faculty of Medicine, Basic Medical Sciences, Memorial University, St. John's, Newfoundland and Department of Anatomy, Dalhousie University, Halifax, Nova Scotia.

Recent hodological, histochemical and immunocytochemical studies have neccessitated a redefinition of the dorsal motor nucleus of the vagus (DMV) in terms of its topography, viscerotopical organization and cytology. Moreover, present evidence, albeit disputed, suggests that there exist subpopulations of efferent catecholaminergic neurons in the DMV. As yet, it is unclear where these neurons project, which catecholamine they utilize as primary transmitter and whether their intranuclear distribution displays a viscerotopic pattern. In view of the prominent connections between the DMV and the upper alimentary tract, we sought to determine (1) if gastric and oesophageal projection neurons of the DMV exhibited immunoreactivity for both tyrosine-hydroxylase (TH) and dopamine β hydroxylase (DBH) or TH only.

Adult male Sprague-Dawley rats were injected with 0.5 µl of a 20% solution of horseradish peroxidase (HRP) in the left or right cervical vagal trunk or 3 µl of a 5% solution of wheatgerm agglutinin conjugated HRP in the cervical or distal oesophagus or stomach wall. Sagittal sections (25 µm) of the brainstem were double-stained for HRP and either TH- or DBH- immunoreactivity. HRP was visualized using the tetramethylbenzidine and diaminobenzidine/$CoCl_2$ method while TH or DBH were detected by means of the peroxidase-antiperoxidase method of Sternberger.

With either vagal trunk or stomach wall injections, double-staining was observed in a small number of DMV perikarya displaying TH-immunoreactivity; however, HRP/DBH-positive perikarya could not be detected. HRP/TH double-stained neurons represented less than 1% of the total population of retrogradely labelled DMV perikarya and were confined to the portion of the DMV contained within the closed portion of the medulla from the level of the obex to beyond the caudal limit of the pyramidal decussation. Although oesophageal efferent neurons present in the rostral DMV intermingled extensively with TH-immunoreactive perikarya, no evidence for double-staining was obtained.

It is concluded that the DMV contains a subpopulation of catecholaminergic gastric efferents which likely utilize dopamine as a neurotransmitter. These neurons could play a role in noncholinergic vago-vagal reflexes, particularly receptive relaxation.

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191.17 ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF SITES OF EXCESS LAMININ ACCUMULATION IN THE PRESUMPTIVE AGANGLIONIC BOWEL OF *ls/ls* FETAL MICE: RELATIONSHIP TO THE MIGRATION OF NEURAL AND GLIAL PRECURSORS IN THE BOWEL WALL. V.M. Tennyson, R.E. Payette*, H.D. Pomeranz, T.P. Rothman and M.D. Gershon, Departments of Anatomy and Cell Biology and Pathology (Neuropathology), Columbia University, College of Physicians and Surgeons, New York, NY 10032.

The enteric nervous system is formed by precursors of neurons and glia that migrate to the bowel from the neural crest. In the *ls/ls* mutant mouse the terminal colon is abnormal because an intrinsic defect of the presumptive aganglionic zone prevents the entry and colonization of this region by migrating neural crest cells. Previous studies have shown that neural crest cells fail to enter the terminal colon both *in vivo* and *in vitro*. Since components of extracellular matrix (ECM) have been implicated in neural crest cell migration, we have examined the distribution of these elements in the bowel wall. In the adult *ls/ls* mouse, smooth muscle cells in the muscularis mucosa of the aganglionic zone are hypertrophic and surrounded by reduplicated basal laminae. In the *ls/ls* embryo on day E 11, a time prior to the formation of ganglia anywhere in the bowel, we have found an increase in Alcian blue staining in the mucosal basal lamina and in the enteric mesenchyme of the terminal colon and tail gut. Immunostaining of laminin and type IV collagen was also increased at this time in the mucosal basal lamina and extended from it as fronds into the surrounding mesenchyme. Enteric ganglia are formed in the terminal colon of control mice on days E 15- E 16. At this age, the aganglionic zone of the *ls/ls* mouse has an abnormal accumulation of laminin in the basal laminae of both the mucosal epithelium and the smooth muscle cells of the circular layer, especially in the innermost zone adjacent to the submucosa. The myoblasts of the circular muscle layer are hypertrophic, but there are fewer of them than in controls. The presumptive myoblasts of the longitudinal layer of smooth muscle of the *ls/ls* mouse are immature and separated by spaces containing laminin immunoreactivity. The bowel wall in this region is circumscribed by a thickened layer of adventitial fibroblasts. Ultrastructural observations following either ruthenium red or tannic acid block staining reveal that basal laminae of both the mucosa and the myoblasts of the circular smooth muscle layer are thickened. In addition, an irregular fibrillar material consisting of 4.5-6 nm filaments associated with 14-20 nm granules is present in lakes of extracellular space between presumptive myoblasts in the longitudinal smooth muscle layer. Utilizing a pre-embedding immunocytochemical procedure with colloidal gold, we have found laminin immunoreactivity on the irregular fibrillar material in the lakes of extracellular space, as well as on filamentous material extending from the lamina densa of the thickened mucosal basal lamina. Our histochemical and ultrastructural data demonstrate that abnormalities of ECM are present in the terminal colon of the *ls/ls* mouse at the time of enteric ganglion formation and are located at sites that might interact with migrating neural crest cells in the bowel wall. The observations are consistent with the hypothesis that the accumulation of these materials is causally related to the development of aganglionosis in the mutant. Supported by grants # HD 17736, NS 15547, HD 21032, The March of Dimes Fdn., the Dysautonomia Fdn., and the Parkinson's Disease Fdn.

191.18 MIGRATION OF SACRAL NEURAL CREST CELLS TO THE AVIAN BOWEL AND GANGLION OF REMAK: RELATIONSHIP TO LAMININ. H.D. Pomeranz, R.E. Payette* and M.D. Gershon, Department of Anatomy and Cell Biology, Columbia University, College of P & S, New York, NY 10032.

The enteric nervous system (ENS) is formed by precursors that migrate to the gut from the vagal and sacral levels of the neural crest. The sacral crest also forms the ganglion of Remak. There appear to be defined pathways of crest cell migration, but the route taken by sacral crest cells to reach the hindgut and ganglion of Remak is not yet known. The current experiments were done to identify the pathway between the sacral crest and the bowel and to examine the relationship of laminin to migrating sacral crest cells. A monoclonal antibody (MAb), NC-1, that recognizes an epitope on migrating crest cells was used for their immunocytochemical identification. MAb EC/8, which recognizes a neurofilament-associated protein, NAPA-73, was employed to define the subset of crest cells expressing a neuronal marker. Dual-label immunofluorescence on single cryostat sections was used to demonstrate the localization of laminin simultaneously with the MAbs described above. Serial sections were traced in chick embryos from stages 16 to 27. At stage 16, in the region of the most caudal somites, NC-1$^+$ cells appeared to migrate ventrally along the basal lamina of the neural tube, laterally to precede the emerging ventral roots through the somites, and dorsally in close apposition to the epidermal basal lamina. EC/8$^+$ cells were far fewer than NC-1$^+$ cells, and were only found at some distance from the neural tube. Caudal to this region the neural tube was not yet closed and there were no NC-1$^+$ cells. Laminin was present in formed basal laminae and in patches and fibrils between mesenchymal cells. Somites were incompletely enclosed by a laminin$^+$ sheath, especially where penetrated by crest cells. By stage 21, two large masses of NC-1$^+$ crest cells could be seen in the mesenchyme near the developing hindgut. One, more caudal, extending from an accumulation of crest cells ventral to the sacral neural tube, approached the gut in a V formation. Cells at the apex of the V entered the cloacal mesenchyme rostral to the allantoic bud and were EC/8$^-$. Relatively little laminin was found among the crest cells, but was abundant in the surrounding mesenchyme. A second, more rostral mass of crest cells, many of which were EC/8$^+$, approached the hindgut ventral to the aorta between the mesonephric ducts. These cells remained in the dorsal mesenchyme of the bowel and did not move ventrally around the gut. At stage 23, both groups of crest cells could still be identified. The group of sacrally derived cells at the mesenteric attachment extended rostrally and were always separate from another group of NC-1$^+$ cells (of truncal origin) that formed a crescent ventral to the aorta. It seems likely that this cluster of crest cells in the dorsal mesentery gives rise to the ganglion of Remak. Crest cells did not migrate into regions densely laminin-immunoreactive, such as the nephrogenic mesenchyme. At stage 27, NC-1$^+$ cells migrate circumferentially beneath the serosal basal lamina. The colonization of the hindgut by sacral crest cells proceeds in a caudal to rostral direction and lags behind the rostral growth of the ganglion of Remak within the dorsal mesentery. Only a subset of the cells that enter the gut are EC/8$^+$, the remainder may be glial precursors or neuronal precursors not yet committed to a neuronal lineage. The relationship of crest cells to the distribution of laminin is compatible with the view that laminin helps to delineate pathways along which neural crest cells migrate. Supported by NIH grants NS 15547, HD 17736, and GM07367.

191.19 EFFECT OF BILATERAL OVARIECTOMY ON THE KINDLING LIKE-CONVULSIVE ACTIVITIES OF THE ISOLATED FEMALE GUINEA PIG ILEUM. M. Luján*, R. Rodríguez*, M. Velasco*, F. Velasco and I. Márquez*. Dept. of Pharmacology. Mexico University, School of Medicine and National Medical Centre, IMSS.

In previous reports (1), (2), (3) we have shown that iterative subthreshold electrical stimulation of the isolated ileum of the male guinea pig, produced in the majority of cases (18/20 ilea 62/80 ileum segments) a progressive increase of the basic muscular tonus, followed by self sustained, high amplitude muscle contractures; and blocked by various anticonvulsant compounds. These changes represent a plastic modification of the male ileum neuromuscular activity; and are equivalent in many respects to the "epilepsy kindling" produced by the electrical stimulation of the CNS. In contrast, intestinal kindling in the female guinea pig was only occasionally observed (2/20 ilea and 2/80 ileum segments). In the present work, the effect of bilateral ovariectomy on the intestinal kindling of 10 female guinea pig was studied in order to determine to what extent the presence of gonadal hormones is responsable to avoid the kindling process of the intact female quinea pig ileum. Bilateral ovariectomy flattened completely the vaginal cytology, and significantly increased number of kindled female intestines ($P < 0.001$) and intestine segments ($P < 0.01$). Velocity of the kindling process however, were in ovariectomized female ilea similar to that of the male ilea.

(1) Luján et al. IPEG International Pharmaco EEG Group 1986, pp. 132
(2) Rodríguez et al. Life Sciences 1986, 39:1037-1041
(3) Luján et al. Exp. Neurol. 1987 (In press)

192.1 **AGE AND SEX DIFFERENCES IN 3H-THYMIDINE LABELING IN THE DEVELOPING ZEBRA FINCH SONG SYSTEM.** J.R. Kirn & T. J. DeVoogd, Psychology, Cornell University, Ithaca, NY. 14853

Zebra Finch song is a learned, sexually dichotomous behavior for which there are parallel dimorphisms in the nervous system: only males sing and song control brain regions are larger in males than in females (reviewed by DeVoogd, J. Neurobiol. 17(3): 177-201). The developmental mechanisms which produce these brain dimorphisms are largely unknown. Moreover, despite reports of adult neurogenesis in one song control region-HVc (Hyperstriatum Ventralis pars caudalis), little is known about the time course and amount of neurogenesis in male and female vocal control nuclei during development. This report examines neurogenesis from hatching to 30 days of age in 4 interconnected telencephalic song nuclei; HVc , RA (Robustus Archistriatalis), Area X, and MAN (Magnocellular nucleus of the Anterior Neostriatum) (Nottebohm et al., J. Comp. Neurol., 207:344-357).

Male and female finches received 3 injections of 3H thymidine (2.5 uCi/g body weight, sc., specific activity 20 Ci/mM) covering one of the following 5 day intervals: post hatch days 2-6; 8-12; 14-18; 20-24; or 26-30. At 60 days, birds were anesthetized, perfused, and 10um coronal brain sections were processed for autoradiography. In representative cresylecht violet stained sections from each brain region, cells with a clear nucleus, 1-2 nucleoli, and darkly staining cytoplasm plus a 10 x background grain count were classified as 3H labeled neurons.

Neurogenesis in HVc and Area X was found to occur throughout the first 30 days post hatch. Across all injection age groups, 1-5% of neurons in male Area X and 1-3% of neurons in male HVc were labeled at 60 days. In addition, a substantial sex difference was found in the number of labeled cells in HVc: total labeled neurons/injection age group for male HVc was 822 $\pm$ 366 compared to 74 $\pm$ 72 in females (also see Nordeen & Nordeen, Soc. Neurosci. Abstr. 12:1214, 1986). It is likely that augmented cell death in females during the interval between thymidine treatment and sacrifice (Kirn & DeVoogd, Soc. Neurosci. Abstr. 11:532, 1985) accounts for some of this difference in labeled cell number. In contrast, differential cell death cannot account for a prominant male Area X and the apparent absence of this nucleus in females (Kirn & DeVoogd, Soc. Neurosci. Abstr. 12:1214, 1986). Present data indicate that Area X neurogenesis overlaps with known sex differences in steroid levels (Hutchison et al., J. Endocr. 103:363-9, 1984). Thus, Area X dimorphisms may result from differential neurogenesis or migration.

No labeled neurons were found either in RA or MAN following any tritium injections, suggesting that the majority of cells present in these nuclei at 60 days are generated prior to hatching. Primary afferents for male RA and MAN may not arrive until 25 and 45 days post hatch, respectively (Konishi & Akutagawa, Nature, 315: 145-7, 1985; Bottjer et al., Soc. Neurosci. Abstr. 12:1213, 1986) suggesting a surprisingly long delay between neurogenesis and the arrival of major afferents in these nuclei. Ongoing research employing embryonic thymidine injections will provide a more complete description of neurogenesis in song control brain regions.

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192.2 DEVELOPMENT OF SYNAPSES IN SONG CONTROL NUCLEI OF THE ZEBRA FINCH. R.P. Clower and T.J. DeVoogd. Department of Psychology, Cornell University, Ithaca, NY 14853.

One of the least understood aspects of the neurobiology of singing in birds is the development of the song system. While auditory experience and prac-tice, important in the "template" theory of song learning, are certainly of fundamental importance, it is as yet unclear precisely how or where in the central nervous system their effects are produced. Clearly, a thorough, multi-level, multi-age description of the anatomy of the brain areas controlling singing in passerines must be made before template formation or song expression can be fully explained.

Song generation is usually traced from hyperstriatum ventrale pars caudale (HVc) to robustus archistriatalis (RA) (both telencephalic nuclei) and then to the brainstem hypoglossal nucleus (nXIIts), both directly and by way of the dorsomedial part of nucleus intercollicularis (ICo), found in the midbrain. These nuclei are well defined in Nissl stained adult tissue from all song birds which have been studied to date. However, RA is just discernible at 5 days post hatching in zebra finches and HVc is still somewhat ambiguous at day 10. Tritiated proline injections have suggested that RA is not innervated by HVc until about day 30 (Konishi and Akutagawa, 1985). It would be useful to know precisely what is taking place at at an ultrastructural level as these nuclei first emerge and as extrinsic innervation occurs.

Male zebra finches are being studied at 5, 10, 30 days post hatching and in adulthood. Birds are anesthetized and their brains are processed according to a standard EM protocol. Approximately 50 - 100 synapses in Hvc, RA and nXIIts are being classified according to synapse class, frequency, length of postsynaptic thickening (PST), size of pre- and postsynaptic processes, and number of synaptic vesicles.

Preliminary data are available for RA. Large numbers of synapses exist in RA in 5 day old birds. Frequency and PST length do not change appreciably over subsequent development (frequency range: 9.9 synapses/100μ at 10 days to 11.9 synapses/ 100μ in adulthood; PST range: .26 μ at 10 days to .34 μ at 30 days). However, synapses in older animals are much more likely to occur on spines than in younger animals (19% of identified synapses on spines at 5 days, 42% at 10 days, 48% at 30 days, and 72% in adulthood). Degenerating profiles are frequently observed at 10 and 30 days, suggesting that synaptic turnover is occuring. Myelinated axons are first seen at 30 days and are frequent in adults. Extracellular space is obvious at 5 days, less clear at 10 days and virtually absent at 30 days and in adulthood.

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192.3 **SENSITIVE PERIODS FOR MASCULINIZATION OF NEURONAL STRUCTURE IN N. ROBUSTUS ARCHISTRIATALIS OF THE ZEBRA FINCH SONG SYSTEM.** T. J. DeVoogd and C. I. Gould*, Dept. Psychology, Uris Hall, Cornell Univ., Ithaca, N.Y. 14853.

There are dramatic sex differences in zebra finches in the appearance of song related brain regions. We have shown that sex differences in neuronal structure in the nucleus RA occur as a result of dendritic spine loss in females as well as neuronal growth and reduced rates of cell death in males (Kirn & DeVoogd, 1985; DeVoogd *et al.*, 1986). These anatomical dimorphisms are probably caused by differing developmental levels of steroid. An adult female zebra finch given testosterone will not sing (Arnold, 1974) unless she has been previously treated with T or E in the first 3 weeks of life (Gurney & Konishi,1980). This increased song capacity in steroid-treated females in response to exogenous androgens during adulthood corresponds to an increase in RA volume (Gurney, 1982) and an increase in mean soma diameter of neurons within RA (Gurney, 1981). Little is known of the timing of endocrine influences on the microanatomy of song control regions.

In this research, endocrine state during development was systematically manipulated to determine sensitive periods for the development of dimorphic features in the neuroanatomy of RA. Females were given subcutaneous T-implants for 5 day intervals over the following ages: 0-4, 5-9, 10-14, 15-19, 20-24, 25-29 days. 25 implanted females and 3 normal males and 3 normal females were perfused at 6 weeks of age. Their brains were processed for Golgi-Cox staining (Glaser & Van der Loos, 1981), dehydrated and sectioned at 120 microns.

We have found that different time periods of gonadal hormone administration to a female zebra finch have different effects on the development of RA. Females implanted with T for 5 days in the interval10-29 days had significantly larger dendritic trees ($p<.025$), greater numbers of dendritic spines ($p<.01$) and larger cell dendritic volumes and Sholl curves than females exposed within the first 10 days of life. Some aspects of RA morphology were restricted in their sensitivity to steroids for a shorter period of time. RA significantly increased in volume only in response to T during 15-19 days of age ($p<.025$); and the number of primary branches on neurons within RA was significantly masculinized in females receiving T within days 10-19 ($p<.05$). Females exposed to androgens before day 10 showed a minimal response in all measures of RA morphology.

Thus, androgen exposure in early life results in masculinization of the appearance and neuronal morphology of RA. The two classes of effects can be dissociated. This suggests that RA size may be more closely tied to number of cells (and therefore directly related to cell death) than to dendritic differentiation. Supported by NICHD 21033

192.4 DEVELOPMENT AND CONNECTIVITY OF SEXUALLY DIMORPHIC SONG NUCLEI. R. Mooney* and M. Rao (SPON: M. Konishi). Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

Nucleus RA of the zebra finch song system is not sexually dimorphic in either cell size or number until approximately 30d posthatch, at which time neurons in the female nucleus begin to undergo extensive atrophy and cell death. The innervation of RA by HVc neurons was studied by injecting 3H-proline into HVc to label HVc axons. Injections at 15-25d in either sex result in a ring of labeled terminals around RA. In the male, labeled fibers first appear in RA at 25d, and by 35d, RA is completely labeled. In the female, RA remains mostly devoid of labeled fibers during development and in adulthood (Konishi and Akutagawa, 1985).

We have begun a physiological study of the HVc projection to RA using in vitro brain slice techniques. We prepared 400 µm thick parasagittal sections of 50-90d male zebra finch forebrain which contained a large portion of RA, HVc and the interposed bundle of HVc axons. Intracellular recordings showed that many RA neurons exhibited EPSPs upon depolarizing stimulation of the HVc fiber tract. Intracellular injection with Lucifer Yellow revealed that the dendrites of RA neurons exhibiting such EPSPs were spinous and confined to RA.

Furthermore, a fluorescent carbocyanine dye (diI) was injected in vivo to allow visualization of HVc axons in the slice preparation. Injecting HVc produced a staining pattern consistent with results achieved with proline autoradiography. Injected birds younger than 25d displayed a ring of label surrounding RA. Neurons in the unstained central portion of the ring exhibited EPSPs upon stimulation of the HVc fiber tract, even though their Lucifer Yellow-labeled spinous dendrites did not arborize outside of RA. Thus, RA neurons appear to be innervated before labeled HVc terminals can be visualized within the nucleus. Either 3H-proline and diI injections made in HVc do not label processes entering RA before 25d, or axons within the HVc fiber tract innervate RA neurons via a polysynaptic input.

192.5 PARADOXICAL HYPERMASCULINIZATION OF THE ZEBRA FINCH SONG SYSTEM BY AN ANTIESTROGEN. G.A. Mathews*, E.A. Brenowitz, and A.P. Arnold. Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Estrogen exerts a potent masculinizing effect on the neural system that regulates song production in oscine birds (Gurney, 1982). Male Zebra Finches (Poephila guttata) show higher circulating levels of estradiol in the first ten days post-hatching than do females (Hutchison, Wingfield & Hutchison, 1984). If estrogen masculinizes the song system, then blocking estrogenic action should prevent this pattern of differentiation. We tested this hypothesis by injecting neonatal finches with tamoxifen, an antiestrogen. Contrary to our expectations, we observed anatomical hyper-masculinization of the male song system and masculinization of the female song system.

Ten Zebra Finches of each sex received daily subcutaneous injections of either 100ug tamoxifen in 3ul propylene glycol or only 3ul propylene glycol for 20 days post-hatching. They were sacrificed at 60 days of age. We measured the cross-sectional areas of neuronal somata in the magnocellular nucleus of the anterior neostriatum (MAN) and robust nucleus of the archistriatum (RA) and total volumes of the caudal nucleus of the ventral hyperstriatum (HVc), RA and Area X.

Somal area in MAN of males was 200 ± 21 um2 (X ± SD, tamoxifen-treated) and 164 ± 9 um2 (control) ($p < 0.001$, t-test). In male RA, somal area was 207 ± 30 um2 (tamoxifen) and 167 ± 25 um2 (control) ($p < 0.001$). For female MAN somal area was 126 ± 13 um2 (tamoxifen) and 102 ± 15 um2 (control) ($p < 0.001$). Somal area in female RA was 139 ± 35 um2 (tamoxifen) and 92 ± 25 um2 (control) ($p < 0.001$).

Total volume of RA in males was 0.47 ± .02 mm3 (tamoxifen) and 0.42 ± .03 mm3 (control) ($p < 0.05$). For Area X of males, volume was 2.32 ± .35 mm3 (tamoxifen) and 1.81 ± .26 mm3 (control) ($p < 0.05$). HVc volume in males was 0.73 ± .10 mm3 (tamoxifen) and 0.63 ± .05 (control) ($p>0.05$). For females, volume of RA was 0.12 ± .04 mm3 (tamoxifen) and 0.09 ± .01 mm3 (control) ($p > 0.05$). HVc in females was 0.20 ± .08 mm3 (tamoxifen) and 0.12 ± .03 mm3 (control) ($p > 0.05$). Tamoxifen-treated females displayed a prominent Area X (volume = 0.39 ± .07 mm3). This region was not discernable in control females.

The hypermasculinization of males and masculinization of females could reflect an estrogenic rather than antiestrogenic action of tamoxifen. However, the hypermasculization of males is paradoxical since administration of exogenous estradiol itself does not have this effect. The consequences of a hypermasculine song system for vocal behavior are under study.
(Supported by NSF grant BNS 86-62917)

192.6 PROJECTIONS OF ANDROGEN-ACCUMULATING NEURONS WITHIN THE ZEBRA FINCH SONG SYSTEM. F. SOHRABJI*, E.J. NORDEEN, AND K.W. NORDEEN.* (SPON: V. Laties) Dept. of Psychology, U. of Rochester, Rochester, N.Y. 14627.

In zebra finches, androgens trigger song development and stimulate growth of the neural regions controlling song. Early exposure to estrogen establishes this ability to respond to androgens probably by increasing the number of androgen target cells in two song regions, hyperstriatum ventralis pars caudalis (HVc) and the magnocellular nucleus of the anterior neostriatum (MAN). Since the neuroanatomical growth induced by androgens extends beyond HVc and MAN, it is likely that some of androgens' effects are mediated indirectly; that is, through connections of these regions with other song nuclei. Powerful, yet indirect support for this idea is that Area X grows substantially in response to androgens, yet does not itself contain androgen-accumulating cells. This song regions does, however, receive a massive projection from HVc, as does the robustus nucleus of the archistriatum (RA), another androgen-responsive region with relatively few androgen-accumulating cells. This study determined whether HVc cells which project to Area X or RA also accumulate androgens. If so, this would be consistent with the hypothesis that the regulation of androgen accumulation in HVc in turn defines the androgenic sensitivity of other song nuclei.

Adult male zebra finches were castrated and injected with fluorescent latex microspheres directed towards Area X (n=3) or RA (n=2). Three to six days later, each bird was injected with 3H-DHT and killed 90 minutes later. Coronal brain sections were processed for autoradiography and HVc cells that accumulated 3H-DHT were identified using the Poisson criterion. Retrogradely-labeled HVc neurons were identified and the density of silver grains over these cells was determined by combining fluorescent and bright field illumination.

In birds in which injections of the retrograde tracer filled most of the intended target, a large number of HVc cells were backlabeled (Area X - 14.3 ± 3.1%; RA - 34.6 ± 6.7%). Many of these projection neurons were also androgen-accumulating. Among HVc neurons projecting to Area X, 38.1 ± 5.2% accumulated 3H-DHT or its metabolites. Similarly, 57.4 ± 3.1% of the HVc neurons projecting to RA accumulated androgens. The overall incidence of androgen-accumulating cells in HVc was 42.6 ± 4.0%. These data imply that androgenic effects on HVc neurons are communicated to Area X and RA, and argues that the masculinization of androgen accumulation in HVc may in turn dictate how other song regions respond to androgenic stimulation. (Supported by USPHS grants HD22160 and the Sloan Foundation).

192.7 NEURONS OF HYPERSTRIATUM VENTRALIS PARS CAUDALIS BORN DURING SONG LEARNING DO NOT PROJECT TO AREA X. E.J. NORDEEN AND K.W. NORDEEN*. Dept. of Psychology, U. of Rochester, Rochester, N.Y. 14627

As juvenile male zebra finches learn song, newly-born neurons insert into the song control nuclei hyperstriatum ventralis pars caudalis (HVc) and Area X. Young females (who do not develop song) produce or incorporate fewer of these neurons, contributing to the development of a marked sex difference in neuron number within HVc and Area X. We are interested in characterizing these sexually dimorphic populations of late-generated neurons to understand better their role in song learning and sexual differentiation. This study examined whether, in males, HVc neurons born during adolescence project to Area X, a principal target of HVc. If so, then sex differences in the production or insertion of these neurons could in turn produce sex differences in the HVc-Area X projection and in the anatomy of Area X.

Beginning at 20 days of age, male zebra finches were injected with ^{3}H-thymidine (2.5 uCi/gm) every 24 hours for 20 days. At 57 days of age, birds were anesthetized and injected bilaterally with .7 ul fluorogold (Flg) directed towards Area X. They were perfused with saline-formalin 7-8 days later, and their brains were removed and embedded in paraffin. Coronal sections (10um) were processed for autoradiography. In 3 birds the Flg injections filled most of Area X bilaterally. Sections through HVc of these birds were first examined under UV illumination to determine the density of Flg-labeled neurons. Then, the silver grain density was determined over each Flg-labeled neuron by combining UV and bright field illumination. Finally, these sections were stained with thionin and the overall neuronal density and incidence of thymidine-labeled neurons were determined. A neuron was considered labeled by thymidine if the density of silver grains over its' soma exceeded 5X background grain density.

A substantial and reliable proportion of HVc neurons were retrogradely labeled by Flg injections into Area X (15.7 ± 2.2%). Of these neurons projecting to Area X only 6.5 ± 1.8% were also labeled by ^{3}H-thymidine. In contrast, thymidine injections between 20 and 40 days of age labeled 18.7 ± 2.1% of HVc neurons overall. Thus, only about 5% of the neurons born and incorporated into male HVc during adolescence project to Area X. The majority of neurons added to HVc during song learning are likely, therefore, to either be interneurons or contribute to the projection of HVc to the robust nucleus of the archistriatum. This latter possibility is presently under investigation.
(Supported by USPHS grants HD21372, HD22160 and the Sloan Foundation).

192.8 DEVELOPMENT OF SEXUALLY DIMORPHIC PHYSIOLOGY IN *XENOPUS LAEVIS* LARYNX; EFFECTS OF ANDROGEN DEPRIVATION. M.L. Tobias, D.A. Sassoon and D.B. Kelley, Dept. Biol. Sci., Columbia Univ., New York, N.Y. 10027; Pasteur Institute, Paris, France

Adult frogs (*Xenopus laevis*) perform sex-typical vocalizations to express reproductive state. The male-specific mate call is more complex in temporal pattern, more rapid (66 vs 6 Hz), and more robust in amplitude and duration than the female-typical call ticking. Male vocal behavior is androgen sensitive; castrated males do not mate call, but calling is reinstated following exogenously administered androgen. Females do not mate call. Two physiological properties of the vocal effector organ, the larynx, have been shown to contribute to the male's unique vocal ability (Tobias & Kelley, J. Neurosci, in press). Male laryngeal muscle, in response to nerve stimulation, generates twitch contractions at rates even greater than those required to produce the mate call; female laryngeal muscle produces only maintained tension at these stimulus rates. Male laryngeal muscle exhibits marked compound action potential (cAP) potentiation in response to laryngeal nerve stimulation at mate call rates; female laryngeal muscle shows little or no potentiation at these rates. At metamorphosis, androgen levels are low and monomorphic. Adult levels are reached by six months p-m in males, but remain low in females (Lambdin & Kelley, Neurosci., 12:332.2, 1986). In this study we examined the electrophysiological properties of male and female larynges during post-metamorphic development. Our aim was to determine when sexually dimorphic properties of laryngeal physiology first appear and whether these properties are controlled by androgen secretion.

Larynges from male and female frogs of different ages were isolated and muscle activity in response to laryngeal nerve stimulation examined. At six months post-metamorphosis (p-m), both contraction rate and cAP potentiation are monomorphic and feminine. At ten months p-m, males begin to demonstrate rapid muscle contractions, but still do not exhibit cAP potentiation. These results suggest that masculinization of these properties occurs relatively late in development, at some time after six months p-m. We next determined whether androgen exposure after six months p-m is necessary for development of male physiological characteristics. Males were either gonadectomized or sham operated at six months p-m and tested at 12 months p-m. Masculinization was completely arrested by gonadectomy. Tension and electromyogram records from gonadectomized animals appeared identical to those of unoperated siblings tested at six months p-m. Sham operated animals were significantly more masculinized than operates ($p<.01$, Wilcoxon Rank Sign Test); the records were indistinguishable from control adult males. Laryngeal weights of sham operated and gonadectomized animals were also significantly different (sham = 0.41 gms, gonadectomized = 0.16 gms). Laryngeal weights of sham operates are equivalent to those of adult males while laryngeal weights of gonadectomized animals are equivalent to those of adult females. Unlike masculinization of fiber number, which increases throughout development in response to gradually increasing androgen titers, (Sassoon & Kelley, Am. J. Anat., 177:457, 1986) masculinization of tension and cAP characteristics occurs relatively late in development and diverges more rapidly. Supported by NS 23684

192.9 CELL BODIES OF ORIGIN OF A SEXUALLY DIMORPHIC ENKEPHALINERGIC FIBER POPULATION IN THE PREOPTIC AREA OF THE RAT. R.E. Watson, Jr., S.J. Wiegand, M.D. Fitzsimmons, and G.E. Hoffman. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642

We have recently identified a sexually dimorphic met-enkephalin (m-ENK) immunoreactive fiber pathway in the periventricular preoptic area (pePOA) of the rat. Expression of the system, normally present only in the female, can be altered powerfully by both an activational and organizational action of the gonadal steroids. In an effort to further characterize this system, we have undertaken experiments aimed at identifying the enkephalinergic cell bodies of origin of this dimorphic system, and of others projecting to adjacent regions in the sexually dimorphic preoptic area.

Pressure injections of the retrograde tracer fluorogold (2% w/v in 0.9% saline) ranging in volume from 3-50 nl were made into the proximity of the pePOA of the female. Two days later, the animals were administered colchicine intracerebroventricularly (100 µg/10 µl) and allowed to survive an additional two days prior to perfusion with saline followed by either Zamboni's fixative or 4% buffered paraformaldehyde. Sections were cut on a freezing microtome at 30 µm thickness, and alternate sections were mounted with no further treatment or were reacted for the immunofluorescent detection of m-ENK. For the latter, following incubation in rabbit anti-m-ENK (ImmunoNuclear) and biotinylated secondary antibody (Vector Labs), immunofluorescence was achieved with reaction with rhodamine- or Texas Red-labelled streptavidin (Amersham).

While many retrogradely filled fluorogold-positive cells and enkephalin immunopositive cells can be identified within many of the same brain regions in the hypothalamus and brainstem, there have been surprisingly few double-labelled cells observed. In many areas, numerous retrogradely labelled cells appear to be in immediate proximity to enkephalin immunoreactive cells. Also surprising are the diverse sites at which double-labelled cells have been detected. These sites include the hypothalamic arcuate nucleus, posterior pole of the ventromedial hypothalamic nucleus, perifornical area, dorsomedial hypothalamic nucleus, lateral hypothalamus, nucleus tractus solitarius, and the lateral parabrachial nucleus.

Presently, these results suggest at least two possibilities regarding the origin of the sexually dimorphic enkephalin system. First, as reflected by the tract-tracing data, widely dispersed cells within the hypothalamus and brainstem may contribute to this dimorphic pathway by converging in the female pePOA. Alternatively, the pathway may be comprised of local circuit fibers originating from enkephalin immunoreactive cells in close proximity to the pePOA, and located within the active zone of uptake of the retrograde tracer. Further experimentation utilizing other techniques, including anterograde tracing methods, will be necessary to conclusively ascertain the location of the enkephalin synthesizing cells which provide the major afferent input to this system. Elucidation of the neural circuitry of this system may help in identifying its potential role in the mediation of a sexually differentiated function.

{Supported by NS-23591}

192.10 ESTROUS CYCLE VARIATIONS IN LEVELS OF CCK IMMUNOREACTIVITY WITHIN CELLS OF THREE INTERCONNECTED SEXUALLY DIMORPHIC FOREBRRAIN NUCLEI. A.E. Oro*, R.B. Simerly and L.W. Swanson. (SPON: D. Simmons). Howard Hughes Medical Inst. and Salk Inst., La Jolla, CA 92037.

The central part of the medial preoptic nucleus (MPNc), the encapsulated part of the bed nucleus of the stria terminalis (BSTe), and the posterodorsal part of the medial nucleus of the amygdala (MeAp) are all thought to be involved in the neural control of female reproductive behavior, as well as other neuroendocrine mechanisms. Although the dependence of the development of these sexual dimorphisms on levels of gonadal steroids early in develpment is well known, the importance of possible activational effects of circulating gonadal steroids in the adult is less clear. Recently, we demonstrated the dependence of CCK levels within these cell groups on circulating gonadal steroids in adult male rats (Simerly and Swanson, PNAS 1987). In the present study we evaluated the number of CCK-immunoreactive cells present within the MPNc, BSTe, and MeAp of regularly cycling female rats over the estrous cycle. Female Sprague-Dawley rats that showed at least two normal 4 day cycles received colchicine injections into the lateral ventricle and were sacrificed 3 days later. The animals were grouped according to the appearance of their vaginal smears on the day of sacrifice and the brains were processed for immunohistochemistry according to procedures detailed elsewhere (Simerly et al., JCN, '86). In addition, the effects of ovariectomy and estrogen replacement on CCK staining in regularly cycling female rats were also examined. The number of CCK-immunoreactive cells that could be detected within each cell group was found to vary over the estrous cycle from a minimum during diestrus, increase 2-4 fold during proestrus, and decline to intermediate numbers during estrus. These changes appear to be due at least in part to changes in levels of circulating estrogen because subcutaneous implants of estradiol prevented the decline in the number of CCK-stained cells within the MPNc,BSTe, and MeAp that was seen in untreated, ovariectomized female rats. Thus, the present findings support the hypothesis that levels of CCK within cells of these three sexually dimorphic cell groups are regulated by circulating gonadal steroids within a physiologically relevant time frame, and may possibly contribute to the activation of female reproductive behavior as well as other neuroendocrine functions.

192.11 CORPUS CALLOSUM: MASCULINIZED VIA PERINATAL TESTOSTERONE. R. H. Fitch*, A. S. Berrebi, and V. H. Denenberg. Biobehavioral Sciences Graduate Degree Program. Univ. of Conn., Storrs, CT 06268.

Previous research on sexual differentiation of the rat brain has focused on measures of adult sexual behavior, hypothalamic cyclicity, the volume of the SDA-POA, and the alteration of these factors through early hormonal manipulation. The differentiation of all factors has been shown to be testosterone mediated (although cellular factors may depend on the estrogen metabolite of testosterone). Recently there has been evidence of a sexual dimorphism at the cortical level. Berrebi et al. (Soc. Neurosci, Abst., 1986, 12:1536)reported that the corpus callosum of the male rat is larger than that of the female. This study was designed to investigate the potential role of testosterone in mediating this dimorphism.

The day after birth, 2 randomly selected males from each of 7 litters were castrated, while 2 others received sham surgery. On Day 4, 2 randomly selected females from these same letters received 1 mg testosterone propionate, while the remaining 2 females received an injection of vehicle only. All litters were handled daily until Day 20.

At 110 days the animals were perfused, brains fixed, and cut sagitally. Morphometric analysis of midline sections yielded 7 width measurements taken at specified percentages of total callosal length: the 8th, 16th, 33rd, 50th, 67th, 84th, and 96th percentiles. In addition, these measures were adjusted for brain weight differences.

Male controls had significantly wider callosa than female controls at the 8th and 16th percentiles (anterior), and near significance ($p<.055$) for the 96th percentile (posterior). When the 84th and 96th percentile widths were combined, the sex difference in the posterior region was significant. These findings confirm our earlier report (Berrebi et al., 1986). Females receiving testosterone in infancy had significantly wider callosal values than control females at the 8th, 84th, and 96th percentile widths. These values were close to, or slightly larger, than sham males. Castration did not affect the widths of the males. These results were not changed after adjusting for brain size.

These data suggest that sexual differentiation of the callosum is testosterone mediated. The failure to obtain an effect from castration is taken to mean that endogenous testosterone had masculinized the callosum during the prenatal period. The sensitivity of the callosum to testosterone is still present at least 4 days postnatally, since the callosa of females receiving TP resembled those of males. Since callosal width measures presumably represent fibers of passage from homotopic cortical regions, these data imply that testosterone affects cortical areas.

192.12 SEXUAL BEHAVIOR OF TESTICULAR FEMINIZED (*Tfm*) MICE: EFFECT OF NEONATAL HORMONE TREATMENTS. K. L. Olsen & K. M. Hock*. Dept. of Psychiatry & Behavioral Science, SUNY-Stony Brook, NY. 11794.

Testicular feminized (*tfm*) rats and mice provide a means to examine the contribution of androgens in the differentiation of neural systems underlying behavior. This X-linked mutation is characterized by defects in the androgen but not estrogen receptor system. As a result, X/Y males are insensitive to androgens secreted by their testes and develop a female phenotype. *Tfm* rats and mice differ in their degree of insensitivity since mutant rats show partial response to androgens. Adult castrated *tfm* rats mount when given testosterone propionate (TP), estradiol benzoate (EB) or EB + dihydrotestosterone (DHT) while *Tfm* mice display little or no male sexual behavior following these hormonal treatments. It is not clear whether this demasculinization results from the inherited androgen insensitivity or from insufficient exposure to testicular hormones during development. Therefore, *Tfm* mice and siblings were given exogenous hormones neonatally and tested for their ability to display adult masculine mating responses.

Mice were bred by mating *Ta+/Y* males and *Tfm/+Ta* females resulting in four genotypes. The neonatal treatments were: (a) TP (500 µg) at birth (D1); (b) TP (500 µg), D1+D2; (c) DHT (250 µg), D1+D2; (d) EB (25 or 250 µg), D1. In adulthood, mice were given eight weekly 1 hr tests for male sexual behavior.

Masculine behavior of *Tfm* mice was not enhanced by neonatal exposure to TP. Like untreated *Tfm* mice, 20% of the androgen-ized mutants displayed sexual behavior but only on three of 80 tests. In contrast, wildtype males exhibited mating behavior and 80% of the tests were positive. Carrier females exhibited variable responsiveness ranging from no effect to showing the ejaculatory pattern. While a single injection of TP had an enhancing effect, TP given on D1 + D2 and EB tended to disrupt the development of male sexual behavior in some genotypes.

These results indicate that *Tfm* mice show deficient levels of male sexual behavior even following excess androgen exposure during development. Similar treatments enhanced masculine behavior of their female siblings suggesting that androgens are involved in behavioral masculinization in mice. We are continuing to examine the differentiating action of other doses of TP, EB and DHT since identifying the hormones that mediate behavioral masculinization and defeminization is necessary for understanding the underlying neural mechanisms. The *Tfm* mutation can serve as a model to identify these hormone(s).

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192.13 EVIDENCE FOR LONG LASTING ALTERATIONS IN THE NEUROENDOCRINE REGULATION OF PROLACTIN SECRETION RESULTING FROM NEONATAL PROLACTIN DEFICIENCY IN RATS. G.V. Shah*, S.W. Shyr*, B.L. Carroll* C.E. Grosvenor, and W.R. Crowley. Depts. of Physiology and Biophysics and Pharmacology, Univ. of Tennessee, Memphis, Memphis, TN 38163.

Previous findings of this laboratory (Endocrinology 119, 1217-1221, 1986) suggest a role for milk-borne prolactin (PRL) in development of neuroendocrine controls over PRL secretion. Thus, offspring of mothers treated from days 2-5 postpartum with the dopamine (DA) agonist bromocriptine, a treatment that lowers the concentrations of PRL in milk, show reduced concentrations and turnover of DA in the median eminence and elevated serum levels of PRL at 30-35 days of age. The present experiments were undertaken to investigate whether neonatal PRL deficiency results in 1) a hyperprolactinemia and reduced median eminence DA turnover that persists beyond the onset of puberty and 2) alterations in pituitary responsiveness to hypophyseotropic factors. Lactating female rats received sc injections of either saline vehicle or bromocriptine (125µg/rat/d) on each of days 2-5 postpartum. Blood samples were obtained from female offspring at various postnatal ages and PRL concentrations were determined by RIA. Serum PRL concentrations in both groups of offspring were low until after weaning, but the offspring of bromocriptine-treated mothers showed significant elevations in serum PRL between days 30 and 90 postpartum. The turnover of DA in the median eminence, estimated from the rate of decline after synthesis inhibition, was significantly reduced in the offspring of bromocriptine-treated mothers at 35 days of age, but was not different from controls at 60 days of age. Ovariectomy on day 30 prevented the elevation of serum PRL on days 45 and 60 in bromocriptine-offspring, and the concentrations of estradiol in serum were elevated in these animals compared to controls. Anterior pituitary cells from 100 day old offspring of control- or bromocriptine-treated rats were dispersed, cultured for 3 days, and PRL release during a 4 h incubation was assessed. Pituitary cells from offspring of bromocriptine-treated mothers were less responsive to the PRL release-inhibiting action of bromocriptine in vitro. The content of PRL mRNA in cytoplasm of cultured anterior pituitary cells from the two groups of offspring was measured by dot-blot hybridization. Basal levels of PRL mRNA did not differ in the two groups. Stimulation of DA receptors with bromocriptine significantly reduced PRL mRNA in the control cells but failed to do so in the cells of bromocriptine-offspring. These results suggest that a deficiency in exposure to milk-borne PRL during a neonatal critical period leads to a long lasting hyperprolactinemia that is the result of reduced release of DA from the hypothalamus, decreased pituitary response to DA and increased secretion of estradiol.

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192.14 ACTIVATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) RELEASE ADVANCES THE ONSET OF FEMALE PUBERTY. H.F.Urbanski and S.R.Ojeda. Neuroscience Division, Oregon Regional Primate Research Center, Beaverton, Oregon 97006.

The juvenile-peripubertal transition period in the female rat is associated with an ovarian-independent afternoon increase in the amplitude of plasma LH pulses. Firstly, to determine if the immature pituitary gland could be activated to cause precocious puberty juvenile female rats were stimulated with a microprocessor driven iv pulsatile pattern of exogenous LHRH. The animals received a 1-min pulse of 50 ng LHRH/kg BW every 30 min during the afternoons (1300 - 1701 h) from days 26 through 29, an LHRH regimen that produced a peripupertal pattern of LH release. Secondly, to determine if the LHRH neurons themselves could be prematurely activated to induce such a peripubertal pattern of plasma LH, and hence lead to precocious puberty, the neuroexcitatory amino acid analog N-methyl-DL-aspartic acid (NMA) was similarly administered (20 mg NMA/kg BW per 1-min pulse); NMA does not act at the pituitary gland to alter LH release. In vehicle treated controls (n=5) vaginal opening occurred at 38.4 ±0.4 days of age and was advanced by 5 and 7 days respectively in the LHRH (n=7) and NMA (n=7) treated groups. On average all animals ovulated within 2 days of vaginal opening; this was confirmed by the detection of corpora lutea within the ovaries at first diestrus. Although ovarian weight at diestrus was similar in all of the animals regardless of treatment, a juvenile body weight was retained by the animals that underwent precocious puberty.

These results indicate: a) that the juvenile adenohypophysis can be driven by the episodic administration of exogenous LHRH to initiate puberty, and more importantly, b) that the juvenile LHRH neuronal system can be precociously activated by the episodic administration of an excitatory amino acid analog known to interact with specific brain receptors. It is quite likely, therefore, that the initiation of puberty in the female rat depends on the development of neuronal inputs to the LHRH secreting neurons, rather than maturation of the LHRH releasing system itself.

(Supported by NIH Grant HD-09988, project IV)

192.15 **SEX DIFFERENCES IN SPATIAL MEMORY OF RATS: EARLY ORGANIZATIONAL EFFECTS OF GONADAL STEROIDS ON BRAIN LATERALIZATION.** C. L. Williams and W. H. Meck. Depts. of Psychology, Barnard and Columbia Colleges, Columbia University, New York, New York 10027.

We have recently demonstrated that in rats, superior performance on a spatial memory task may be due to exposure to high levels of gonadal hormones during the first postnatal week of life. All subjects were gonadectomized at 45 days of age and began behavioral testing at 75 days of age. Females treated with 10 ug estradiol benzoate on postnatal days 1, 3, 5, 7, & 9 (n = 9) and control males (n = 11) showed faster acquisition of a 12-arm radial maze task (with 8 baited and 4 unbaited arms) compared to males castrated within 24 hr of birth (n = 8) and control females (n = 12).

Magnitudes of left and right turning biases were used as an index of brain lateralization. During the acquisition phase, lateralization was highly correlated with choice accuracy across all treatment groups ($r = .39$, $p < .01$), while direction of turning was not reliably correlated with performance ($r = .15$, ns). Further analysis revealed that brain lateralization differed reliably as a function of early steroid exposure (brain sex) but not genetic sex, with early steroid exposure leading to increased lateralization and a greater degree of reliance on geometry cues as opposed to landmark cues. After the rats had reached asymptotic performance a test of cue usage was conducted by systematically altering either geometry or landmarks. Lateralization was highly correlated with the use of geometry cues as opposed to landmark cues as a function of brain sex ($r = .42$, $p < .01$), while choice accuracy and use of geometry during acquisition were also highly correlated ($r = .64$, $p < 001$).

These data suggest: 1) The more lateralized the brain of a rat, the better its performance on a spatial memory task due to selective use of geometry cues, and 2) Exposure to gonadal steroids during the first week of life leads to increased lateralization. Moreover, the observed correlations between brain lateralization and choice accuracy across all treatment groups strongly suggests that lateralization is the optimal brain organization for spatial memory in both male and female rats.

(Supported by NINCDS grant NS 20671 to CLW.)

192.16 PRE- AND POST-NATALLY ADMINISTERED ACTH, ORGANON 2766 AND CRF FACILITATE OR INHIBIT ACTIVE AVOIDANCE TASK PERFORMANCE IN YOUNG ADULT MICE. L.C. Honour*§ and M.H. White*¶ (SPON: J. Matochik). §Department of Psychology and the Neurobiochemistry Group, Mental Retardation Research Center, Neuropsychiatric Institute, Univ. of California, Los Angeles, CA. 90024 and ¶Department of Psychology, California State University, Fullerton, CA. 92634.

The purpose of this study was to investigate the effect of learning/memory-related neuropeptides and their ontogenetic effect on neural processes associated with behavioral task performance in later life. A 1 mg./kg. dosage of adrenocorticotropic hormone (ACTH) 4-9, Organon 2766; ACTH 4-10; ACTH 1-24; corticotropic hormone releasing factor (CRF); or diluent (0.9% physiological saline) was subcutaneously injected into either pregnant females or into newborn pups during specific neural developmental windows. Each of the progeny was later trained in an active-avoidance task and tested for acquisition on postpartum days 35-37. The mice were then tested for memory task performance and reacquisition on days 42-44 postpartum using the identical experimental paradigm as that used in the training sessions.

Pre-natal treatment with these learning/memory-related neuropeptides resulted in significant facilitation of learning/memory-related task performance in male and female mice treated with Organon 2766 ($p< 0.001$), and a significant inhibition of learning/memory-related task performance in males and females treated with ACTH 1-24 ($p< 0.01$). Additional sex-specific performance facilitations and inhibitions resulted from the pre- or post-natal administration of the various neuropeptides used in this study.

These results suggest that neuropeptides, when available in increased amounts during specific neural developmental windows, can significantly improve or suppress related behavioral performance capability in later life.

193.1 PATERNAL ETHANOL ALTERS BEHAVIOR OF MOUSE PROGENY. G. Friedler*, D.R. Brown*, V. Wooten* and M-E. Meadows* (SPON. M. Oscar-Berman). Boston University School of Medicine, Boston, MA 02118.

This study evaluated the effect of limited paternal exposure to ethanol (ET) on the motor activity of his offspring. Two groups of sexually mature male mice (CD-1, Chas. River) received either ET (3.5 g/kg, 17.5% w/v) or isocaloric sucrose (CON) by gastric gavage twice daily (8½ dys). CON were pairfed to ET males who received ground mouse chow and water ad libitum. Following the final intubation day, food and water were freely available for 7 additional days prior to pairing of males (10/grp) with untreated females for a single estrous cycle. Births were recorded within 12 hrs and litters reduced to 8 (4M, 4F). A blind procedure was used for all behavioral testing and data analysis.

Pivoting locomotion was assessed at 10 days in individual offspring from 6-8 litters/paternal treatment grp. Using a 30 sec period of observation, a significant decrease in both time spent pivoting (13.6±1.6 sec,ET; 19.3 ± 1.4,CON) and number of pivots(90°) (8.8 ± 1.2,ET; 13.5 ± 1.2,CON) occurred in ET grps ($P<.05$). At 12 weeks, individual males (6/grp) representing 6 separate litters/paternal treatment grp were tested for patterns of motor behavior in a plexiglass chamber (15"x15"x9")with midline divider. Pairs of ET and CON progeny were tested simultaneously between 6-8 P.M. in an isolated room under red light. Behavior was recorded (10 min) by videotape for subsequent analysis of quantitative changes in activity patterns and interactions between individual behaviors as detailed elsewhere (Norton, S. Handbook of Psychopharm.7:83, 1977; Brown, D.R. et al, Toxicol. Industr. Health 1:81, 1985). No changes in overall activity or repertoire of behaviors were observed between ET and CON progeny. The distribution of behaviors between grooming, exploratory and attention modes also did not differ for the two grps. Interactions between individual behaviors, reflective of the activity profile during exploration of the test chamber, differed markedly for the two grps. ET mice showed a significant ($P<.05$) increase in number of interactions (70%) when compared with CON (44%). However, the pattern of interactions was more restrictive in individual ET offspring than in CON. An increase ($P<.05$) in 180° turns was also evident in ET mice.

The results indicate that paternal exposure to ethanol, in the absence of maternal exposure, can alter the behavior of both young and adult offspring.

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193.2 EFFECTS OF PRENATAL EXPOSURE TO ETHANOL ON THE GLUCOSE UTILIZATION OF MOTOR AND SOMATOSENSORY CORTICES IN THE RAT. M.W. Miller and D.L. Dow-Edwards. Dept. of Anatomy, Sch. of Osteopathic Med. and R.W. Johnson Med. Sch., UMDNJ, Piscataway, NJ 08854 and Dept. of Neurosurgery, Health Sci. Ctr., SUNY, Brooklyn, NY 11203.

Gestational exposure to ethanol causes multiple behavioral abnormalities such as hyperactivity, learning deficits, and difficulty in response inhibition. Such abnormalities indicate that the function of cerebral cortex is altered. In the present study, 2-deoxyglucose autoradiography was used to determine whether ethanol exposure alters cortical metabolic requirements. Pregnant hooded rats were fed ad libidum a diet consisting of 35% (v/v) ethanol in vitamin fortified Sustacal. Others were pairfed an isocaloric diet. Rats were fed these special diets from gestational day 7-21. Postnatally, mothers and weanling pups were fed chow and water. Plasma samples and frozen coronal sections from 105 day old pups were processed and analyzed by the quantitative autoradiographic technique of Sokoloff et al. (J. Neurochem. 28:897). Rates of glucose utilization were determined in five cortical areas, primary somatosensory area 3, secondary somatosensory area 2, primary motor area 4, and rostral and caudal secondary motor area 6/8. The identity of the cytoarchitectonic areas and the thicknesses of the cortical layers were determined on alternate thionin-stained sections. Despite finding no differences in the thicknesses of the corresponding laminae in each segment of somato-motor cortex of control and ethanol-treated rats, there were striking group differences in the rates of cerebral glucose utilization. The overall glucose utilization of controls was similar in all five regions ranging from 82-88 mol/100 g/min. Specific laminae had different rates of glucose utilization, layer IV had the greatest and layers I and VI the lowest. In ethanol-treated rats, overall glucose utilization in areas 3, 2, 4, and rostral 6/8 was statistically significantly less (-23-24%; t test, $p<0.01$) than in corresponding sites in controls. In each of these four regions, layers IV and V were affected most severely (-21 to -29%; $p<0.01$) and layer I the least (-14 to -19%). Caudal area 6/8 differed from the other somatosensory and motor cortices in that overall, glucose utilization was not significantly different ($p>0.02$) in both groups and that layer I was the most affected stratum. These data corroborate anatomical data which show structural abnormalities in deep cortex and suggest that ethanol exposure preferentially affects a major afferents system (thalamic input to layer V) and two major efferent systems (callosal and subcortical projections from layer V).

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193.3 PRENATAL ALCOHOL EXPOSURE ALTERS SEIZURE AND THERMOREGULATORY RESPONSE TO HEAT STRESS IN NEONATAL RATS. B. Zimmerberg, C.D. Khazam*, and E.P. Riley*. Center for Behavioral Teratology, Dept. of Psychology, SUNY-Albany, Albany, N.Y. 12222.

Prenatal alcohol exposure delays the ontogeny of thermoregulation in neonatal rats exposed to cold stress. In this experiment, the effects of prenatal alcohol exposure in neonatal rats exposed to heat stress was investigated using a hyperthermia-induced seizure paradigm. Two-day-old male and female rat pups were chosen from independent litters with one of three prenatal treatment histories: 35% ethanol-derived calories (35% EDC), pair-fed control (0% EDC) or lab chow control (LC). Subjects (n=6 per sex per group) were continuously monitored for rectal temperature while in a chamber which was heated to 47°C. Subjects were observed for loss of righting reflex with onset of tonic-clonic convulsions (hyperthermia-induced seizure). There was a significant effect of prenatal treatment on body weight, the latency to the first seizure, the number of total seizures, and the duration of seizure activity (p's <0.01). The 35% EDC pups seized sooner than control groups (p's<0.05), which did not differ from each other. Alcohol-exposed pups also had more seizures and a greater total duration of seizure activity than pups from both control groups (p's<0.01), which did not differ from each other. However, rectal temperatures at the first seizure and at the peak of heating did not differ by prenatal treatment. This suggests that while the body temperature seizure threshold is not altered by prenatal alcohol exposure, the rate of temperature rise to reach this threshold is markedly increased for alcohol-exposed pups at this age. In addition, 35% EDC pups' body temperatures remained above this threshold for a longer period of time after the first seizure compared to control pups (p's<0.05), which did not differ. This suggests that body weight differences are not solely responsible for these effects, since the smaller 35% EDC pups, with their larger surface/weight ratio, should lose heat faster. It appears, therefore, that prenatal alcohol exposure may cause some deficit in thermoregulation. This altered response to heat stress may have implications for deficits in neuronal function, brain growth and/or behavior in fetal alcohol exposure. (Supported by grants from NIAAA #AA07009 to BZ and #AA03249 to EPR)

193.4 BRAIN/BODY WEIGHT REDUCTION AFTER ACUTE PRENATAL ALCOHOL EXPOSURE IN C57 MICE: EFFECTS OF ASPIRIN PRETREATMENT. C.L. Randall, R.F. Anton, H.C. Becker, and C.K. Williams*. VA Medical Center and Medical University of SC, Charleston, SC 29403.

Acute alcohol administration on a single day of pregnancy has been shown to result in anomalous morphologic development in mice (Sulik et al., 1981; Webster et al., 1983) and suppressed cell division in rat embryos (Pennington et al., 1984). In chicks, alcohol-induced growth retardation was antagonized by the prostaglandin synthetase inhibitors aspirin (ASA) and indomethacin (Pennington et al., 1985). Since previous work from our laboratory (Randall and Anton, 1984) showed a significant reduction in alcohol-induced birth defects in C57BL/6J mice pretreated with ASA, the purpose of this study was to determine the effect of ASA pretreatment on alcohol-induced body and brain weight deficits in mice. C57BL/6J mice were time bred (plug day= gestation day 1). On day 15 of gestation, mice were injected s.c. with 150 mg/kg ASA or phosphate buffer. One hour later, 5.8 g/kg alcohol (or isocaloric sucrose) was administered by gavage. On day 19 of gestation, fetuses were removed from the uterus. Body and brain weights were recorded. The results confirmed a marked reduction in brain ($F= 15.09$, $df= 1/36$, $p< .01$) and body ($F= 16.20$, $df= 1/36$, $p< .01$) weight in fetuses prenatally exposed to alcohol. The brain/body ratio was significantly greater in alcohol-exposed animals, as well, confirming a brain "sparing" effect ($F= 14.84$, $df= 1/36$, $p< .01$). The main effect of aspirin and the alcohol-aspirin interaction terms were not statistically significant. These data suggest that aspirin pretreatment does not antagonize alcohol-induced growth deficits incurred late in gestation in C57BL/6J mice.

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193.5 A FURTHER EXAMINATION OF THE EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA OF THE HYPOTHALAMUS IN MALE AND FEMALE RATS. S. Barron[1], S.B. Tieman[2], and E.P. Riley[1] (SPON: R. Oesterreich). [1]Center for Behavioral Teratology, [2]Neurobiology Research Center, S.U.N.Y., Albany, NY 12222.

In rodents, prenatal alcohol exposure alters a number of sexually dimorphic reproductive and non-reproductive behaviors that are influenced by perinatal sex steroids. It has been hypothesized that alcohol-induced hormonal alterations might be one mechanism by which prenatal alcohol exposure affects these behaviors. Recently, we examined the effects of in utero alcohol exposure on the sexually dimorphic nucleus of the preoptic area of the hypothalamus (SDN-POA) which is also sensitive to perinatal sex steroid levels. This nucleus is typically larger in males than females. Following prenatal alcohol exposure, the volume of the SDN-POA in male rats, but not female rats, was significantly reduced. To examine the relative specificity of this effect, we have now examined a second nucleus, the nucleus of the anterior commissure (NAC) which is found within the same sections as the SDN-POA but which does not appear to be sexually dimorphic in size. To further examine alcohol-related alterations in the SDN-POA, cell sizes were examined. Subjects were adult male and female rodents who were exposed prenatally to either a 35% ethanol-derived calorie liquid diet or a 0% isocaloric pair-fed control diet on gestation days 6-20. An ad libitum lab chow group was also included. There were no group differences in NAC volume suggesting that the effects previously reported are relatively specific to a sexually dimorphic nucleus. Average SDN-POA cell size was markedly smaller in alcohol-exposed offspring relative to controls in males (p's <0.05) but not in females. This decreased cell size in alcohol-exposed males may be one mechanism by which alcohol alters SDN-POA volume. These findings provide neuroanatomical support for the hypothesis that prenatal alcohol exposure alters sexual differentiation in males and that females may be less sensitive to alcohol's effects on the SDN-POA. (This work was supported, in part, by grants from NIAAA to E.P.R.)

193.6 PRENATAL EXPOSURE TO ETHANOL. EFFECTS ON SYNAPTOSOMAL UPTAKE OF SEROTONIN IN THE CORTEX: Mary J. Druse-Manteuffel and Leo H. Paul* (SPON: J. McNulty) Department of Biochemistry, Loyola University Stritch School of Medicine, Maywood, IL 60153.

Previous studies from this laboratory have shown that the developing offspring of ethanol-fed rats have a deficiency of cortical serotonin (5-HT) and 5-hydroxyindoleacetic acid. In addition, recent studies from this laboratory have found that there is a decreased B_{max} for the binding of [3H]-5-HT to the 5-HT_{1B} receptor (the presumed presynaptic autoreceptor). In an attempt to determine whether other presynaptic components of the serotonergic system are similarly affected by prenatal exposure to ethanol, we studied the uptake of [3H]-5-HT by cortical synaptosomes.

Female, Sprague-Dawley rats were pair-fed using control or 6.6% (v/v) ethanol liquid diets on a chronic basis prior to parturition. Synaptosomes were isolated from whole cortex, motor cortex, and somatosensory cortex from the 19- and 37-day-old offspring of control and ethanol-fed rats. The uptake of 0-1000 nM 5-HT by synaptosomes was determined in the presence and absence of 10 µM fluoxetine.

The results of these experiments demonstrated that the developing offspring of ethanol-fed rats had decreased uptake of serotonin by synaptosomes from the cerebral cortex and cortical regions. These results support our hypothesis that prenatal ethanol exposure has an adverse effect on the development of presynaptic components of the serotonergic system in the cortex.

This research was supported by a grant from the USPHS (AA 03490).

193.7 EFFECTS OF LIMITED POSTNATAL ETHANOL EXPOSURE ON THE RAT CEREBRAL CORTEX. D.E. Phillips and E.A. Harper*. Dept. of Biology, Montana State University, Bozeman, MT 59717.

Although it is known that ethanol exposure during gestation produces behavioral changes and learning and motor deficits in humans, studies of the specific cellular effects of ethanol in the developing sensory-motor cerebral cortex are limited. Most experimental studies have used exposures to rodents during stages of brain development that correspond to the first two trimesters in the human while few studies have examined the effects of exposures during developmental stages equivalent to the third trimester. In this study a limited postnatal ethanol exposure in rats was used to parallel a partial third trimester exposure in human brain development.

Animals were provided controlled exposures by using artificial rearing techniques similar to those of Samson and Diaz (Fetal Alcohol Syndrome, ed., E.L. Abel, 1982, pp 131-150) and West et al.(Alcohol, 1:213-222, 1984). Anesthetized four day old rat pups from normal dams were fitted with a gastrostomy tube and were then provided an artificial diet fed from syringe pumps. Experimental animals received a diet identical to control animals with the exception of 4% ethanol (v/v) added on postnatal days 5 through 9. On day 16 the animals were anesthetized, perfused with aldehydes, and one millimeter slices of the frontoparietal motor cortex at the level of the anterior commissure were prepared for plastic sectioning for light and electron microscopy. Sections (1-2 microns thick) were cut with glass knives, stained with toluidine blue and studied by light microscopy. Measurements of distances and areas were made on a computerized bit pad, either from photomicrographs or directly from the microscope with a drawing tube.

The overall thickness of the cerebral cortex was decreased in ethanol exposed animals to approximately 80% of control values. There were approximately 40% fewer dendrites (greater that 1.3 microns in width) per unit area in experimental animals at a depth corresponding to layer III. The area occupied by dendrites in the same unit area was also decreased a corresponding amount. The number of neurons per unit area at a standardized depth corresponding to layer V were increased in the experimental animals to 145% of control values, probably indicating a slower migration through this layer of later developing neurons. Thus limited postnatal ethanol exposure in the rat causes a delay in maturation of the 16 day frontoparietal motor cortex as evidenced by a probable delay in neuronal migration, a delay in the development of larger dendrites, and an overall decrease in the cortical thickness. (Supported by NIAAA 1 RO-1AA07042, NIH MBRS S0608218,

193.8 ULTRASTRUCTURAL STUDY OF LAYER V IN SOMATOSENSORY CORTEX OF MATURE RATS PRENATALLY EXPOSED TO ETHANOL. S. Al-Rabiai and M.W. Miller (SPON: H.J. Gould, III). Dept. of Anatomy, Sch. Osteopathic Med. and R.W. Johnson Med. Sch., U.M.D.N.J., Piscataway, NJ 08854.

Prenatal exposure to ethanol produces abnormalities in the structure of cortical neurons. For example, pyramidal neurons in layer V of ethanol-treated rats have shorter dendrites and dysmorphic spines. Moreover, cortical projection patterns are changed in ethanol-treated rats, e.g., the numbers of cortical neurons projecting to the spinal cord are increased. In this study, we examined the effect of prenatal exposure to ethanol on the ultrastructural organization of layer V. Two groups of rats were fed liquid protein-enriched diets (BioServ) from gestational day 5 to the day of birth. One group was fed a diet containing 37.5% (v/v) ethanol and another group was pair-fed an isocaloric control diet. Postnatally, all mothers and weanlings were fed chow and water. Thirty day old pups were perfused with an aldehyde fixative and somatosensory area 3 was processed by standard electron microscopic techniques. Point counts of the ultrastructural contents of pyramidal and local circuit neurons (e.g., rough endoplasmic reticulum, Golgi apparatus, free ribosomes, mitochondria, and nucleus) were made. For all of these features, no significant differences between the dietary groups were found. The composition of the neuropil also was examined. No significant differences in the percentage of space occupied by dendritic shafts, dendritic spines, axonal varicosities, axonal shafts, myelinated axons, and glia were observed. Comparable numbers of symmetrical and asymmetrical synapses were counted in samples from control and ethanol-treated rats. Analyses of 1.0 µm thick sections revealed that in ethanol-treated rats the numbers of neurons and the space occupied by them was greater, the space occupied by blood vessels was less, and the space taken by the neuropil was less than those in controls. The smaller volume of neuropil coupled with no change in the density of synapses means that the total number of synapses in layer V was less in ethanol-treated rats. This difference may contribute to documented physiological changes such as the lower rate of glucose utilization in layer V of somatosensory cortex of ethanol-treated rats. These changes may underlie clinical characteristics of fetal alcohol syndrome such as mental retardation.

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193.9 ASPIRIN AUGMENTS ALCOHOL IN RESTRICTING BRAIN GROWTH IN THE NEONATAL RAT. D.J. Bonthius and J.R. West. Department of Anatomy, University of Iowa, Iowa City, Iowa 52242.

Aspirin has been reported to protect the mouse fetus against the deleterious morphological effects of prenatal alcohol exposure (Randall and Anton, Alcoholism: Clin. Exp. Res., 8:513, 1984), but others have found that aspirin worsens the teratogenic effects of alcohol (Guy and Sucheston, Teratology, 34:249, 1986). The purpose of this study was to determine whether the microencephaly in rats resulting from early postnatal alcohol exposure is altered by a concurrent administration of aspirin. Neonatal rats were gastrostomized on gestational day (GD) 26 (postnatal day 4) and artificially reared from GD 26-32. This includes the period of the brain growth spurt in the rat and is similar to the third trimester of human brain development. The ethanol-treated groups received 6.6 g/kg/day of ethanol and either 0, 12.5, 25 or 50 mg/kg/day of aspirin in a milk solution. The alcohol was administered in 4 of the 12 daily feedings, and the aspirin was administered in all 12 feedings. Control groups received either 0 (gastrostomy control), 12.5, 25 or 50 mg/kg/day of aspirin in a milk solution to which maltose-dextrin had been added, making it isocaloric with the alcohol solutions. Each group consisted of 6 male and 6 female pups. On GD 32, the pups were perfused with a glutaraldehyde/paraformaldehyde fixative, and the brains were removed. Brainstem, cerebellum and total brain weights were measured. Alcohol alone significantly reduced the mean total brain weight, cerebellum and brainstem weight by 20.8%, 22.1% and 12.3%, respectively, relative to gastrostomy controls. A significant interaction [$F(3,80)=4.27$, $p<.01$] between ethanol and aspirin was observed for total brain weight. The mean total brain weight of the group receiving both alcohol and 50 mg/kg/day aspirin was significantly lower than all other experimental groups ($p<.01$), and was reduced 28.9%, relative to gastrostomy controls. The lower doses of aspirin did not significantly alter the effects of alcohol on total brain weight. No interaction occurred between alcohol and aspirin in the measures of cerebellum or brainstem. However, the highest dose of aspirin alone significantly reduced cerebellar weight relative to gastrostomy controls ($p<.05$), but had no significant effect on brainstem or total brain weight. No sex differences were seen in any measurements, nor were body weights significantly different among the groups. No differences in mean peak blood alcohol concentrations were observed among the alcohol-treated groups. In conclusion, 50 mg/kg/day aspirin restricts cerebellar growth and augments the effects of alcohol in restricting brain growth during the brain growth spurt in the neonatal rat. (Supported by NIAAA grant AA05523 to J.R.W. and NIH training grant GM07377 to D.J.B.).

193.10 THE EFFECTS OF RESTRAINT STRESS COMBINED WITH PRENATAL ETHANOL EXPOSURE ON BEHAVIOURAL DEVELOPMENT IN MICE. G.R. Ward*[1] and P.E. Wainwright[2]. [1]Dept. of Psychology, and [2]Dept. of Health Studies, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Both prenatal ethanol consumption and prenatal stress have been shown to produce behavioural alterations in rodent offspring (Abel, E. Psych. Bull., 90:564, 1981; Ward, I.L., Psychoneuroendocr., 9:3, 1984). Since ethanol consumption increases both the basal levels of corticosterone and the adrenocortical response to stress in pregnant dams (Weinberg, J., & Clendenning, S., Alcohol. Clin. Exp. Res., 8:126, 1984) it is possible that a combination of the two treatments will increase the adverse effects on the offspring. Therefore the present study used a 2 x 2 factorial design to investigate the effects of restraint stress combined with ethanol consumption during the last week of pregnancy on the behavioural development of mouse pups. On day 12 of pregnancy (conception = day 0), female B6D2F mice were fed either of two liquid diets: one in which 25% of the calories were in the form of ethanol, and one in which maltose/dextrin was substituted isocalorically for ethanol. In addition, half the mice in each dietary condition were assigned to a group receiving two daily restraint sessions while the other half remained in their cages. Both treatments were carried out from days 12 to 17 inclusive, and all groups were pair-fed on these days to the group receiving both ethanol and restraint stress in order to control for differences in nutritional intake.

None of the groups differed in terms of pup weight at day 22 or day 32, post-conception, but there were main effects for both ethanol and restraint stress on day 32 behavioural development. While prenatal ethanol exposure retarded development at this stage, restraint stress accelerated behavioural development in the offspring, regardless of the dam's diet or treatment.

In a second study, the animals were sacrificed at day 15 post-conception, the fetuses extracted, and maternal blood assayed for plasma corticosterone and blood alcohol levels. Preliminary analyses of these data support an effect of ethanol on fetal body weight but not of stress. A third study in progress controls for postnatal maternal factors by using a surrogate fostering technique and extends the results to include myelination of forebrain fibre tracts.

193.11 ONTOGENY OF THE BETA-ENDORPHIN RESPONSE TO STRESS: EFFECT OF PRENATAL EXPOSURE TO ETHANOL. P. Angelogianni* and C. Gianoulakis, Department of Psychiatry, McGill University, Douglas Hospital Research Centre, 6875 LaSalle Blvd, Verdun, Quebec, Canada H4H 1R3

Studies on the ontogeny of the hypothalamic-pituitary-adrenal axis in the neonatal rat have shown a reduced response of ACTH and corticosterone to stress in the first 2 weeks of life. Furthermore, prenatal exposure to ethanol was shown to alter the pituitary-adrenal response to stress in adult life. Since in the adult rat ACTH and B-Endorphin (B-EP) are co-released in response to stress, the objective of the present studies was to investigate the ontogeny of the B-EP response to stress and the effect of the prenatal exposure to ethanol. Pregnant rats were fed with an ethanol diet from day 1 of pregnancy till parturition. Control rats were either pair-fed with an isocaloric sucrose diet, or were fed ad lib with lab chow. The response of B-EP to cold and ether stress was investigated in the offspring on days 1, 3, 8, 10, 14 and 22 of life, by estimating the content of B-Endorphin-Like Immunoreactivity (B-EPLIR) in the plasma, the pituitary gland and the hypothalamus at 5 minutes after the onsent of the stress period. All the offspring exhibited either no response or a small response to stress on days 1, 3, 8 and 10 postnatally. A significantly enhanced response of B-EP to stress was noticed on days 14 and 22. On day 14 the release of B-EP in response to cold stress was more pronounced than in response to ether stress. However, on day 22 the responses of B-EP to both cold and ether stress were similar. Ethanol treatment delayed the maturation of the neural pathways mediating the B-EP response to stress as was indicated by the smaller increase of plasma B-EPLIR content on day 14 postnatally, in the offspring exposed to ethanol in utero. However, on day 22 higher responses of B-EP to both cold (40%) and ether (70%) stress were observed in the ethanol treated offspring. In all the offspring the increased plasma levels of B-EPLIR were associated with a decreased content of pituitary B-EPLIR. A small decrease in the hypothalamic content of B-EPLIR following stress was noticed only on day 22 of life. In conclusion the present results indicated the existence of a stress hyporesponsive period for B-EP, and a delay in the maturation of the neural pathways mediating the response of B-EP to stress, by the prenatal exposure to ethanol.

Supported by the Medical Research Council of Canada.

193.12 EFFECTS OF PERINATAL EXPOSURE TO ASPARTAME ON RAT PUPS. M.D. Holder* (SPON C. Harley). Psychology, Memorial University of Newfoundland, St. John's, NFLD Canada A1B 3X9.

Possible side effects of perinatal exposure to L-aspartyl-L-phenylalanine methyl ester (aspartame) on rat pups were investigated. Aspartame (.007%, .036%, .18% or .9% w/v), or phenylalanine (.45%), or nothing was added to the only water source of adult females and later their pups for 12 days prior to conception until the pups were 36 days of age. Compared to the pups that were exposed only to plain water, no effects of aspartame or phenylalanine were detected on two measures of morphological development. Latencies to pinnae detachment and eye opening were similar for all groups. There was also no effect on two tests of reflex development. Latencies for surface righting and negative geotaxis at 7 and 8 days of age respectively did not differ between groups. All groups were similar in spatial memory as assessed with two different mazes with pups 30-36 days of age. The number of arms chosen before reentry in an 8-arm radial-arm maze and the acquisition curves from a Morris milk maze did not differ between groups. Furthermore, the latencies of mothers to retrieve their litters was also unaffected by the aspartame and phenylalanine. These results suggest that perinatal exposure to aspartame does not have side effects on rat pups as assessed with a wide range of measures.

Group	water	aspartame .007%	aspartame .036%	aspartame .18%	aspartame .9%	phenyl
Measure						
Pinnae detachment	4.00	3.79	4.20	4.20	3.50	3.83
litters	11	7	10	5	6	6
Eye opening	15.35	15.57	15.40	15.75	15.25	15.08
litters	10	7	10	4	6	6
Righting Reflex	5.70	4.86	4.70	3.50	2.67	3.67
litters	10	7	10	4	6	6
Negative Geotaxis	27.7	31.4	31.1	28.0	22.5	25.8
litters	10	7	10	3	6	6
Maternal Retrieval	326	478	407	299	513	391
litters	10	7	10	4	6	6
Radial Arm Maze						
arms to reenter	3.22	3.04	3.05	3.08	3.32	3.92
litters	10	7	10	4	6	6

Latencies for pinnae detachment and eye opening are reported in days and for righting reflex, negative geotaxis and maternal retrieval in seconds. Values in table including the number of arms entered before reentering an arm in the 8-arm radial maze were determined by first calculating a mean for each litter and then a mean for each group was calculated using these litter means.

193.13 PRENATAL NICOTINE EXPOSURE AFFECTS DEVELOPMENT OF NORADRENERGIC SYSTEMS. H.A. Navarro*, F.J. Seidler*, W.L. Whitmore* and T.A. Slotkin (SPON: L. Schweitzer). Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Smoking during pregnancy is associated with low birth weight and neurobehavioral alterations in the offspring. Pregnant rats injected with nicotine have been used as a model for smoking, specifically because acute nicotine administration results in hypoxia and ischemia, which are features common to smoking. To distinguish between effects due to hypoxia/ischemia vs. those due solely to nicotine, we implanted pregnant rats with osmotic minipumps on the 4th day of gestation, to deliver a dosage rate of 6-8 mg/kg/day through gestational day 20. Development of catecholamine systems was then evaluated in three brain regions with different cellular maturational profiles (midbrain + brainstem, cerebral cortex, cerebellum) as well as in the peripheral sympathetic nervous system. In the CNS, prenatal nicotine exposure produced alterations which were both regionally-targeted and transmitter-specific. In the midbrain + brainstem and cerebral cortex, neonates exhibited a generalized deficit in norepinephrine levels which resolved by the second to third postnatal week. A similar pattern was seen for dopamine levels in the cerebral cortex, but not in the midbrain + brainstem. No deficits of catecholamine levels were seen in the cerebellum. Because levels of transmitters do not necessarily indicate neural activity, we also assessed turnover rates for the catecholamines after acute administration of α-methyl-p-tyrosine. An initial deficit in transmitter turnover was seen only for the cerebral cortex, where again the effect disappeared by the second to third postnatal week. A different effect was evident in the midbrain + brainstem, where norepinephrine turnover tended to be elevated initially and depressed in young adulthood, with no apparent changes in dopamine turnover. In the cerebellum, only a late increase in turnover was apparent in the nicotine-exposed animals. To rule out the possibility that changes in turnover reflected primary actions on the number of nerve terminals, we also measured synaptosomal uptake of norepinephrine, which was generally unaffected by nicotine exposure; in contrast, tyrosine hydroxylase activity, which is reactive to nerve impulse activity, showed a deficit in the region (cerebral cortex) in which turnover was most affected. These data are consistent with a slowing of development of neural activity in the cerebral cortex of animals exposed to nicotine via maternal infusions. The results can be contrasted with earlier work with injected nicotine (nicotine plus hypoxia/ischemia), where evidence for cortical hyperinnervation and increased turnover was obtained. However, at least some features of the nicotine infusion effect resemble those of injected nicotine, including late elevations in cerebellar noradrenergic activity and suppression of development of peripheral sympathetic innervation of the lung. These data support the view that both nicotine and hypoxia/ischemia contribute to neurobehavioral alterations caused by tobacco consumption. (Supported by a grant from the Smokeless Tobacco Research Council and by USPHS NS-06233).

193.14 THE INFLUENCE OF PRENATAL PHENOBARBITAL EXPOSURE ON THE GROWTH OF DENDRITES IN THE HIPPOCAMPUS. C. D. Jacobson, L. L. Antolick*, R. Scholey* and E. Uemura (SPON: P. S. Lacy). Dept. of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

Barbiturates, such as phenobarbital, are often used during pregnancy and early neonatal life to prevent epileptic seizures, hyperbilirubinemia and the stressful effects of labor. However, the long term consequences of barbiturate exposure during the prenatal and neonatal period have not been fully investigated. Several studies have indicated that phenobarbital does effect the resulting morphology and neurochemistry of various components of the central nervous system. In the present study we have investigated the effects of three days of prenatal phenobarbital administration on the growth and development of dendrities within the CA1 region of the hippocampus. Timed pregnant females obtained from our animal colony were used. The presence of sperm in the vaginal smear on estrus was defined as day 1 postfertilization (pf). On days 18, 19 and 20 pf pregnant females were given a subcutaneous injection of sodium phenobarbital (40 mg/kg body weight; PHB) or vehicle. All females delivered on day 23 pf which is defined as day 1 of postnatal life. Pups were sacrificed on days 5, 10, 23 and 35 of postnatal age and the brains were processed for golgi impregnation of neurons. The apical and basilar dendrites of neurons within the CA1 region of hippocampus were analysed with the aid of a scanning stage on a Zeiss universal photomicroscope and a PDP 11/23 microcomputer. In general, results indicated that three days of prenatal PHB severely suppresses the development of the dendritic tree which normally takes place during the first 35 days of postnatal life. There are significantly less branch points and the overall dendritic length of both apical and basilar dendrites is reduced. These results indicate that prenatal PHB, even for short periods of time, affects the normal morphological development of the hippocampus. Thus, the utilization of PHB during the gestational period should be questioned. Supported by NIH grant HD 16148 and the March of Dimes Birth Defects Foundation grants # 1-974 and 15-56.

193.15 EFFECT OF CLONIDINE TREATMENT DURING PREGNANCY ON MATERNAL BLOOD PRESSURE AND THE SENSITIVITY TO SALT-INDUCED HYPERTENSION IN THE OFFSPRING. J.A. McCaughran, Jr. and C.J. Juno*. Department of Psychiatry and Behavioral Science, SUNY @ Stony Brook, Stony Brook, NY 11794.

Hypertension (HT) during pregnancy is a problem that affects a significant number of women each year. The disease presents a threat to maternal health and can compromise fetal development. Although anti-hypertensive drug therapy can reduce maternal mortality and morbidity, there is a risk that the drug may cross the placental barrier and enter fetal circulation. In this study, the effect of chronic administration of the antihypertensive α_2 agonist clonidine on blood pressure (BP) in pregnant inbred Dahl HT-sensitive (S/JR) and -resistant (R/JR) rats maintained on a low salt diet (0.15% NaCl, w/w) was investigated. An Alza miniosmotic pump (#2ML4), containing 0.66 mg/ml clonidine·HCl or the distilled water vehicle, was implanted under the skin of the back in timed-pregnant or age-matched non-pregnant S/JR and R/JR dams on the first day of gestation. BP, heart rate (HR), and body weight (BW) were monitored at 4 day intervals until the removal of the pump on day 20 of gestation. BP, HR, and BW were then monitored once daily until parturition. Overall, BP was greater in the S/JR strain than the R/JR strain. Chronic administration of clonidine to non-pregnant S/JR rats caused a significant reduction in BP and HR relative to vehicle-treated controls. In the same R/JR groups, the drug caused significant bradycardia but did not reduce BP. These contrasting effects suggest that BP in S/JR strain is more sensitive to the anti-hypertensive effects of the drug than the R/JR strain. In pregnant rats from each strain, HR was lower in drug-treated rats than vehicle-treated rats. Interestingly, BP was similar which suggests that antihypertensive effects of the drug did not exceed the reduction in BP that normally accompanies pregnancy in either strain. Treatment with clonidine during pregnancy did not affect fetal mortality or morbidity in the S/JR strain. However, the fetotoxicity of the drug was greater in the R/JR strain, as indicated by a significant reduction in the number of pups/litter relative to vehicle-treated dams. Growth rates within each strain were similar between drug- and vehicle-exposed offspring until weaning at 25 days of age, although BW in S/JR off-spring was consistently greater regardless of the prenatal treatment. At 25 days of age, offspring were weaned to low (0.15%) or high (2.0%) salt diets and BP, HR and BW were monitored. Thus far, the data suggest that prenatal exposure of either strain to clonidine does not effect BP in the R/JR rat or the development of HT in the S/JR rat weaned to the high salt diet.

193.16 EFFECTS OF PRENATAL COCAINE EXPOSURE: I. INITIAL OBSERVATIONS AND REFLEX DEVELOPMENT. C.L. Kirstein, R. Greenbaum*, H. Hoffmann*, N.E. Spear & L.P. Spear. Dept. of Psych. and Centers for Neurobehav. Sci. and Developm. Psychobiol., SUNY-Binghamton, Binghamton, NY 13901.

Offspring of Sprague-Dawley dams that were either: (a) injected s.c. daily on gestational Days 8-20 (E8-20) with 40 mg/kg cocaine HCl, (b) injected daily with the saline vehicle solution and pair-fed to cocaine-treated dams; or (c) not injected and allowed ad lib access to lab chow and water were used in this experiment. All dams were weighed daily from E6-20. On postnatal Day 1 (P1), litters were counted, sexed and examined for gross morphological alterations prior to culling each litter to 10. One female and one male from each litter were examined daily from P3-20 on a test battery including physical maturation measures (body weight at P1,7,14,21; age of eye opening and upper and lower incisor eruption) and sensorimotor reflex development (righting reflex, cliff aversion, horizontal screen, vertical screen and inclined plane tests). No significant differences in maternal weight gain during gestation were observed among the treatment groups. Similarly, the three treatment groups did not differ in number of pups born per litter nor in male/female ratios of offspring. No body weight differences were observed among the treatment groups at P1, although a sex difference in body weight was detected. While no body weight differences were observed in offspring at P7 and 14, at P21 cocaine-exposed offspring exhibited slightly reduced body weights when compared with pups in the control conditions. Other than these body weight differences at weaning, cocaine-treated offspring exhibited very similar patterns of physical maturation and reflex development as offspring in the control groups. Thus, it appears that this dose of cocaine does not have notable adverse effects on pup integrity and maturation, and thus appears to be a suitable dose for further neurobehavioral assessment. Indeed, as is discussed in the following abstract, offspring so exposed prenatally to cocaine did exhibit alterations in both aversive and appetitive conditioning during infancy.

193.17 EFFECTS OF PRENATAL COCAINE EXPOSURE: II. BEHAVIOR, LEARNING AND RETENTION OF APPETITIVE AND AVERSIVE ODOR CONDITIONING IN INFANCY. L.P. Spear, J. Bell*, J. O'Shea*, V. Yootanasumpun*, H. Hoffmann*, N.E. Spear & C.L. Kirstein. Dept. of Psych. and Centers for Neurobehav. Sci. and Developm. Psychobiol., SUNY-Binghamton, Binghamton, NY 13901.

Offspring of Sprague-Dawley dams injected s.c. with 40 mg/kg cocaine HCl daily from gestational Days 8-20, pair-fed dams injected daily with the vehicle alone and ad lib control dams were used as subjects in these experiments. Three offspring per litter were examined in an appetitive odor conditioning experiment on postnatal day 7 (P7). Animals were given either 3 paired or 3 explicitly unpaired presentations of the CS+ odor and milk infusion, together with 3 exposure trials to the CS- (no milk) odor. Pups in a third group were exposed to only the milk infusion. Immediately following training, pups from the pair-fed and lab chow groups displayed evidence of conditioning, whereas prenatal cocaine-treated pups did not. 24 hr. later, retention was evident in the lab chow and pair-fed groups, whereas no significant retention was observed in the cocaine-exposed group. Three additional offspring per litter were examined in an aversive conditioning experiment, using analogous conditioning groups and training procedures as outlined above. Two trials were given per day on P8 and 10. A reversal of the CS+/CS- odors was introduced prior to training on P12, 14 and 16. Conditioning was assessed immediately after each conditioning session and also 24 hr later. On P8, conditioning exhibited on an immediate test was impaired in the pups prenatally exposed to cocaine. Cocaine exposed animals, however, tended to exhibit facilitated 24 hour retention both for the original odor discrimination and its reversal. When compared with the control offspring, cocaine-exposed offspring also appeared to display an earlier ontogenetic peak in wall climbing and matrix crossings in this testing situation. These observations support the suggestion that there are behavioral consequences of prenatal cocaine exposure which are evident ear in the postnatal period.

193.18 **PRENATAL COCAINE EXPOSURE AND DEVELOPMENT OF STRIATAL DOPAMINE RECEPTORS.** **W. Zeng* and L.C. Murrin.** Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE 68105

The recent epidemic in cocaine abuse in this country has led to increasing concerns about the effects of prenatal exposure to cocaine on the fetus. A major concern is how this exposure affects development of organ systems, and particularly the central nervous system. Because cocaine has well-defined effects on dopamine neurons, they are a likely site for developmental effects that may be produced by cocaine. We began studies to examine this possibility. Pregnant Sprague-Dawley rats were administered cocaine hydrochloride by one of two routes, i.p. injection or osmotic mini-pump. At 14 and 21 days of age postnatally, striata were dissected and analyzed for D1 and D2 receptors using standard membrane binding techniques. Ligands used were ^{3}H-SCH 23390 + 30 nM ketanserin for D1 receptors [1 uM piflutixol added for blanks] and ^{3}H-spiperone + 30 nM ketanserin for D2 receptors [1 uM (+)butaclamol for blanks]. In initial studies we found that a dose of 40 mg/kg administered i.p. on days E15 and E18 led to small litter sizes and significantly reduced weights in the pups from day 0 to day 7 postnatally. A dose of 30 mg/kg on the same schedule did not significantly reduce litter size but it did produce lower weights in the first week postnatally. A dose of 20 mg/kg i.p. on E15 and E18 produced no significant effect on pup weight from birth through 21 days of age. At 14 days of age this dose of cocaine produced no significant changes in D1 or D2 receptors but at 21 days of age there was a significant increase in the number of both receptors.

	^{3}H-SCH 23390 binding cpm/mg protein	^{3}H-SP binding cpm/mg protein
14 Day		
Control	1772 ± 198	2211 ± 184
20 mg/kg i.p.	1437 ± 200	2048 ± 191
21 Day		
Control	4407 ± 334	3586 ± 146
20 mg/kg i.p.	5794 ± 403*	4864 ± 260*

*** - Significantly different from control, p<.02, ANOVA followed by Scheffe's test. n=13 to 15. Data are mean ± SEM.**
Administration of cocaine via osmotic mini-pump at a dose of 30 mg/kg per day had no significant effects on pup weight or development of D1 and D2 receptors, probably due to low plasma levels of cocaine. These data indicate that exposure to cocaine prenatally at doses that do not significantly affect the weight of pups can produce alterations in the normal development of dopamine receptors. Presumably this reflects alterations in the level of dopamine activity in the synapse. E15 and E18 represent times early in the development of the nigrostriatal dopamine neurons. While preliminary, these data indicate prenatal cocaine exposure may alter normal development of the brain. **Supported by NS23975.**

NEUROTOXICITY: METALS

194.1 TRIETHYLTIN NEUROTOXICITY IN AREA CA1 OF THE HIPPOCAMPAL SLICE. S.B. Fountain, Y.L.T. Ting, S.K. Hennes*, J.A. Zuga*, and T.J. Teyler. Department of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Triethyltin (TET), an industrial plasticizer and biocide, induces reversible edema and splitting of myelin. We examined the effect of TET on the electrophysiology of area CA1 of hippocampus. Hippocampal slices 400-450 um thick were obtained from adult hooded rats and maintained in vitro at the interface of a medium pool containing artificial cerebrospinal fluid and an atmosphere providing a suitable oxygen content. Stimulation of Schaffer collaterals was used to evoke field potentials which were recorded from CA1 pyramidal cells. For each slice, an input/output (I/O) profile was obtained before TET exposure; the I/O profile characterized the CA1 population EPSP and spike responses produced by a wide range of stimulus intensities. In addition, a "paired-pulse" (or "double-shock") procedure was used to assess the status of the recurrent inhibitory system which normally modulates the CA1 pyramidal cell response. Slices that failed to show a stable baseline response to a constant stimulus or that failed to demonstrate significant inhibition prior to TET exposure were excluded from the experiment.

After these baseline measures were obtained, slices were exposed to either 1, 3, or 10 uM TET in the incubating medium. Acute TET exposure was accomplished by medium exchange using a push-pull pump. Following the onset of TET exposure, the timecourse of changes in slice excitability was observed by recording the CA1 population response to a "standard" stimulus every 5 min; the "standard" stimulus was one that produced a 1 mV population spike preexposure. The monitoring of changes in slice excitability was interrupted at the end of each hr postexposure to obtain an I/O profile and to administer a paired-pulse test. Slice excitability was monitored in this way for 3 hr post-exposure.

TET exposure suppressed population EPSP slope and spike amplitude at each concentration tested. In addition, the timecourse of effects was dose-dependent; the population spike elicited by the "standard" stimulus was nearly completely suppressed by 2-3 hr postexposure for 1 and 3 uM exposures, and by 1-2 hr postexposure for the 10 uM exposure. The effects of TET on inhibitory systems assessed by the paired-pulse test were much less pronounced. Additional results will be reported that assess the specificity of TET effects on axonal conduction of Schaffer collaterals.

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194.2 SERIAL AUDITORY BRAINSTEM RESPONSES AND NEUROPATHOLOGY IN GUINEA PIGS DRINKING TRIETHYLTIN SULFATE. A.A. Lidsky and E.R. Popovitch*. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314

While triethyltin-induced neuropathology has been described in detail for the visual system of several species this paper uniquely presents progressive neurophysiological effects on, and pathology of, the auditory system in guinea pigs. Hartley animals were exposed ad libitum to triethyltin sulfate (TET), a dysmyelinating agent, in drinking water (30 mg per liter). Auditory Brainstem Responses (ABR) to clicks were recorded twice weekly with permanently implanted skull screw electrodes (Cz-Fz) in restrained awake animals, beginning before exposure to TET, to monitor sequential changes in respective ABR components. Significant delays in all ABR peaks were identified in animals receiving TET, in relation both to their pre-TET scores and to repeated tests in Control animals. However, delays of individual peaks - which were most consistent for PIII and PIV - were found to vary as functions of several factors, including number of days on TET (DoT), auditory stimulus rates (5 vs 10 Hz), and the animals' ages at the start of TET ingestion (30 - 90 days).

Morphological analyses of the auditory pathway white matter supported the observations suggested by ABRs. Induced pathological changes consisted most prominently of vacuolation, the severity of which was rated from mild to extensive. While delayed peaks in ABR waves I through IV were evident within three DoT, the most prolonged latencies of components I through V were seen at 18-21 DoT. Until 14 DoT vacuolation was only mild or moderate, with ratings of severe or extensive limited to animals evaluated after 20-54 DoT. Youngest TET animals were affected earliest with delays in ABR components PII, PIII and PV, while more mature animals were initially most delayed at PIV and PIII. The hematoxylin-eosin and myelin stains revealed widespread vacuolation, with the most severe histopathological changes at the levels of the superior olivary complex and trapezoid bodies. The data are also contrasted with sequential results from guinea pigs with experimental allergic encephalomyelitis, in which local inflammatory activity, demyelination, and more selective involvement of ABR peaks are described.

194.3 *EFFECTS OF TRIMETHYLTIN ON CA1 PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICES.*

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Pyramidal cells of the hippocampus are among the neurons that display pathological responses following exposure to low levels of the neurotoxin trimethyltin (TMT). The mechanisms underlying this selective toxicity is unknown. We have previously studied the effects of TMT on evoked potentials and spontaneous activity of hippocampal pyramidal cells using extracellular techniques. In the present study intracellular recordings of pyramidal cell responses were made before, during and after TMT application to determine the toxin's affect on membrane parameters.

Transverse rat hippocampal slices were placed in a constant perfusion recording chamber mounted on a microscope stage. A miniature concentric bipolar electrode, positioned in the Schaffer collaterals, was used to provide orthodromic stimulation of pyramidal cells in the CA1 region. Intracellular recordings were made from CA1 pyramidal cells to assess the effects of TMT on: (1) resting membrane potential, (2) input resistance, (3) orthodromic synaptic input, (4) action potential amplitude and (5) spike size. Data was used only from cells with resting membrane potentials greater than -50 mV, input resistances >35 Meg ohms, and spike amplitudes greater than 55 mV. Thirty three cells successfully fulfilled all three criteria.

In 18% of the cells tested (n=6), a 10 min exposure to a low concentration of TMT (1 to 2 um) produced a 10 to 15 mV membrane depolarization. The level of depolarization was sufficient to initiate spike activity in previously quiescent cells. These excitatory effects were reversible after superfusing the slice with normal saline for a 20 min period. Despite the change in membrane polarization and firing activity, we did not observe significant effects of TMT on either input membrane resistance, action potential duration or spike amplitude in these neurons. In the remaining CA1 pyramidal cells tested (n= 27), we did not observe any significant change in their electrophysiological parameters with TMT exposure up to periods of 30 min.

The TMT-evoked activity in 18% of the cells was unexpected since previous extracellular experiments in our laboratory have demonstrated a small, but significant decrease in population spike amplititude under similar exposure conditions. It is possible that the excitatory responses produced in one subpopulation of cells is masked by other events occurring during the simultaneous firing that generates a population spike. Higher concentrations of TMT are currently being tested to determine threshold differences between cells. Suported by NIH Grant RR08194.

194.4 DISTINCT RESPONSES OF CHOLINERGIC FOREBRAIN AFFERENTS TO A SINGLE ADMINISTRATION OF TRIMETHYLTIN CHLORIDE IN RATS. J.A.D.M. Tonnaer, D.C.E. De Goey*, J.J. Hagan* and P. Room*. CNS Pharmacology Department, Organon International B.V., 5340 BH Oss, The Netherlands.

Trimethyltin chloride (TMT) is an organotin compound which, following acute administration, induces severe neuropathological changes in the brain's limbic system. In rats, hippocampal CA_3 and CA_4 pyramidal cells and the dentate gyrus afferents from the entorhinal cortex appear particularly vulnerable to TMT. Little information is available, however, on the effects of TMT on an other major hippocampal input system: the cholinergic afferents entering via the fimbria-fornix. In the present study, therefore, the long-term consequences of TMT on post- and presynaptic cholinergic markers were evaluated in the hippocampus and compared to those in other brain regions. For this purpose, adjacent cryostat brain sections from control or TMT-treated Wistar rats were used for histological staining and quantitative autoradiography employing [^{3}H]quinuclinidyl benzilate ([^{3}H]QNB) and [^{3}H]hemicholinium-3 ([^{3}H]HC) as the radioligands. These ligands are considered as post- and presynaptic cholinergic markers, as they specifically bind to postsynaptic muscarinic binding sites and presynaptically located high-affinity choline carriers, respectively.

Rats were treated once by gavage with vehicle or TMT at a dose of 7.5 mg/kg or 10 mg/kg, and sacrificed after time intervals varying from 1 to 12 weeks. Three weeks after 7.5 mg/kg TMT, rats were impaired in a spatial learning task. This points to hippocampal dysfunctioning. Indeed, pyramidal cell atrophy in the CA_3 and CA_4 region was observed, whilst atrophy was extended to other hippocampal areas at the higher dose. Granular cells in the dentate gyrus were preserved but their density exhibited a more condensed package, possibly due to changes in the neuropil. TMT caused a gradual impairment of muscarinic receptor binding, especially in CA_3 and CA_4. At the dose of 7.5 mg/kg, TMT induced a 5 weeks persisting enhancement of [^{3}H]HC binding to cholinergic terminals of both the supra- and infrapyramidal afferents. After 12 weeks, [^{3}H]HC binding in CA_2, CA_3 and CA_4 was decreased below control values whilst binding to CA_1 and the dentate gyrus still was above control values. At the higher dose a much stronger immediate enhancement of [^{3}H]HC binding was observed, whereas this binding started to decline from week 1. Different effects on [^{3}H]HC binding, with respect to magnitude and time course, were evident in the amygdala and neocortex. The present data illustrate distinct responses to TMT of cholinergic projections innervating particular brain structures. The responses observed may be secondary to the typical neurotoxic effects of TMT on hippocampal or cortical neurons. Alternatively, TMT may have a direct action on particular cholinergic neurons in the forebrain.

194.5 SUSCEPTIBILITY OF RAT MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC NEURONS TO CHRONIC LEAD EXPOSURE. S.M. Lasley and J.D. Lane. Dept. of Basic Sciences, U. of Illinois Coll. of Med., Peoria, IL 61656 and Dept. of Pharmacology, Texas Coll. of Osteopathic Med., Ft. Worth, TX 76107.

In previous work a decreased radiolabelled dopamine (DA) turnover in nucleus accumbens (NAc) has been observed after chronic Pb exposure as well as associated Pb-induced alterations in the DA response to d-amphetamine in striatum and NAc. Further evidence suggests that Pb results in decreased GABA activity in striatal and NAc efferents. These studies indicate that the neurotoxic action of Pb on DA neurons is largely presynaptic in origin and at least partially related to changes in DA synthesis. A primary form of DA synthesis regulation is the inhibition exerted on synaptic tyrosine hydroxylase activity via dopaminergic autoreceptors. This study assessed the functional status of this mechanism employing a pharmacological model.

At parturition dams received 0.2% Pb acetate in the drinking water while control dams received distilled water. Offspring were weaned to and maintained on the same solution given their dam until sacrifice at 125 days. Forty min before sacrifice rats were given saline or a DA agonist (2.5-20 mg/kg i.p.), 6,7-dihydroxy-2-dimethylaminotetralin (TL-99), followed 10 min later by 750 mg/kg i.p. of gamma-butyrolactone (GBL) or saline. NAc, caudate-putamen (C-P), substantia nigra (SN), and ventral tegmental area (VTA) were analyzed for content of DA and its metabolites.

The ability of TL-99 to prevent the GBL-induced increase in DA content was significantly diminished in NAc of exposed rats compared to controls. A similar result in C-P was not statistically significant. This finding suggests that chronic Pb exposure impairs receptor-mediated regulation of synthesis in mesolimbic DA nerve terminals. Concentrations of DA and dihydroxyphenylacetic acid were significantly decreased by Pb exposure by 23 and 25%, respectively, in VTA - but not SN - from animals receiving only saline injections. In this latter group homovanillic acid content was significantly diminished by exposure in NAc(21%), SN(21%), and VTA (29%).

The basis of the differential effect of Pb exposure on the mesolimbic and nigrostriatal dopaminergic systems is not known. However, other studies have shown the mesolimbic DA system to be more sensitive to treatment with ethanol, opiates, and psychomotor stimulants than the nigrostriatal system. The question as to whether the Pb-induced changes in NAc and VTA are related or discrete is currently under investigation. (Supported by ES04359).

194.6 EFFECTS OF CHRONIC EXPOSURE TO LEAD ON A TERRESTRIAL MOLLUSK FAST BURSTER NEURON. A. Camara*, G. Daston*, and J. Copeland. Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53201 and Department of Biology, Swarthmore College, Swarthmore, PA 19081.

Lead is a common environmental pollutant. We have studied the effects of chronic exposure to lead on the spiking of an autoactive burster neuron and the survivorship of a terrestrial slug.

Limax maximus is a terrestrial pulmonate gastropod mollusk, a herbivore commonly found in moist woods, hedgerows, and gardens. It possesses a circumesophageal nerve ring and paired buccal ganglia. Each ganglion contains numerous large re-identifiable neurons, some of which are autoactive.

The salivary burster neuron (SB) is a fast burster neuron found in each buccal ganglion. A process of SB innervates the musculature of the ipsilateral salivary duct and initiates duct peristalsis. SB has been shown to be autoactive, and its autoactivity can be altered by excitatory and inhibitory synaptic drive from other buccal and cerebral ganglion neurons.

Using atomic absorbance spectrophotometry, we found that environmental lead entered the hemolymph of the slugs. Slugs were exposed for 12 weeks to lead ($0.01 - 2 \times 10^4$ ug/l) placed in their food and water. Hemolymph showed a positive dose-response relationship for the presence of lead. However, lead was present at considerably less than environmental levels.

Survivorship of slugs after 12 weeks exposure to all concentrations of lead was significantly reduced. Additionally, survivorship was significantly reduced after 8 weeks of exposure to lead (2×10^4 ug/l). However, there was no reduction in survivorship after 4 weeks of exposure to the same concentration of lead.

We looked for evidence of toxicity at the neuronal level by monitoring the axon spike of SB during five minute recording sessions in all animals chronically exposed to lead. The number of bursts which occured were counted, and the interburst intervals (IBI) were measured.

Lead exposure at the highest concentration had an excitatory effect on burst activity with 12, 8, and 4 week exposure. Both IBI and number of bursts were significantly changed versus controls. Thus, at 4 weeks exposure, when suvivorship was high, a significant toxicological effect could be seen with chronic exposure to lead.

194.7 A NEW MODEL OF GESTATIONAL LEAD TOXICITY: FETAL BRAIN LEAD ACCUMULATION. E. J. Kasarskis and T. M. Forrester*. Depts. Neurology and Toxicology, VA and Univ. Kentucky Med. Ctrs., Lexington KY, 40536-0084.

Skeletal lead (Pb) has been considered to represent a relatively inert depot for this element, with minimal neurotoxicologic consequences. A chance observation indicated significant mobilization of maternal Pb during gestation several months following Pb exposure, accompanied by CNS hemorrhages and Pb accumulation. We report the results of a systematic investigation of this phenomenon.

Groups of Sprague-Dawley dams were exposed to inorganic Pb for 21 days during lactation (0.2% - 2.5% Pb acetate in drinking water) according to the classic postnatal Pb toxicity paradigm of Pentschew and Garro (Acta Neuropath 6:266-78, 1966). Rats were subsequently offered Pb-free commercial chow and distilled water for 5 months, followed by mating to non-Pb exposed males. At 21 days gestation, fetuses were delivered by Caesarian section, weighed, and observed for congenital malformations. After dissection (into femur, kindney, liver, spleen, heart, cortex, brainstem, hippocampus, pons, and cerebellum), pooled fetal tissue samples from a given litter and maternal specimens were analyzed for Pb content by graphite furnace atomic absorption spectrophotometry using the method of standard additions. NBS certified liver was simultaneously analyzed as a secondary comparator standard.

Although estrous cycles and mating behaviors were unaffected, prior Pb exposure was associated with an increased number of fetal resorptions and stillbirths in a dose-related fashion. In viable fetuses, widespread hemorrhages were observed throughout the CNS. Lead, presumably mobilized from the maternal skeleton, was deposited in the developing fetus. Of the tissues sampled, Pb accumulation was greatest in fetal hippocampus and cerebellum (11.0 and 56.9 ug Pb/g dry matter, respectively).

The results of this study indicate that Pb, once deposited in the skeleton, remains a latent toxin which can be mobilized during subsequent pregnancies, even in the absence of overt maternal toxicity in the interim. The mobilized Pb appears to preferentially accumulate in the hippocampus and cerebellum of the developing fetus. By employing cross-fostering techniques, the present model offers the potential to investigate the differential effects of pre- and postnatal Pb exposure upon subsequent behavior. (Suported by the VA Research Service, the MDA, and NIH grants NS00768 and AA05931).

194.8 NEUROTOXICITY OF LEAD IN IMMATURE GUINEA PIGS. T. Rowles*, A.J. Castiglioni, G. Miller*, C. Womac*, G. Wall*, J.-N. Wu*, G.R. Bratton and E. Tiffany-Castiglioni. Dept. Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

Studies have shown behavioral deficits and lower I.Q. in children with moderately elevated blood or dentine Pb levels who are asymptomatic otherwise. These findings are supported by numerous animal studies. However, these studies usually use the suckling rat model and employ much larger Pb doses than children receive, because rats are very resistant to Pb toxicity. A more Pb-sensitive animal model is needed in order to better define the cellular effects of Pb. We evaluated the immature guinea pig as a model because the adult guinea pig is very sensitive to Pb neurotoxicity. In Trial I, Pb acetate (0, 5, or 20 mg/kg body weight) was given orally for 5 consecutive days to 10-day-old guinea pigs. With this regimen, Pb had no effect on body and organ weights. Tissues were collected 24 days after Pb treatment was discontinued. Blood lead levels (ppm) were significantly ($p<0.01$) elevated in the 5 mg (0.02) and 20 mg (0.06) treatment groups over the control value (0.01). Liver and kidney Pb values in the 20 mg group (0.19 and 0.18) were double the Pb values of the control group (both 0.07). In contrast, Pb levels (mg/kg) were only slightly ($p<0.05$) elevated in the cerebrum (0.08 control vs. 0.10 treated). Zinc values in blood were unaffected by 20 mg Pb treatment, but were almost doubled in both cerebrum and kidney, e.g. 2.75 mg/kg in control vs 5.76 mg/kg in treated cerebra. On the other hand, zinc levels in liver were significantly reduced by 20 mg Pb treatment. Copper levels were elevated in blood, but unchanged in kidney, liver and cerebrum. Blood zinc protoporphyrin (ZPP) levels and blood ALAD activity were normal in all groups. The finding that zinc was elevated in brain and kidney indicates that potentially toxic cellular changes may occur that are undetectable by measurements of ALAD and ZPP. Trial II guinea pigs were treated with Pb (0, 10, 20 mg/kg) for two periods of 5 days (10-15 and 18-23). Tissues were collected on day 103. Blood Pb ($p<0.05$) increased in the 20 mg group, but no differences in blood copper, zinc, ZPP, or ALAD values were found. However, image analysis of Golgi-stained brain tissues revealed abnormalities in astroglia and neurons. In particular, maximum diameters (measured from the tips of the processes) of astroglia in the parietal lobe were reduced in the 20 mg group (60.8 um) relative to the control group (60.8 um). The functional significance of these alterations is unknown. Funded by the Center for Energy and Mineral Resources, TAMU and EPA R811500.

194.9 EFFECTS OF *IN VITRO* AND *IN VIVO* LEAD EXPOSURE ON VOLTAGE-DEPENDENT CALCIUM CHANNELS IN CENTRAL NEURONS OF *LYMNAEA STAGNALIS*. Gerald Audesirk. Biology Department, University of Colorado at Denver, 1100 14th Street, Denver, CO 80202.

Currents through calcium channels of members of the B cell cluster of neurons in the brain of the pond snail *Lymnaea stagnalis* were studied under voltage clamp. To maximize the visibility of voltage-dependent calcium currents and minimize contamination by other currents, the normal physiological saline was modified by substituting tetraethyl ammonium for sodium (to eliminate sodium currents and to suppress voltage-dependent potassium currents) and barium for calcium (to suppress calcium-activated potassium currents). Brains were removed from animals never exposed to lead *in vivo*, a B cell was voltage clamped and the brain was perfused with modified saline. Cells were stepped from -100 to 0 mV to obtain a control I-V curve. Depolarizing voltage steps induce an inward current, the magnitude of which varies with the barium concentration. This current is presumably carried by barium and flows through voltage-dependent calcium channels. The same cell was then perfused with modified saline with lead acetate added in concentrations of 0.25 to 14 uM and the I-V curve rerun. Each cell thus served as its own control. Lead acetate exposure *in vitro* inhibits the barium current by 59 $\pm$ 14 % (mean $\pm$ s.d.). The magnitude of barium current inhibition is independent of the lead concentration over this concentration range, although the residual current still varies with the barium concentration. The voltage dependence of the current appears to be unaffected by lead. In contrast to many other calcium-channel blockers, such as cobalt, the inhibition of barium currents by *in vitro* lead exposure is irreversible, at least in short-term experiments of 30 to 60 minutes.

In a separate set of experiments, snails were exposed to either 0 or 5 uM lead for 6 to 12 weeks *in vivo*. Brains were removed and B cells voltage clamped in modified saline without lead. Any differences in barium currents between lead-exposed and control cells must therefore have been due to the residual effects of the *in vivo* lead exposure. B cells from *in vivo* lead-exposed animals showed barium currents approximately twice as large as those of the controls. It is possible that chronic *in vivo* lead exposure may cause an increase in the number of calcium channels in these neurons.

This research was supported by a grant from the National Institute of Environmental Health Sciences.

194.10 DETERMINATION OF POTENTIAL TOXICITY USING A NEURON CELL CULTURE MODEL Charles A. Ferguson* and Gerald Audesirk. (SPON: T. Audesirk) University of Colorado at Denver;Denver, Colorado 80202

Isolated neurons from the mollusc *L.stagnalis* and embryonic chicks were cultured to determine the effect on neurite outgrowth of two insecticides, permethrin and DDT.

We isolated neurons of the pond snail *L. stagnalis* and embryonic chickens. These were then exposed to concentrations of 25uM to 500uM permethrin dissolved in alcohol. *L. stagnalis* and embryonic chick cells were exposed to DDT in concentrations of 500nM to 25uM as well. The effect of these concentrations of toxins on the ability of the neuron to extend new neurites was studied.

The number of *L. stagnalis* cells with neurites as compared to two controls decreased as concentrations of permethrin increased from 25uM to 250uM. An LC_{50} of 50uM for permethrin was found. Cells exposed to 25uM showed an 82% growth rate as compared to controls, and cells exposed to levels of 500uM or greater did not survive.

L. stagnalis cells exposed to DDT showed a decrease in neurite growth as concentrations increased from 500nM to 10uM. An LC_{50} of 750nM was found. Cells exposed to 500nM showed a 93% growth rate as compared to controls. Cells did not survive concentrations greater than 10uM.

Embryonic chick cells showed a sensitivity to these two toxins as well. Cells exposed to permethrin showed a decreased growth rate as concentration increased from 25uM to 250uM with permethrin and 10uM to 500nM with DDT.

Both of these toxins have been shown to exert their effect electrophysiologically in axons by increasing the sodium currents (Narahashi, T. 1985). Activity of neurons of *L. stagnalis* was recorded after exposure to toxins. These exposures ranged from 20 minutes to 16 hours. It was found that as concentration increased, a significantly shorter exposure time was necessary to elicit repetitive spontaneous action potentials with an eventual total depolarization of the cell for several seconds before returning to the original resting potential. Repeated action potentials were also generated following a single stimulus. Exposures to concentrations as low as 1uM permethrin and 750nM DDT elicited this response.

These studies indicate the potential usefulness of these models as assays for the determination of neurotoxicity of different substances in the environment. Further, our data suggest that in vitro models may be useful in determining response between species to specific environmental contaminants. Electrophysiological data support previous work that Na+ channels are being affected by these two toxins.

194.11 TELLURIUM INDUCED HYPOMYELINATION IN THE NEONATAL RAT CENTRAL AND PERIPHERAL NERVOUS SYSTEMS. J.P. Hammang*, S.F. Worth* and I.D. Duncan. (Spon: H.S. Schutta) University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI 53706.

The effect of elemental tellurium (Te) on the PNS of rats has been well described, but little information is available on the effects of Te on the CNS. Previous work has shown that while extensive Schwann cell and myelin changes occur in the PNS, CNS myelin and oligodendrocytes appear to remain unaffected. It has been reported that there are no neurotoxic effects on either PNS or CNS in rats fed Te from the day of birth to 15 days of age (Duckett, S., et. al., Neuropathol. and Appl. Neurobiol., 5:265, 1979.) This study was designed to determine the effect of Te on both the CNS and PNS of neonatal rats. At the time of whelping, rat dams were fed a 1.25% Te diet, and pups received Te via the mother's milk (Agnew, W.F., et. al., Exp. Neurol. 21:120, 1978). Normally fed rats served as controls. Forty rats were used in this study. Five each of normal and Te fed rat pups were sacrificed at 1,2,3 and 4 weeks of age. Animals were pulse-labelled with ^{3}H-thymidine, anesthetized and perfused with a modified Karnovsky's fixative. The entire CNS and a section of sciatic nerve was removed and embedded in epon for light and electron microscopy.

CNS - The results of these experiments indicate that by 14 days of age, the optic nerves and to a lesser extent, the spinal cords of Te fed rats were notably hypomyelinated and exhibited abnormalities of myelination when compared to age-matched controls. At 21 and 28 days of age this trend continued as the optic nerves of Te fed rats showed marked hypomyelination.

PNS - In sciatic nerves of Te fed rats, Schwann cell changes and myelin sheath abnormalities were evident by 7 days of age. After 14 days on the Te diet, numerous unensheathed axons remained and Schwann cell and myelin sheath changes were evident. At 21 and 28 days of age, there was a marked hypomyelination, with numerous naked axons and abundant Schwann cell abnormalities in the Te fed rats. The effect that Te has on the development of the nervous system in the rat has been clearly illustrated in this experiment. Whether the abnormality of myelination described in the CNS is a result of oligodendrocyte impairment or death, or whether axons fail to mature requires further study. The effect of Te compounds on human CNS development remains an important, unanswered question and it may be that this element should be considered a more important neurotoxic substance.

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194.12 EFFECTS OF CHROMIUM (Cr^{3+}) ON NEUROMUSCULAR TRANSMISSION IN THE FROG. G. P. Cooper, Dept. of Environ. Health, University of Cincinnati, Col. of Med., Cincinnati, OH 45267-0056.

In the experiments described here we examined both the pre- and post-synaptic actions of Cr^{3+} on neuromuscular transmission. Conventional microelectrode and electrophysiological techniques were used in experiments on the isolated sciatic nerve-sartorius muscle preparation of the frog Rana pipiens. Preparations were mounted in a suitable chamber which permitted the temperature to be maintained at 16^{o}C and which allowed easy introduction and removal of physiological solutions. Ringer solutions contained 111 mM NaCl, 2.5 mM KCl, 4 mM tris-maleate, adjusted to a pH of 7.0-7.2, and 1 μg/ml neostigmine bromide. Chromium was added as $CrCl_3$. Average endplate potential (EPP) amplitude was obtained by electronically averaging individual responses. Miniature endplate potential (MEPP) frequency was determined as a measure of spontaneous quantal ACh release. The postsynaptic effects of Cr^{3+} were determined by iontophoretically applying acetylcholine (ACh) to the endplate from an extracellular microelectrode filled with 1 M ACh. In some experiments microelectrodes filled with 1 M NaCl were used to record extracellular action potential (AP) and endplate currents. Exposure to 10-50 μM Cr^{3+} irreversibly reduced ACh release resulting from nerve terminal depolarization produced by APs or by exposure to high potassium (20 mM) Ringer's solution. Cr^{3+} did not directly block exocytosis since exposure of preparations to Ringer's solutions containing $PbCl_2$ still produced large increases in MEPP frequency even after all AP-evoked release of ACh had been blocked by Cr^{3+}. Cr^{3+} concentrations of 50-200 μM reduced MEPP amplitude and the endplate response to iontophoretically applied ACh without altering the resting muscle membrane potential or the input resistance. Simultaneously recorded extracellular AP and endplate currents revealed that Cr^{3+} reduced the endplate current without affecting AP current. These results indicate that acute exposure to Cr^{3+} has little or no intracellular effect and that it probably acts mainly to interfere with membrane receptors or channels to block the normal actions of Ca^{2+} and ACh. (Supported by NIH grants ES-00159 and ES-03992.)

194.13 BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF ORAL ALUMINUM ADMINISTRATION TO RATS. D.J. Connor, R.S. Jope and L.E. Harrell. Departments of Psychology, Pharmacology and Neurology. University of Alabama at Birmingham, Birmingham, AL 35294.

Aluminum toxicity has been associated with disorders manifesting memory dysfunction and dementia such as Alzheimer's disease and dialysis encephalopathy. Cholinergic hypofunction has also been associated with Alzheimer's disease and memory impairment. To examine the effects of orally administered aluminum on behavior and cholinergic function, aluminum sulfate was added to the drinking water of rats for one month (0.3% aluminum). The behavioral tasks included open field activity, radial arm maze performance, active avoidance acquisition and retention, and passive avoidance acquisition and extinction. Biochemical measures of choline acetyltransferase activity (ChAT), inositol phospholipid hydrolysis and muscarinic receptor binding of tritiated quinuclidinyl benzilate (^{3}H-QNB) and pirenzepine (^{3}H-PZ) were made in the cortex and hippocampus.

No differences between the aluminum treated animals and controls were observed in open field activity, radial eight-arm maze performance, active avoidance acquisition/retention, or on a standard seven-day extinction passive avoidance task. However, in two paradigms utilizing an extended passive avoidance extinction task (with and without pre-habituation to the apparatus), the aluminum treated animals demonstrated a faster rate of extinction than controls ($p<.0001$ and $p<.01$ respectively).

The biochemical measures indicated no significant effect of aluminum treatment on choline acetyltransferase activity in the cortex or hippocampus. Inositol phospholipid hydrolysis was measured by incubation of brain slices with tritiated myo-inositol and exposure to agonists (Berridge et al., Biochem. J. 206:587-595; 1982). Incorporation of myo-inositol into lipids was not different between groups. Levels of basal, carbachol (100 μM), norepinephrine (200 μm), and K^+ (25 mM) stimulation of hydrolysis showed no significant difference between controls and the aluminum treated animals in the cortex or hippocampus. The B_{max} of muscarinic receptors (^{3}H-QNB) was slightly increased in the hippocampus, but not in the cortex of the treated animals. Pirenzepine binding did not differ between the two groups in either brain region.

Our results demonstrate that oral aluminum administration disrupts some types of learning behavior. The neural substrate of this disruption is unknown, however we found no indication of cholinergic hypofunction from presynaptic (ChAT) or postsynaptic (^{3}H-QNB, ^{3}H-PZ, inositol phospholipid hydrolysis) cholinergic markers.

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194.14 ALUMINUM NEUROTOXICITY: ALTERATIONS IN GENE EXPRESSION OF CYTOSKELETAL PROTEINS. N.A. Muma, J.C. Troncoso, P.N. Hoffman and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The neuronal cytoskeleton exhibits abnormalities in several human disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. In order to better understand perturbations of the neuronal cytoskeleton, we are investigating an animal model of pathology of the neuronal cytoskeleton. When rabbits are given intrathecal injections of aluminum salts, several populations of neurons develop striking neurofibrillary pathology. It is possible that the appearance of accumulations of neurofilaments is related to the amplified expression of neurofilament genes. To test this hypothesis, we examined the expression of genes encoding for several cytoskeletal proteins in two neuronal populations in aluminum-intoxicated animals. One cell population, motor neurons, showed neurofibrillary pathology; the other, neurons of the dorsal root ganglia, do not. Animals were sacrificed immediately upon the appearance of neurological signs, i.e., between 7 and 14 days after the first of three injections of aluminum lactate. Using Northern blots to examine spinal cord tissue, we found reductions in the expression of genes encoding for high and low molecular weight neurofilament proteins, tubulin, and actin. The magnitude of the reduction of neurofilament protein and tubulin mRNAs was greater than that of actin mRNA. In contrast, neurons of the dorsal root ganglia, which do not display aluminum-induced neurofibrillary pathology, do not show reduced levels of mRNA encoding the low molecular weight neurofilament protein and tubulin. Thus, reductions in the gene expression of cytoskeletal proteins appear to occur in nerve cells that display neurofibrillary pathology. These studies suggest that neurofilamentous abnormalities in this model are associated with reductions of, and not amplifications of, genes encoding for neurofilaments.

195.1 IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUCOCORTICOID RECEPTORS IN ADRENERGIC DERIVATIVES OF NEURAL CREST DURING POSTNATAL DEVELOPMENT OF THE RAT. G.Yang*, M.F.Matocha* and S.I.Rapoport. Lab. Neurosciences, NIA, NIH, Bethesda, MD 20892.

Differentiation of neural crest results in several adrenergic derivatives, which include adrenal and extra-adrenal chromaffin cells and sympathetic neurons. Of these, the chromaffin cells share morphological and functional characteristics, and it is established that glucocorticoids are essential for maintaining their phenotypes. In vivo, however, adrenal chromaffin cells are exposed to a much higher concentration of glucocorticoids than extra-adrenal chromaffin cells, suggesting that the local hormonal environment is not a determinant of chromaffin cell phenotype. Alternatively, regulation of the cell type may be intrinsic. We investigated this possibility by examining glucocorticoid receptor (GR) localization in the adrenergic derivatives of neural crest during postnatal development.

The adrenal medulla, extra-adrenal chromaffin tissues, and celiac-superior mesenteric ganglia were removed from male Fischer-344 rats, aged 2, 8, 15, and 90 days and were processed for immunocytochemistry. Immunoreactivity was monitored by an avidin-biotin peroxidase complex using a monoclonal antibody against DNA-binding sites of the rat liver GR. In the adrenal chromaffin cells, weak immunoreactivity was detected at day 2. At day 8, immunoreactivity was concentrated in the nucleus. The nuclear immunoreaction intensified markedly at the 15th day and remained high at day 90. Also, immunoreactivity in the nucleus was unchanged following i.p. injection of dexamethasone (DEX, 0.1 mg/kg b.w.) at 30 minutes before killing the 15- and 90-day-old animals. Extra-adrenal chromaffin cells showed a similar time course of GR immunoreactivity and responsiveness to DEX, with the exception that, at day 2, only a subpopulation of these cells was immunoreactive. In contrast, immunoreactivity in the sympathetic neurons was localized in the nucleus at day 2 and was intensified at day 8. Thereafter, heterogeneity in nuclear staining became apparent. Also, nuclear immunoreactivity was enhanced after administration of DEX to animals aged 15 and 90 days.

These results indicate that adrenal and extra-adrenal chromaffin cells have similar temporal patterns of GR localization and responsiveness to DEX, and that these characteristics are not found in sympathetic neurons. The correlation of cytological traits with developmental expression of the GR suggests that this receptor helps to establish phenotypes of adrenergic derivatives of the neural crest.

195.2 DEVELOPMENT OF NEUROPEPTIDES AND CATECHOLAMINE SYNTHETIC ENZYMES IN RAT ADRENAL CHROMAFFIN CELLS. P.D. Henion* and S.C. Landis. (SPON: T.E. DICK). Depts. of Developmental Genetics and Anatomy and Pharmacology, Case Western Res. Univ., Cleveland, OH 44106.

The developmental mechanisms responsible for the determination of phenotype of neural crest derivatives are not fully understood. Adrenal chromaffin cells have proven to be a useful system to study the factors involved in expression and regulation of catecholamine synthetic enzymes. Considerably less is known about the development of neuropeptides in these cells. As a first step in studying the regulation of peptide expression during development we have used an immunocytochemical approach to examine the expression of neuropeptide Y (NPY-IR) and leucine enkephalin (LE-IR) and compared that with the expression of tyrosine hydroxylase (TH-IR) and phenylethanolamine N-methyltransferase (PNMT-IR). Double label indirect immunofluorescence was used to determine the proportion of chromaffin cells containing PNMT, NPY, or LE. Consistent with previous reports, approximately 90% of TH-IR chromaffin cells in the adult rat exhibited PNMT-IR. Approximately 40% had 10% of TH-IR cells were NPY-IR and LE-IR respectively.

Cells from the sympathoadrenal region of the neural crest invade the forming adrenal at 13.5 days of gestation (Bohn et al, Dev. Biol. 82, 1-10, 1981). On embryonic day 15 (E15), TH-IR, NPY-IR, and LE-IR cells were present in small aggregates dispersed throughout the gland and in extra-adrenal chromaffin tissue. In contrast, small numbers of PNMT-IR cells were first detectable on E16 and were present only in the adrenal proper. These observations raise the possibility that the mechanisms responsible for initial expression of NPY and LE are different from those required for PNMT but not necessarily different than those responsible for TH. The relative proportions of chromaffin cells which expressed PNMT-IR, NPY-IR, and LE-IR increased until birth. Postnatally, the proportion of PNMT-IR cells remained constant while the proportions of NPY-IR and LE-IR cells decreased to adult values. This suggests that the postnatal development of the adrenal medulla may involve continued chromaffin cell division without concomitant peptide expression or the loss of peptide expression in post-mitotic cells, possibly due to the absence or alteration of the signals involved in the initial expression of these peptides during prenatal development. (Supported by NS 02378).

195.3 MIGRATION AND DIFFERENTIATION OF NEURAL CREST AND NON-NEURAL CREST CELLS FROM QUAIL NEURAL TUBES IN VITRO. J.F. Loring, D.L. Barker, and C.A. Erickson*. Dept. of Zoology, Univ. of California, Davis, CA 95616 and Protein Databases, Inc., Huntington Station, N.Y. 11746.

During vertebrate development, neural crest cells migrate from the dorsal neural tube and give rise to pigment cells and most peripheral ganglia. To study these complex processes it is helpful to make use of in vitro techniques, but the transient and morphologically ill-defined nature of neural crest cells makes it difficult to isolate a pure population of undifferentiated cells. We produced cultures from quail neural tubes (embryonic spinal cord) containing neural crest cells and non-neural crest cells and compared their subsequent differentiation by HPLC and immunohistochemistry. The cultures examined were: 1) cells that emigrate from explanted thoracic neural tubes (neural tube outgrowths); 2) neural crest clusters, believed to contain exclusively neural crest cells [Loring et al (1981), Dev. Biol. 82:86-94]; 3) dorsal and 4) ventral portions of neural tubes. Both neural tube outgrowths and neural crest clusters contained catecholamines after 5 days in culture. However, 5-HT was only detected in outgrowths, not in cultures of neural crest clusters. Immunohistochemistry confirmed that 5-HT-containing cells were not present in cultures of neural crest clusters, and furthermore that 5-HT cells arise from precursors in the ventral, not dorsal neural tube (see Table). Ventral neural tube cultures did not contain neural crest cells since no pigment cells appeared in these cultures. Therefore, the 5-HT containing cells are not, by definition, neural crest cells. The demonstration that cells from the ventral (non-neural crest) part of the neural tube migrate in vitro suggests that the same phenomenon may occur in vivo. We propose that the embryonic "neural trough", as well as the neural crest, may contribute to the peripheral nervous system of vertebrates. (Supported by NIH Grant DE06530 to CAE and Air Force Grant AFOSR-86-0076 to Dr. G. Mpitsos).

Culture	Pigment Cells	5-HT
1. Neural Tube Outgrowths	+	+
2. Neural Crest Clusters	+	-
3. Dorsal Neural Tube	+	-
4. Ventral Neural Tube	-	+

195.4 CELLULAR HETEROGENEITY IN THE NEURAL CREST AND ITS POSSIBLE DEVELOPMENTAL SIGNIFICANCE. M.E. Forbes*, D.S. Christie, and G.D. Maxwell (SPON. Y. Grimm-Jorgensen). Department of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

One important issue in neural crest cell differentiation concerns the extent to which developmental potential is limited in individual neural crest cells. We have begun to approach this problem by analyzing operationally the developmental capabilities of cellular subsets present in the early neural crest. Primary quail trunk neural crest outgrowths after 2 days in vitro were stained with the monoclonal antibodies HNK-1 and R24 by double label indirect immunofluorescence using subclass-specific secondary antisera. The percent of cells with a given phenotype was:

HNK-1+ R24+	HNK-1+ R24-	HNK-1- R24+	HNK-1- R24-
12 ± 4	30 ± 3	7 ± 3	54 ± 6

When primary neural crest cultures were treated with HNK-1 and complement at 2 days in vitro and then allowed to grow until 9 days in vitro the number of catecholamine-positive (CA+) cells observed was reduced to 10% of the control number which developed after treatment with antibody alone, complement alone, or no addition. Numerous melanoctyes and unpigmented cells were present in the cultures treated with HNK-1 and complement. This is consistent with the precursors of CA+ cells being HNK-1+ and/or any necessary accessory cells required for CA+ development being HNK-1+.

Fluorescence activated cell sorting was used to isolate HNK-1+ and HNK-1- cells from primary neural crest outgrowths after 2 days in vitro. These isolated cell populations were then grown in culture for one week. Analysis of these cultures showed that the cell population isolated as HNK-1+ gave rise to melanocytes, unpigmented cells, and CA+ cells. The cell population isolated as HNK-1- gave rise to melanocytes and unpigmented cells, but not CA+ cells. These results indicate that the precursors of the CA+ cells as well as any necessary accessory cells existed in the HNK-1+ isolated population. Since other growth conditions may elicit different phenotypic responses one cannot conclude on the basis of these data that HNK-1- cells cannot become CA+ cells, but only that they did not do so under the conditions of these experiments. The development of melanocytes from the HNK-1- population shows that the HNK-1- cells are of neural crest origin and are not a non-neural crest cell type which emigrated from the neural tube.

These experiments demonstrate the feasibility of using positive and negative selection methods for the isolation of defined subsets of neural crest cells and the analysis of the developmental capabilities of these cell populations. Supported by NIH grants NS 16115 and RCDA NS 00696 to GDM and NRSA NS 17841 to DSC.

195.5 CELL AUTONOMOUS EXPRESSION OF LINEAGE-SPECIFIC NEURAL FUNCTIONS IN DROSOPHILA MELANOGASTER. R. Huff* and A.P. Mahowald* (SPON: R. Ruff). Department of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

The embryonic central nervous system of Drosophila first becomes apparent at gastrulation when a population of neuroblasts segregates from the ventral ectoderm. These neuroblasts can be isolated and cultured in vitro to produce clonal neural cell lineages. The neural cultures differentiate such that a subpopulation of cell lineages contains cells that produce dopamine and serotonin, as observed by immunohistochemistry. The ratio of serotonergic and dopaminergic neurons in culture correlates well with that described in vivo. In addition, the pattern of expression of these transmitters within their cell lineages is similar to the pattern which is seen in vivo. The production of these neurotransmitters correlates with a subpopulation of cell lineages that transcribe dopa decarboxylase (DDC), based on in situ hybridization analysis. We have also shown that the actual cells responsible for DDC transcription and transmitter synthesis arise at different times within the cell lineage depending on whether the lineage is serotonergic or dopaminergic.

These results indicate that the determination of serotonergic and dopaminergic functions in Drosophila is an early event which occurs at or near the time of neuroblast formation. The program of differentiation then proceeds autonomously within a given neural cell lineage in vitro. Furthermore, the data suggest that the neural regulation of DDC transcription has two distinct patterns depending on whether a cell lineage is determined to produce serotonin or dopamine.

195.6 THE DROSOPHILA S8 GENE PRODUCT IS A NUCLEAR PROTEIN WITH HOMOLOGY TO THE PER PROTEIN. John B. Thomas, Stephen T. Crews* and Corey S. Goodman. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305.

Mutations of the S8 locus alter the precise spatial pattern of neuronal precursor cells which give rise to the CNS. In S8 mutant embryos, a subset of precursor cells which lie at the midline fail to emerge. These cells normally delaminate from the ectodermal epithelium along with the rest of the neuroblasts (NBs) and give rise to MP1, the MNB and its progeny, and a set of specialized ectodermal cells at the midline of the CNS.

We have sequenced what is likely to be all of the S8 coding region, and have raised antibodies to portions of the S8 protein fused to B-galactosidase. The S8 gene encodes a predominantly hydrophilic protein of at least 604 amino acids. The protein contains a 51 a.a. repeat separated by 115 a.a. This repeat, as well as the overall spacing between the two repeats is also found in the per protein. Homologies between the four repeats of the two proteins are approximately 25% (without introducing gaps). The significance of this homology is at present unknown.

Staining of embryos with antibodies to the S8 gene product demonstrates that the S8 protein is localized to the nuclei of those midline cells which fail to emerge in the mutant. This suggests that the S8 protein plays a controlling role in the expression of downstream genes involved in the emergence and differentiation of these midline cells.

195.7 USE OF RETROVIRAL VECTOR-MEDIATED GENE TRANSFER TO STUDY CELL LINEAGE AND DIFFERENTIATION IN RAT RETINA IN VIVO. D.L. Turner* and C. Cepko (SPON: R. Masland). Dept. of Genetics, Harvard Medical School, Boston, MA 02115

The mechanisms underlying the generation of different cell types in the CNS, including the role of cell lineage, remain unknown. To begin to investigate these mechanisms, we have developed a cell lineage marking system based on retroviral vector-mediated gene transfer. We have employed a replication-incompetent retroviral vector to stably introduce the E. coli β-galactosidase gene into rat retinal progenitor cells in vivo. Histological detection of β-galactosidase activity allows us to identify individual infected cells and their descendents. The integrated vector is inherited only by cells descended from infected progenitor cells. Clones of cells derived from single progenitors are used to determine cell lineage relationships among different cell types (Price, Turner & Cepko, PNAS 84:156-160, 1987).

We have chosen the postnatal rat retina for our initial experiments since it is easily accessible and it remains mitotic for about a week after birth. Injection of concentrated virus between the retina and pigment epithelium of newborn animals labels numerous clones of retinal cells. These clones contain one to three cell types, with the most complex clones including rods, bipolar cells, and amacrine cells, or rods, bipolar cells and Muller glia. Infections on postnatal days 4 and 7 show that two cell clones, probably generated from terminal mitoses, can give rise to two different cell types, including a rod and a Muller glial cell. This indicates that several types of retinal neurons and Muller glia can arise from common precursor very late in development, probably at or after its final division. We think that the most plausible model for these results is the presence of a multipotential precursor cell which differentiates in response to environmental cues.

In addition to lineage analysis, we are using this system to study retinal differentiation by observing the composition of clones after perturbing retinal development. One approach is to introduce vectors which express the β-gal marker gene and also expresses an additional gene, which may affect differentiation. We are also using various cell type-specific toxic substances in combination with the β-gal marker virus, to see if the depletion of one cell type during development influences the subsequent differentiation of other cells.

195.8 ESTABLISHMENT OF NEURAL CELL LINES USING RETROVIRUS-VECTOR MEDIATED ONCOGENE TRANSFER. E. Ryder*, E. Snyder*, and C. Cepko, Department of Genetics, Harvard Medical School, Boston, Ma. 02115.

We are attempting to establish neural cell lines through the introduction of immortalizing oncogenes into primary cultures via retrovirus vector-mediated gene transfer. These experiments have resulted in the characterization of the viral gene transfer system for transduction of exogeneous genes into primary neural tissue in vitro, as well as provided a series of neural lines. (For information on viral gene transfer into the nervous system in vivo, see the abstract by Turner, D. and Cepko, C.). The lines originated as mouse or rat primary cultures from the olfactory bulb, cerebral cortex, or cerebellum. We used several oncogenes, including Adenovirus E1A, polyoma large T, SV40 large T, N-myc, avian myc, and mouse p53, along with the dominant selectable marker for resistance to the drug, G418 (neo gene), to establish a large number of primary colonies. Infection with a retrovirus vector containing only the neo gene, without an oncogene, was also performed. All of the above tissue sources proved to be readily infectable, in that many G418-resistant colonies were observed after infection with any of these constructs. However, the ability to pass the colonies beyond 1 passage required the presence of an oncogene. All of the oncogenes listed above had activity in prolonging the life of the colonies in vitro. Due to the magnitude of effort required to test the number of passages that any given colony could produce, we do not know if all of the oncogenes can truly "immortalize" in every case. However, most of the colonies which have been carried for several months, and up to 1.5 years, have given very rapid and reproducible growth, without undergoing crisis.

To date, most of the characterization of the nature of these colonies has been with antisera that are defined as markers of the main neural cell types. By far the vast majority (several hundred) of the colonies were quite boring, exhibiting a flat morphology and no staining with many different neural antisera. However, a minority of colonies have proven to be reproducibly positive with classic antisera for the main neural cell types. We have lines that contain the markers for all of the neural cell types; neurons, astrocytes, and oligodendrocytes. Some lines are positive with antibodies which recognize a set of antigens consistent for a given cell type. For example, lines which bind tetanus toxin are also positive with neurofilament antibodies and a variety of glycolipid antisera (e.g. A2B5, R24), and are negative for staining by antisera against GFAP and galactocerebroside. In order to confirm the histochemical identification of neurofilament expression, Northern blot analysis of selected lines was performed, and found to be positive. Interestingly, within many clonal lines, there is heterogeneity of expression for any given marker (e.g. A2B5, R24, GFAP), although certain lines exhibit almost 100% staining for their most characteristic marker (neurofilament or GFAP). In addition, some lines have not maintained their original differentiated status. For example, an galactocerebroside$^+$ line, during routine culturing, developed subclones which are positive for neuronal or astrocytic markers, while losing the expression of galactocerebroside. We are currently attempting to further define the activity and behavior of selected lines, both in vivo and in vitro.

195.9 GENERATION OF PERMANENT CELL LINES DERIVED FROM POSTNATAL SEPTAL AND HIPPOCAMPAL REGIONS. H.J. Lee, D.N. Hammond, and B.H. Wainer. Com. on Neurobiology, The University of Chicago, Chicago, IL 60637.

In order to better understand cholinergic neurotrophic mechanisms, we have generated hybrid cell lines from the postnatal septal area and from a principal target of its cholinergic efferents, the hippocampus. Septal and hippocampal cells from postnatal day 21 C57BL/6 mice were fused with murine neuroblastoma N18TG2 cells using a phytohemagglutinin and polyethylene glycol-mediated somatic cell fusion technique. The fusion products were plated in monolayer with medium containing hypoxanthine, aminopterin, and thymidine (HAT) to select against hypoxanthine phosphoribosyltransferase-deficient N18TG2 cells. Similarly treated septal cells, hippocampal cells, or N18TG2 cells alone produced no viable colonies. Viable fusion colonies were subsequently expanded in medium without HAT and subcloned or frozen.

Characteristics typical of septal cholinergic neurons but not of N18TG2 neuroblastoma cells were examined in several colonies. Twenty-two parent septal colonies were radiochemically screened for choline acetyltransferase (ChAT) activity and yielded specific activities up to 260 pmol acetylcholine formed/min/mg protein. Several first generation subclones have ChAT specific activities over 500 pmol/min/mg. Long neuritic processes more than 1 mm in length were observed in monolayer culture. However, no correlation between the level of ChAT specific activity and morphology was noted. Two first generation subclones have been further characterized immunocytochemically. Both stained positively for ChAT, nerve growth factor receptor, and neurofilament proteins but not for glial fibrillary acidic protein using the antibodies Ab8, alpha 192, TA.51, and 2.2 B10.6 respectively. Fourteen hippocampal derived lines were frozen for later analysis.

These hybrid cell lines are the first permanent cell lines to be derived from normal postnatal central nervous system tissue. Since they are derived from the basal forebrain and from one of its major projection targets, they may provide a controlled system to examine the factors modulating central cholinergic neuronal development and the formation and maintenance of basal forebrain connections.

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195.10 PATTERN OF EXPRESSION OF A NEURON-SPECIFIC BETA-TUBULIN IN EARLY DEVELOPMENTAL STAGES OF CHICK EMBRYO. M. LEE*, J.B. TUTTLE, A. FRANKFURTER*, Depts. of Neuroscience, Physiology, and Biology., Univ. of Virginia, Charlottesville, VA 22901.

A beta-tubulin specific monoclonal antibody (TuJ1) has been isolated (Frankfurter et al., Soc. Neurosci. Abstr., 12:1123, 1986). Partial characterization of TuJ1 indicates this tubulin antigenic site is expressed in rat, human, and chicken brain and in rat testis. The binding site in brain is expressed exclusively in neurons. Neither the functional significance nor the time of expression of this tubulin is known. The chick embryo was examined immunocytochemically to determine initial expression and distribution of this beta-tubulin isotype.

Stage 17-26 chick embryos (Hamburger and Hamilton) were fixed, frozen sectioned (12-16 um), and immunocytochemically stained via indirect immunofluorescense. At all stages, intense unequivocal immunoreactivity is associated with neurons and their processes in germinal epithelia (brain,retina, and spinal cord) and ganglia. The number of immunoreactive neurons and the intensity of staining increases with development and is related to rostral-caudal position in the neural tube. Somal staining is prominent in the marginal zone, while germinal regions appear free of immunoreactivity. Stained cells most often have processes extending out of the epithelium with early fiber tracts clearly demonstrated. Staining of variable intensity is seen dorso-lateral to the spinal cord before the formation of the dorsal horn. The bipolar aspect of these cells suggests that they are neurons derived from neural crest in various stages of differentiation. No staining is apparent in neural crest prior to migration. A similar pattern is observed during development of the ciliary ganglion: early variable immunoreactivity during coalescence into a definitive ganglion is followed by intense staining at a slightly later stage.

For all stages examined, there is a notable absence of staining in non-neural elements. However, low levels of equivocal reactivity were present in the early mesonephric duct, lens, aorta, and perhaps erythrocytes. Apparently specific staining occurs in the amnionic ectoderm. Whether this reactivity represents transient expression of the antigen or non-specific binding is unclear.

This study indicates that TuJ1 recognizes an antigenic determinant expressed in post-mitotic neurons. Expression of this beta-tubulin isotype correlates with the stage of neuronal development associated with neurite outgrowth.

Supported by grants from NIH and NSF.

195.11 THE CHARACTERIZATION OF A MOUSE NEUROBLASTOMA-DERIVED CDNA CLONE THAT HYBRIDIZES TO AN RNA SPECIES THAT IS INDUCED UPON TRANSFER OF CELLS FROM SUSPENSION TO SURFACE CULTURE. Eugene T. Butler, III and Bruce K. Schrier. Molecular Neurobiology Unit, Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD. 20205

The NS20Y mouse neuroblastoma cell line was adapted for growth in suspension culture in order to enhance phenotypic differences between undifferentiated, suspension cell cultures (S cells) and differentiated cell cultures (P cells) that were grown as surface-attached cultures in the presence of dibutyryl cAMP. Complimentary DNA (cDNA) libraries were constructed from S and P polyA-selected RNA fractions following the application of subtraction hybridization steps to increase the representations of sequences that are differentially expressed under the two sets of growth conditions. A number of cDNA clones have been isolated that correspond to mRNAs that exhibit either increased or decreased levels of expression in cells that have been grown under the two sets of conditions. Via RNA blot-hybridization analyses with probes prepared from two cDNA clones, A216 and F31, we have identified an RNA species, ca. 3 kilobases (Kb) in length, that is expressed in surface-grown NS20Y cells but is virtually undetectable in cells that have been grown in suspension culture. The addition of dibutyryl cAMP to suspension cultures of NS20Y cells does not result in the appearance of the 3 Kb RNA species. While the A216 and F31 cDNAs appear to hybridize to the same RNA species, the two clones do not cross-hybridize. The A216 cDNA insert is 1345 bp in length and its nucleotide sequence has been determined; an open reading frame of significant length is not found in the sequence. Determination of the nucleotide sequence of the F31 cDNA and identification of the protein that may be encoded by the polyadenylated RNA species to which the clone hybridizes are in progress.

195.12 DIFFERENTIAL EXPRESSION OF THE RAS ONCOGENE IN CLONAL RAT GLIAL CELLS. K.A. Zito and J.A. Connolly, Dept. of Anatomy, University of Toronto, Toronto, Canada M5S 1A8.

When C6 glial cells progress from a logarithmic to a stationary phase of growth they begin to differentiate as measured by an initiation of synthesis and accumulation of the brain specific S-100 protein (Labourdette et al, 1977). Both intercellular contact (Labourdette et al, 1975; 1977) and/or the presence of factors present in normal growth media which suppress the ability of these cells to differentiate (Edstrom et al, 1973) have been suggested as possible mechanisms for the control of differentiation in this glial tumor cell line. Treatment with colchicine, which disrupts microtubules, selectively inhibits S-100 synthesis.

The protein ras has been suggested to play an intimate role in the differentiation of certain cell types. Employing the technique of immunofluorescence microscopy we have used a monoclonal antibody to ras to examine its distribution in normal, serum-starved or colchicine treated differentiating C6 cells. Both subconfluent and confluent C6 cells were grown in alpha MEM containing 5% fetal bovine sera. Some cells in both groups were switched to either serum-free media for 48 hrs or treated with 1 uM colchicine prior to being processed for immunohistochemistry.

C6 glioma cells stain positively for ras. In subconfluent cells grown in normal serum conditions the protein is diffusely distributed throughout the cell cytoplasm and additionally can be localized to the plasma membrane. After 48 hrs of serum starvation these cells begin to extend long processes which also stain positively for ras. In serum starved cells ras becomes more intimately associated with the plasma membrane where it appears to be non-uniformly distributed. When cells are treated with colchicine cytoplasmic staining of ras increases to a level above that seen in untreated subconfluent cells. When these cultures are carried to confluency the staining pattern of ras becomes more diffuse and punctate.

These preliminary findings lend support to the notion that ras may be involved in the differentiation of C6 glioma cells and their subsequent expression of S-100. We are currently examining the expression of S-100 in ras transfected glial cells in addition to the localization and expression of other oncogenes in these cells.

195.13 EXPRESSION OF HOMEOBOX-CONTAINING GENE PRODUCTS BY NEURAL CREST-DERIVED CELLS OF EARLY AVIAN EMBRYOS. P. Hill*, O. Civelli* and G. Ciment. Dept. of Cell Biology & Anatomy and Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences Univ., Portland, OR 97201.

The neural crest is a transient structure seen during early vertebrate embryogenesis. Neural crest cells originate within the neural tube, but leave the tube to migrate extensively in the embryo, eventually localizing at various sites and giving rise to a wide variety of cell types.

Crest cells which migrate from rhombencephalic regions of the neural tube colonize a set of structures known as the branchial arches (BAs). Previously, we found that the crest-derived cells within the posterior BAs of avian embryos behaved like their crest cell antecedents. That is, posterior BA cells expressed the neuronal marker NAPA-73 and gave rise to connective tissue elements, endocrine cells, as well as neurons and glial cells in heterospecific grafting studies (Ciment and Weston, Develop. Biol. 111:73,1985). Little is known, however, about the gene products responsible for this generation of cell type diversity, these morphogenetic movements, or other developmental behaviors observed with these crest-derived cells.

In Drosophila, on the other hand, a number of gene products have been identified which clearly govern morphogenesis. Many of these gene products, moreover, contain translated regions which share striking sequence homologies with genes found in vertebrates, including birds. The regions of sequence homology are termed "homeoboxes" and have been proposed to play a role in both vertebrate and invertebrate development.

In order to determine whether homeobox-containing gene products are expressed during neural crest development, a cDNA library was made from mRNA transcripts isolated from dissected posterior BAs of 400 3.5d quail embryos. This library was constructed in lambda gt10 and contained roughly a million clones. This posterior BA cDNA library was then screened using a cDNA insert containing the 180 base pair Drosophila "antennapedia" homeobox sequence (p903g; kindly provided by Wm. McGinnis). We found that 0.018% of the plaques showed strong and reproducible signals when probed under medium-stringency conditions (37°C, 50% formamide), suggesting that homeobox-containing gene products are moderately abundant in posterior BA cells are this developmental stage.

We present here the initial characterization of the tissue-specific and developmental stage-specific expression of these homeobox-containing gene products. Using RNA blotting and in situ hybridization procedures, we show the developmental appearance of homeobox-containing mRNA transcripts in (1) neural crest cells of the posterior branchial arches, (2) various other neural crest-derived cell types, and (3), various non-crest cell types.

195.14 EXPRESSION OF THE MURINE HOX 1.3 HOMEO DOMAIN PROTEIN IN THE DEVELOPING CEREBELLUM VARIES WITH NEURON TYPE AND AGE. M. Tani.* V. L. Friedrich, Jr., W. Odenwald.* and R. A. Lazzarini.* Laboratory of Molecular Genetics, NINCDS, NIH, Bethesda, MD 20892.

First discovered in embryonically active pattern formation genes of Drosophila melanogaster, the homeo box is a highly conserved 180 bp segment of DNA which occurs in genes of diverse species including mammals. Using mouse brain cDNA and genomic libraries, we isolated the Hox 1.3 homeo box gene and found its message in both embryonic and adult tissues (W. Odenwald et al., *Genes and Development*, in press, 1987.) We prepared antisera specific for an oligopeptide predicted from a non-homeo box coding region of the DNA sequence. Immunolocalization studies have shown that the Hox 1.3 protein is present in neurons in many regions of the adult CNS. In the cerebellum, the Purkinje cell layer stains prominently for the protein, but the internal granule cell layer appears largely unstained. Here we examine the distribution of Hox 1.3 immunoreactivity in detail and compare the adult pattern of cerebellar staining with that in early postnatal mice, in which major developmental changes are occurring.

In the newborn cerebellar cortex, the post-mitotic Purkinje cells exhibit intense nuclear staining without distinct cytoplasmic staining. In the adult, they exhibit much reduced nuclear staining but are distinguished by the appearance of cytoplasmic staining extending into the primary dendrites at an intensity matching or often greater than the nuclear staining. Early in postnatal life, the cerebellar granule cells are generated from mitotic precursor cells in the external granular layer (EGL) and migrate after their last mitosis to their final position in the internal granular layer (IGL.) Neither the proliferative cells of the EGL nor the post-mitotic migratory cells show immunoreactivity. The granule cells in the IGL remain unstained in the adult. The molecular layer stellate and basket cells also arise from the EGL, but in contrast to the granule cells they do exhibit moderate nuclear staining in the adult. Golgi type II interneurons scattered throughout the IGL and neurons of the deep cerebellar nuclei exhibit intense nuclear staining at all ages examined.

Our results identify several patterns of Hox 1.3 immunoreactivity characteristic of mature cerebellar neurons and indicate that immunoreactivity can vary systematically during development. Substantial expression of this protein is evidently not necessary for cell division nor for the migration or maturation of all neurons.

195.15 TRANSIENT EXPRESSION OF AN INSULIN GENE CONSTRUCT IN CATECHOLAMINERGIC PRECURSORS OF TRANSGENIC MOUSE EMBRYOS. S. Alpert*, D. Hanahan*, and G. Teitelman. (SPON: A. Suburo). Div. of Neurobiol., Cornell Univ. Med. Ctr., N.Y..N.Y. 10021 and *Cold Spring Harbor Lab., Cold Spring Harbor, N.Y. 11724.

Tyrosine hydroxylase (TH), the first enzyme of the catecholamine (CA) biosynthetic pathway, has been previously shown to be expressed during development of the insulin producing (β) cells of the islets of Langerhans (Teitelman, 1987). This finding raises the question as to whether CA neurons express endocrine traits at any time. It is, therefore, possible that the developmental regulation of insulin is such that it can be expressed in any cell type that can produce the CA biosynthetic enzymes. To examine this issue we used immunocytochemical procedures to examine the nervous system of a line of transgenic mice. These mice, RIP-Tag #2, which contain a hybrid gene consisting of the 5' flanking region of the rat insulin II gene linked to the protein coding sequences for the Simian Virus 40 large T antigen, were examined to detemine the pattern of expression of both Tag and insulin in neural tissues during development. These animals are known to express Tag in the β cells of their islets (Hanahan, 1985). No Tag or insulin containing cells were seen in the locus coeroleus, substantia nigra, sympathetic ganglia, or adrenal glands of adult RIP-Tag #2 mice. This suggests that in those CA cells the insulin gene is inactive.

Next we examined transgenic embryos of embryonic day 10 (e10) and e11. At e10, although no insulin immunoreactivity was detected, Tag was found in migrating neural crest cells from the rhombencephalic to the caudal region. The antigen was expressed only in neural crest cells that were distributed along the ventral pathway of migration, close to the neural tube. At e11 neural crest cells containing Tag were also seen migrating laterally between somites and ectoderm and then aggregating in areas where the sympathetic ganglia will form. In brain, cells containing Tag were seen in the basal plate of the germinal layer of the neural tube. Cells of the mantle layer did not contain Tag. At these stages a large number of Tag cells were seen in the di- and rhombencephalon, but not in mes- or telencephalon. When histological sections of RIP1-Tag #2 embryos were processed for the localization of two antigens in the same section it was found that neural crest cells foresake expression of Tag when TH first appears. Thus neuroblasts that contain TH did not express Tag. Likewise, in brain, cells containing TH present in the mantle layer did not contain Tag. In contrast, pancreatic cells containing TH also express Tag. We conclude that Tag is transiently expressed by cells of the peripheral and central nervous system. These cells, by their location, are identified as CA precursors. Since the expression of Tag is regulated by the insulin promoter the results suggest that the endogenous mouse insulin gene may be active in CA precursor cells and that the activity of the gene is turned off with the initiation of TH synthesis.

196.1 NEUROTENSIN BINDING IN RAT NUCLEUS BASALIS: EFFECTS OF LOCAL IBOTENIC ACID INJECTIONS. E. Szigethy, G.L. Wenk, J. Epelbaum and A. Beaudet. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4, The Johns Hopkins University, Baltimore, Maryland 21218, INSERM U-159, 75014 Paris, France.

Previous findings in our laboratory have indicated that the distribution of ^{125}I-NT binding sites in rat basal forebrain corresponds to that of magnocellular cholinergic neurons documented in this region. The aim of the present study was twofold: (1) to quantitatively determine the degree of overlap between cholinesterase-reactive and ^{125}I-NT labeled neurons in nucleus basalis magnocellularis (NBM) using adjacent section cholinesterase pharmacohistochemistry-radioautography in animals pretreated with bis-1-methylethylphosphofluoridate (DFP) and (2) to demonstrate that ^{125}I-NT labeled receptors are directly associated with these putative cholinergic cells, rather than with afferent inputs, by examining the effects of unilateral lesions of the NBM with the selective excitotoxin, ibotenic acid on ^{125}I-NT binding. For quantification, rats were injected with 2 mg/kg DFP and sacrificed by decapitation 10 hours later. For lesion studies, rats were stereotaxically infused with 1.0 µl ibotenic acid (25 nmol in 1 µl PBS) into the NBM and sacrificed 14 days later. In both cases, serial 10 µm thick frozen sections were incubated for 1 h at 4°C with 0.1 nM ^{125}I-NT (2000 Ci/mmole) in Tris buffer in the presence (non-specific binding) or absence (total binding) of excess native NT. After several buffer rinses, sections were fixed with 4% glutaraldehyde and processed for light microscopic radioautography using either film or conventional dipping techniques. Alternate sections were fixed by immersion in Zamboni's fixative and processed for AChE histochemistry according to the procedure of Genneser-Jensen (1971). Densometry measurements were obtained from film radioautographs using a bioquant computerized image analysis system. Between 75-90% of intensely AChE-reactive neurons in the NBM exhibited dense ^{125}I-NT binding in DFP pretreated animals while none of the cells having little or no AChE staining had such discrete cellular labeling. Ibotenic acid selectively abolished cholinesterase staining in the NBM and resulted in a concommitant decrease in ^{125}I-NT binding density in the ipsilateral NBM with respect to the lesion as compared to the contralateral side. In addition, a disappearance of individual ^{125}I-NT-labeled cells was observed in the lesioned area. These results suggest that the vast majority of cholinergic neurons in rat NBM possess NT receptors. It would be of interest to examine if similar changes in NT binding are observed in patients with Alzheimer's disease which involves degeneration of the basal forebrain cholinergic system. Knowledge of such alterations may provide insight into the pathogenesis of AD and offer new avenues for diagnosis and therapy. Supported by the Medical Research Council of Canada.

196.2 MONOAMINERGIC AND CHOLINERGIC SYSTEMS IN THE ROSTAL FOREBRAIN L. Heimer, L.F. Snavely, V.N. Luine, and L. Zaborszky. (Spon: R. Michaelis) Dept. of Otolaryngology and Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908; Rockefeller Univ., New York, NY 10021

In preliminary studies it has been shown that unilateral removal of the ascending brainstem pathways (Zaborszky et al., Soc. Neurosci. Abst. 12:571, 1986), or injection of 6-hydroxydopamine in the substantia nigra (Slevin et al., 1986) or the ascending catecholaminergic pathways (Zaborszky and Luine, J. Cell. Biochem., Suppl. 11D:187, 1987) cause significant reductions of choline acetyltransferase (ChAT) immunoreactivities in forebrain areas containing the cholinergic projection neurons. Moreover, 5,7-DHT lesions of the ascending monoaminergic bundles resulted in similar reductions of ChAT levels in the basal forebrain (Zaborszky and Luine, 1987). The present study was designed to investigate the topographic relationship between cholinergic cells and monoaminergic fibers in the rostral forebrain.

Rats were perfused by a buffered picric acid-paraformaldehyde fixative (Somogyi and Takagi, 1982). Adjacent sections of the rostral forebrain were processed for the simultaneous visualization of ChAT/tyrosine hydroxylase or ChAT/serotonin using the DAB-intensified/DAB technique (Gorcs et al., 1986) or the technique developed by Levey et al., utilizing two different chromagens (DAB/BDHC). A significant number of TH-labeled fibers surround cholinergic cell bodies and dendrites in the sublenticular substantia innominata, and globus pallidus. Furthermore, a few TH-containing fibers are in close proximity to cholinergic neurons in the dorsal part of the horizontal limb and the vertical limb of the diagonal band (DB). In addition, cholinergic cells of the dorsal and ventral striatum are embedded in massive TH-terminal arborizations. Successfully stained sections were embedded in resin. Preliminary EM studies revealed a few TH-terminals synapsing on cholinergic dendrites. Serotonin-labeled terminal varicosities were especially abundant around cholinergic cells in the dorsal part of the horizontal limb of the DB. In addition, a few terminal like processes were seen along the cholinergic cells in the vertical limb of the DB. An electron microscopic analysis is now being performed in order to determine if cholinergic cells indeed receive serotonin-containing terminals.

These findings support the idea that the monoaminergic afferents transynaptically modulate the expression of ChAT in the basal forebrain and this may be particularly relevant to Alzheimer's disease in which a marked loss of noradrenergic (locus coeruleus) and serotoninergic (raphe) neurons are reliably observed. Supported by USPHS Grant NS.23945,17743 and by a Grant from the American Health Association Foundation.

196.3 CHOLINERGIC CONTROL OF AMINO ACID AND MONOAMINE TRANSMITTERS IN SLICES OF NUCLEUS ACCUMBENS. M. Gongwer*, P. Robinson* and W.J. McBride. Depts. Psych. & Biochem., Inst. Psych. Res., Indiana Univ. School of Medicine, Indianapolis, IN 46223.

The effects of two cholinergic agonists, oxotremorine (OXO) and nicotine (NIC), on the K^+-stimulated endogenous release of dopamine (DA), norepinephrine (NE), serotonin (5-HT), glutamate (GLU), GABA and aspartate (ASP) from slices of nucleus accumbens were used to assess cholinergic control of the amino acid and monoamine transmitters in this CNS region. The nucleus accumbens was dissected from adult male Wistar rats and sliced into 300 x 300 um sections with a McIlwain tissue chopper. For each experiment, slices from 3 animals were pooled and distributed among four columns (control, 50, 100 and 200 uM agonist). Slices were superfused for 30 minutes each, first with LKE (in mM : 120 NaCl, 4.75 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 D-glucose and 1.0 EGTA equilibrated with 95% O_2/5% CO_2), followed with HKE (same as LKE except for 35 mM KCl and 90 mM NaCl) and then with HKC (same as HKE except for 2.5 mM $CaCl_2$ and no EGTA). Initial studies, using 20, 35 and 60 mM K^+, demonstrated that 35 mM K^+ produced a significant, but submaximal, endogenous release of the amino acids and monoamines above resting efflux values.

Using the above protocol, it was determined that DA, NE, 5-HT, GLU, GABA and ASP were released by 35 mM K^+ in a Ca^{2+}-dependent manner. The finding that 16 mM Mg^{2+} inhibited the K^+-stimulated, Ca^{2+}-dependent release provided further support for a transmitter-like release for these monoamines and amino acids. The endogenous release of alanine and glycine was also measured. The release of these two amino acids was not stimulated by elevated K^+ in either the presence or absence of Ca^{2+}. Furthermore, 16 mM Mg^{2+} had no affect on the efflux of alanine and glycine, thereby indicating that these two amino acids are not released in a transmitter-like manner.

OXO and NIC (at a concentration each of 200 uM) potentiated ($p < 0.05$; N=10) the 35 mM K^+-stimulated, Ca^{2+}-dependent release of 5-HT 180 and 90% above control levels, respectively. NIC (200 uM), but not OXO, also potentiated ($p < 0.05$; N=12) the K^+-stimulated, Ca^{2+}-dependent release of GLU 63% above control values. No effects were observed for these cholinergic agents on the K^+-stimulated, Ca^{2+}-dependent release of NE, DA, GABA or ASP. The data suggest both a muscarinic and nicotinic stimulation of the serotonergic system in the nucleus accumbens and only a nicotinic action on the glutamatergic system in this region. (Supported in part by AA-03243 and AA-07462).

196.4 **INVESTIGATION OF A MONOAMINERGIC ROLE IN THE MODULATION OF [3H]HEMICHOLINIUM-3 BINDING SITES IN RAT BRAIN.** M. A. Feldman*, M. D. Saltarelli, and J. T. Coyle (SPON: M.C. Rogers). Depts. of Anesthesiology and Critical Care Medicine and Neuroscience, The Johns Hopkins University School of Medicine, 600 N. Wolfe St., Baltimore, MD 21205.

Monoaminergic systems are attractive candidates as regulators of central cholinergic activity. The role of dopaminergic fibers as inhibitory inputs regulating cholinergic function in the striatum is well established. Evidence from diverse studies suggests modulatory influences of norepinephrine, dopamine, and serotonin on acetylcholine turnover and release.

Changes in cholinergic neuronal activity are associated with parallel increases or decreases in the capacity of the sodium-dependent high affinity choline uptake (SDHACU) system. SDHACU activity *in vitro* has been shown to serve as an index of the antecedent function of cholinergic neurons *in vivo*. Hemicholinium-3 (HCh-3) is a potent, selective, competitive inhibitor of SDHACU. Alterations in the velocity of SDHACU are correlated with changes in the actual number of carrier sites as measured by high-affinity binding of [3H]HCh-3. We have investigated the effects of monoaminergic modulation on cholinergic activity as measured by *in vitro* high-affinity binding of [3H]HCh-3.

To investigate the effect of release of endogenous catecholamines, rats received amphetamine sulfate (10 mg/kg I.P.). [3H]HCh-3 binding was assayed *in vitro* after 60 minutes. No statistically significant changes were observed in cortex (N=3), hippocampus (N = 3), or striatum (N = 8). The monoamine uptake inhibitor cocaine (30 mg/kg I.P.), however, increased cortical [3H]HCh-3 binding from 45 ± 4 to 57 ± 3 fmol/mg protein (X ± S.E.M., N = 7, 30% increase, $P < 0.05$). The discrepancy between cocaine and amphetamine effects may represent differences in the profile of monoaminergic responses or in the response of other neuromodulatory systems *in vivo*.

To investigate the influence of ascending catecholaminergic systems on the choline carrier, the adrenolytic drug 6-hydroxydopamine was injected bilaterally into the medial forebrain bundle. After 7 days, the animals were sacrificed, and specific regional [3H]HCh-3 binding was measured in crude membrane homogenates. When compared to saline-injected controls (237 ± 5fmol/ mg protein, X ± S.E.M., N = 3), a modest (12%) reduction in binding was observed in striatum (209 ± 4 fmol/mg protein, N = 4, $p<0.01$). Examination of neurotransmitter levels, *in vivo* drug stimulations, and *in vitro* regulation experiments will be necessary for interpretation of these preliminary data.

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196.5 PEPTIDERGIC REGULATION OF CATECHOLAMINE SECRETION IN EMBRYONIC SYMPATHETIC NEURONS.

D.C. Valenta, J.E.G. Downing*, and L.W. Role. Columbia Univ.P & S, Dept. of Anat. & Cell Biol. & Ctr. Neurobiol.& Behav., NY, NY 10032.

The neuropeptide substance P (SP) is present in embryonic chick sympathetic ganglia (Hayashi et al, 1983) and is released by ganglionic depolarization (L.Role and J.Krause, unpublished observations). SP enhances the decay of ACh-evoked currents in voltage-clamped chromaffin cells and sympathetic neurons (Clapham & Neher 1984; Role, 1984a). Preliminary studies also indicate that SP increases the decay of synaptic currents in innervated sympathetic neurons *in vitro* (Role1984b). We have investigated the relationship between SP modulation of acetylcholine receptor (AChR) function and neurotransmitter release in sympathetic neurons by measuring both ACh-evoked currents and catecholamine release in the presence and absence of SP.

Dispersed ED10-11 chick sympathetic neurons voltage-clamped to rest potential were exposed to ACh, carbachol, or 1,1-dimethyl-4-phenyl-piperazinium (DMPP) with or without SP. Macroscopic currents decay in the continued presence of these agonists in a manner that is dependent on agonist concentration. The rate of decay of currents evoked by desensitizing concentrations of ACh, carbachol and DMPP are greatly enhanced by co-application of SP. In contrast, SP has no effect on the rate of decay of macroscopic currents evoked by low (non-desensitizing) concentrations of agonist.

Complimentary experiments were conducted to examine the effects of SP on transmitter release from sympathetic neurons. Cultures of ED 10-13 sympathetic neurons were prelabeled with ^{3}H-NE, and then incubated with cholinergic agonists in the presence and absence of SP. ^{3}H-NE remaining in the cells was determined and net stimulated ^{3}H-NE release was quantitated as a percent of total intracellular content.

Stimulation of sympathetic neurons with nicotinic (DMPP, nicotine), muscarinic (methacholine, muscarine), and mixed cholinergic agonists (ACh, carbachol) elicits significant ^{3}H-NE release. The effects of carbachol are dose-dependent, and are blocked by hexamethonium + atropine. Maximal concentrations of carbachol (0.5-1 mM) stimulate net release of up to 30.2 ± 0.86% of total intracellular ^{3}H-NE. While SP alone (10-20 μM) has no effect on catecholamine release, SP significantly inhibits ^{3}H-NE secretion elicited by intermediate to maximally effective concentrations of ACh, carbachol and DMPP (up to 90%, $p < 0.001$). In contrast, SP has little or no effect on NE secretion evoked by non-desensitizing concentrations of DMPP and carbachol.

The transmitter release and electrophysiological data presented here are consistent with the hypothesis that SP enhances agonist-induced desensitization of the neuronal AChR. The observation that SP can inhibit NE release from sympathetic neurons *in vitro* also suggests that SP may modulate lumbar sympathetic outflow *in vivo*.

Refs: Hayashi et al.,1983 Neurosci 10: 31; Clapham and Neher 1984 J. Physiol. 347: 255; Role 1984a 81: 2924 PNAS; Role, 1984b Soc NS abst 10: 1117.

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196.6 SINGLE CHANNEL PROPERTIES OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS: MODULATION BY SUBSTANCE P.

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The modulatory peptide Substance P (SP) specifically enhances the rate of ACh-elicited current decay in voltage clamped chromaffin cells (Clapham and Neher, 1984) and sympathetic neurons (Role, 1984). These and other studies indicate that SP enhances agonist-induced desensitization (see Valenta et al., this volume). Here we report preliminary data that: 1) describe the single channel characteristics of the sympathetic neuronal acetylcholine receptor (AChR), and, 2) suggest that SP enhances AChR desensitization via a second-messenger pathway.

Sympathetic ganglia were dissected from embryonic chickens (ED 9-11), dispersed to single cells and maintained in tissue culture from 4-14 days prior to use. Single channel currents were recorded at room temperature (22-27 °C) using either the cell-attached (with 10-20 μM ACh in the patch pipette) or the outside-out configuration (with ACh applied onto the isolated membrane from a pressure ejection pipette).

The predominant ACh-elicited current observed in cell-attached patches has a unitary current amplitude of 2.1 ± 0.06 pA (n=17 patches) at rest, a single channel conductance of 34 ± 2 pS (n=12), and an extrapolated reversal potential 30 mV depolarized from rest. The mean channel open-time is 4.8± 0.46 msecs (n=5 patches, 24-25 °C) at the resting potential and, unlike muscle AChRs, either remains constant or decreases slightly with hyperpolarization. Single channel currents with comparable conductances and kinetics were observed in outside-out patches in response to pressure-ejected ACh.

We used the cell-attached configuration to determine if the SP-induced increase in AChR desensitization depends upon a second-messenger pathway. Single channel currents were recorded before and after a 10 second application of 20 μM SP to the remainder of the cell. In two preliminary experiments the frequency of channel openings decreased by 4-7 fold within 5-40 secs following SP application. The single channel current amplitude remained unchanged but the mean open-time of the channels decreased by 2-3 fold (eg. from a control $\tau_o = 3.65 \pm 0.27$ to 1.3 ± 0.18 msecs, following SP). Because the patch pipette physically isolates the AChRs being monitored from the applied SP, these results suggest that SP can modulate AChR desensitization via a second-messenger system. Preliminary evidence indicates that a protein kinase C-dependent phosphorylation may be involved in enhancing neuronal AChR desensitization (see Downing et al., this volume).

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196.7 MODULATION OF NEURONAL ACETYLCHOLINE RECEPTOR DESENSITIZATION BY GANGLIONIC PEPTIDES AND AGENTS THAT ACTIVATE PROTEIN KINASE C.

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In addition to acetylcholine (ACh), avian lumbar sympathetic ganglia contain a number of peptides including substance P (SP) and somatostatin (SS; Hayashi, M. et. al. Neurosci. 10: 31-39, 1983). We are interested in the interaction of these peptides with ACh in a possible modulation of synaptic function. In voltage clamped sympathetic neurons inward current through the AChR channel decays in the continued presence of agonist, and this decay is dependent on agonist concentration. Both SP and SS enhance the rate of ACh-induced current decay. The increase in the rate of ACh-induced current decay with both peptides is manifest only at concentrations of agonist that evoke noticable desensitization on thier own (see abstract by Valenta et al., this volume), suggesting that the peptides potentiate agonist induced desensitization.

Recent observations from cell attached patch recordings (Simmons et al, this volume) demonstrate that the behavior of single neuronal AChR channels can be modulated by SP. This recording configuration indicates some second messenger involvement. In order to pursue the second messenger mechanism underlying this neurotransmitter modulation of excitability, complimentary biochemical and biophysical studies were performed. Our preliminary studies demonstrate that both SP and nicotine cause a rapid and transient rise in phosphatidylinositol (PI) turnover. An approximately two fold increase in the production of inositol phosphates (IP_1, IP_2, & IP_3) is seen in response to maximal concentrations of either nicotine or SP. Since this enhancement of PI metabolism may result in activation of protein kinase C (PKC) we examined whether PKC might regulate nicotinic AChR function in neurons. We have compared the effects of SP, and several activators of PKC (diglyceride 1-oleyol-2-acetyl glycerol, phorbol 12,13-diacetate and phorbol 12,13-dibutyrate). Neurons were voltage clamped at the resting potential and the response to ACh tested before and after treatment with SP or various activators of PKC. We find that all of these agents enhance the rate of decay of ACh-induced current without affecting peak current amplitude or cellular input resistance. The PKC activators were ineffective if applied concurrently with ACh: Significant effects could be detected only after 60 seconds of pretreatment. A phorbol that does not increase PKC activity (4β phorbol) was ineffective in enhancing the decay of ACh-induced current. Thus, the effects of these agents on AChR function are likely to be mediated via their interaction with PKC, rather than by direct interaction with the AChR channel. More detailed analysis reveals that both SP and the PKC activators enhance the decay of the ACh-induced current by decreasing the time constant of the slow component and decreasing the contribution of this component to the overall current decay. In addition to the mimicry of SP effects by activators of PKC, preliminary results indicate partial occlusion of SP's effect when applied to neurons pretreated with PKC ativators. Our data, therefore, suggest that activation of PKC may mediate a slow phase of neuronal AChR channel desensitization. Experiments to determine if this kinase mediates SP's action are underway. *Supported by awards to LR from the NIH (NS22061), the Sloan Fnd. and the Klingenstein Fdn.*

196.8 ACETYLCHOLINE AND FMRFamide, ACTING THROUGH DIFFERENT RECEPTORS, BOTH ACTIVATE 'S'-LIKE K CURRENT AND SUPPRESS Ca CURRENT IN APLYSIA NEURONS.

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In certain neurons in the abdominal ganglion of Aplysia californica, the endogenous neuropeptide FMRFamide induces a slow K current resembling the serotonin-sensitive 'S' current (Brezina et al. 1987, J. Physiol. 382, 267), and simultaneously suppresses the Ca current, and consequently the Ca-dependent K current (Brezina et al. 1987, J. Physiol. 388). These effects of FMRFamide may be mediated by a GTP-binding protein, since they are mimicked by injection into the cell of the non-hydrolyzable GTP analog GTP-γ-S (Brezina & Eckert, 1986, Neurosci. Abstr. 12, 1341). Further experiments (using two-electrode voltage clamp of cells L2-L6, and puffed or bath application of neurotransmitter) reveal that acetylcholine (ACh) also has very similar effects in these cells. Thus, ACh activates a slow K current, most likely the current responsible for the slow phase of the two-component cholinergic inhibition studied by Kehoe (1972, J. Physiol. 225, 85), that resembles the FMRFamide-induced 'S'-like current; in particular, the ACh-induced current is also suppressed by injection of cAMP into the cell, and activated by injection of GTP-γ-S. Furthermore, the K-current responses to FMRFamide and ACh are not additive, supporting further the conclusion that ACh, like FMRFamide, activates the 'S'-like current. FMRFamide and ACh act, however, through independent receptors, since the response to either agent can be desensitized without affecting the response to the other, and the response to FMRFamide is unaffected even when the response to ACh is completely blocked by superfusion of 0.2-1 mM arecoline or phenyltrimethylammonium. These findings provide some support for the theory of Swann & Carpenter (1975, Nature 258, 751) that the various neurotransmitter receptors are linked to only a few types of ion channels in Aplysia. In addition to activating the K current, ACh also suppresses both the early inward and the late outward currents elicited by depolarizing voltage steps in Ca-containing solution, an effect indistinguishable from the action of FMRFamide analyzed previously as suppression of the Ca and Ca-dependent K currents. All of these effects of ACh make it, like FMRFamide (Brezina et al. 1987, J. Physiol. 388), a candidate for an agent of presynaptic inhibition in Aplysia. Interestingly, while ACh mimics the effects of FMRFamide on the 'S'-like, Ca and Ca-dependent K currents, it does not mimic yet another effect of FMRFamide in these cells, activation of a relatively fast Na current (Ruben et al. 1986, J. Neurosci. 6, 252). Instead, ACh activates a fast Cl current (Kehoe, 1972, J. Physiol. 225, 85); significantly, neither fast response is affected by GTP-γ-S.

196.9 SELECTIVE DEPRESSION BY ADENOSINE OF CHOLINOCEPTOR RESPONSES IN RAT HIPPOCAMPAL NEURONES. T.W. Stone and P.A. Brooks*, Dept of Physiology, St. George's Hospital Medical School, London University, London, SW17, UK.

We have recently reported an inhibitory effect of adenosine and related purines on the carbachol induced suppression of evoked potentials in the rat hippocampal slice. (J. Physiol. in press). In the present work we have attempted to confirm the selectivity of that effect by comparing neuronal sensitivity to carbachol and excitatory amino acids on single cells in these slices.

Slices were prepared from the hippocampi of adult male rats and stored at room temperature in a humidified atmosphere of 5% CO^2 in oxygen until required. Single slices (400μm thick) were then transferred to the recording chamber and superfused with Krebs-bicarbonate medium at 30°C. Carbachol and amino acids were applied by microiontophoresis from five-barrelled pippettes and cell recordings made via single electrodes glued alongside. Adenosine and theophylline were added to the perfusing medium.

To date 8 cells have been tested in the CA1 region. Carbachol, N-methyl-aspartate (NMA), kainate and quisqualate all excited each of these cells via accepted receptor populations as indicated by selective antagonists. Adenosine 1-10uM selectively depressed sensitivity to carbachol on 6 of these cells with significantly less effect on NMA responses and little change of kainate or quisqualate responses. The reduction of NMA sensitivity may reflect some inhibition of background cholinergic tone which thus lowers excitability and increases the block of NMA activated channels by magnesium. Atropine (1μM) produced a similar effect. At adenosine levels of 30μM or higher sensitivity to kainate was also diminished though quisqualate remained relatively unaffected even at 100μM adenosine. The inhibitory effect of adenosine could be prevented by theophylline, 10-50μM.

The results support the view that adenosine may exert a selective regulation of acetylcholine receptor sensitivity in the CNS.

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196.10 AUTOCRINE EFFECTS OF ADENOSINE IN GH_3 CELLS ARE MEDIATED VIA THE A1 RECEPTOR. T.M. Delahunty*, J. Linden*, E.L. Hewlett* and M.J.Cronin. (SPON: M. Merickel). Univ. Virginia, Charlottesville, VA 22908.

The purine nucleosides adenosine and 2'-deoxyadenosine can regulate the activity of a number of cell types, including those of the nervous and endocrine systems. High concentrations (2 mM) of adenosine inhibited by 30% pituitary secretion and synthesis of growth hormone and prolactin (Endocrinology 99:1612, 1976). Adenosine at more physiological concentrations (μM) inhibited by 40% growth hormone and prolactin release from GH_4C_1 and GH_3 pituitary tumor cells (Endocrinology 117:2330, 1985). Two functionally and pharmacologically distinct adenosine receptors have been identified, the A1 and A2 receptors, which inhibit and stimulate adenylate cyclase activity, respectively. No one, to our knowledge, has characterized the receptor subtype or measured cyclic AMP (cAMP) metabolism associated with adenosine in pituitary cells. Accordingly, studies were carried out on plated GH_3 cells for 10-20 min at 37°C, and cellular c-AMP was measured by radioimmunoassay. All studies were independently repeated at least twice, and significant differences were determined by ANOVA. The adenosine agonists R-phenylisopropyladenosine (PIA), 5'-N-ethylcarboxamide adenosine (NECA) and adenosine all significantly inhibited cAMP accumulation from 0.1 nM to 0.1 mM; all agonists produced the same maximal inhibition of 50% in basal levels and a maximal 75% inhibition of vasoactive intestinal peptide (VIP, 100 nM) stimulated levels. The order of potency was PIA > NECA > adenosine, typical of A1 receptors. The potent and selective A1 antagonist BWA 1433u blocked the inhibitory action of PIA. Pertussis toxin (50 ng/ml) blocked the adenosine-mediated inhibition of cAMP, while somatostatin (100 nM) and carbachol (1 μM), agents which also inhibit cAMP in GH_3 cells, added to the reduction in cAMP imposed by adenosine. In the presence of RO72956, a phosphodiesterase inhibitor devoid of adenosine receptor antagonist activity, addition of adenosine deaminase (Adase, 1 U/ml) to destroy endogenous adenosine significantly increased cAMP content by 50%. This suggests that physiologically active concentrations of adenosine are secreted by GH_3 cells. In contrast, the Adase inhibitor EHNA (erythro-9-2-hydroxy-3-nonyl adenine HCl) decreased basal cAMP levels by 20%. Adase (0.6 U/ml) and BWA 1433u (1 μM) increased the efficacy of VIP by 26% and 180%, respectively. These results indicate that adenosine is produced in and acts on GH_3 cells via an A1 receptor to inhibit cAMP accumulation. A2 receptor activity was not observed. We recommend the use of an adenosine receptor antagonist or Adase to aid in characterizing the role of other agents that modify cAMP levels in GH_3 cells by attenuating the inhibitory action of endogenous adenosine. (supported by NIH DK37490 & 535269)

196.11 GLYCINE POTENTIATES GLUTAMATE ENHANCEMENT OF [3H] TCP BINDING IN RAT HIPPOCAMPAL MEMBRANES. D. W. Bonhaus and J. O. McNamara. V. A. Medical Center and Departments of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27705.

The NMDA-subtype of excitatory amino acid receptors has been implicated in several forms of neuronal plasticity. Glycine, traditionally thought to be an exclusively inhibitory neurotransmitter, has been recently demonstrated to selectively enhance responses to NMDA in cultured mouse neurons (Johnson and Ascher, Nature, 235:529-531, 1987). The molecular basis of this enhancement is unclear, but an allosteric modulation analogous to the GABA receptor-channel complex is one possibility.

To test this idea, we measured the effects of glycine and glutamate on the binding of the phencyclidine analog, [^{3}H]-N-(1-[2-thienyl]cyclohexyl)piperidine (TCP). Electrophysiologic studies suggest that phencyclidine binds to the open configuration of the channel coupled to the NMDA receptor; we therefore used [^{3}H]TCP as a biochemical index of an NMDA receptor coupled response.

Specific [^{3}H]TCP binding (difference in absence and presence of 10^{-6}M phencyclidine) was measured in a Tris pH 7.7 buffer with well-washed membranes prepared from rat hippocampus. Glycine alone had no effect on [^{3}H]TCP binding at concentrations below 5 uM. Glutamate alone produced a 140% increase of [^{3}H]TCP binding with EC50 of 0.1 uM, thereby confirming the results of Loo et al., E.J.P. 123:467-468, 1986. Inclusion of glycine (1 uM) potentiated the effect of glutamate (basal [^{3}H]TCP binding was 66 fmol/mg; with addition of 5 uM glutamate TCP binding was 92 fmol/mg; with addition of 5 uM glutamate plus 1 uM glycine TCP binding was 130 fmol/mg). The effect of glycine was not inhibited by 10^{-6} M strychnine.

These biochemical findings support the hypothesis of a direct physical coupling among an NMDA receptor, a strychnine insensitive glycine receptor and a cation channel in mammalian hippocampal membranes. This may provide a rapid in vitro assay for agonists and antagonists of the strychnine insensitive glycine receptor.

196.12 BEHAVIORAL EVIDENCE FOR INTERACTIONS BETWEEN PHENCYCLIDINE AND NMDA RECEPTORS. G.E. Handelmann, L.J. Christine*, L.L. Mueller*, P.C. Contreras, and T.L. O'Donohue. Central Nervous System Diseases Research, G.D. Searle & Co., St. Louis, MO 63198.

Phencyclidine (PCP) has been shown to be a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor (Martin, D., Lodge, D., Neuropharmacol., 24:999, 1985), and the distribution of PCP binding sites closely parallels those for NMDA (Yound, A., Eur. J. Pharmacol., 123:173, 1986). A relationship between PCP and NMDA receptors has therefore been proposed which would allow PCP an inhibitory influence on the NMDA receptor gated ion channel. If such a relationship exists, PCP should produce the same effects on neural function as an NMDA competitive antagonist. We have therefore compared the behavioral effects of low doses of PCP to those of (D,L)2-amino-7-phosphonoheptanoic acid (APH) on several behavioral measures known to be sensitive to AHP.

APH has been shown to have anxiolytic activity (Stephens, D.N. et al., Psychopharmacol., 90:166, 1986; Bennett, D., Amrick, C., Life Sci., 39:2455, 1986), to produce muscle relaxation (Turski, L., et al., Neurosci. Lett., 53:321, 1985), and to impair spatial memory (Morris, R.G.M., et al., Nature, 319:774, 1986). We found PCP to be more potent than APH in producing anxiolytic effect in two types of anticonflict test paradigms. PCP increased exploration of a "plus maze", and increased punished licking in the Vogel paradigm at lower doses than APH. Similarly, PCP was more potent than APH in producing muscle relaxation. Finally, PCP (1 mg/kg IP) impaired long-term memory of a learned spatial discrimination in a t-maze, analogous to APH.

These data, indicating similar behavioral effects of PCP and APH, support the hypothesis that there are interactions between PCP and NMDA receptors, and that PCP may produce inhibitory modulation of the NMDA receptor and associated sodium channel.

196.13 DIGITAL BRAIN ATLAS FOR ANATOMICAL DATA COMPARISON AND INTEGRATION. A.R. Moser*, W.T. Rogers*, L. Rinaman* S.M. Diamond* and J.S. Schwaber (SPON: K.M. Spyer) Med. Prod. Dept. (LR, SMD & JSS) and Eng. Dept. (ARM & WTR) E.I. du Pont de Nemours & Co., Wilmington, DE 19898

Stereotaxic atlases are commonly used for comparison and integration of data from many animals by plotting data onto standard atlas plates. This function has inherent limitations since the plane of section in every experiment is unique and only approximates that of the atlas. In addition, atlas plates represent only a sampling of tissue sections, thus experimental sections can only be approximately matched to corresponding atlas plates. Even if the experimental tissue section falls in the plane and level of an atlas plate, it is distorted with respect to the atlas plate due to processing.

We have developed a digital brain atlas which overcomes these limitations. This atlas consists of a three dimensional image acquired from unstained serial coronal sections. The sections were floated on surfactant to avoid distortion artifacts, and were imaged using a high resolution solid state video camera. The 3-D image, obtained by reconstruction from the serial sections, fully represents the brain of a 300 gm male Sprague-Dawley rat at a resolution of approximately 30 microns. The existence of the 3-D image in digital form allows resectioning of the image in arbitrary planes by defining an origin and a normal vector to those planes. In addition this data can be scaled and warped, resulting in electronic images that geometrically match the experimental tissue sections. This match defines a coordinate transformation, allowing anatomical data from any number of animals to be integrated into a database relevant for the species rather than for the individual. The strength of this approach is that it permits high resolution comparison to be made across treatments, and the integration of multiple data sets in a common coordinate framework.

We are testing the extent to which neuronal structures are conserved and thus the limits of resolution with which data matches can be made. This is being done by matching two reconstructed high resolution mappings of the entire population of neurons efferent to the vagus nerve.

196.14 MOLECULAR SANDWICHES: BINDING SITES ON PEPTIDES AND PROTEINS FOR AROMATIC NEUROTRANSMITTERS AND DRUGS. R. S. Root-Bernstein. Neurobiochemistry, T-85, Veterans Administration Hospital, Brentwood, CA 90073.

"Molecular sandwich" is a term referring to the interaction of aromatic molecules with the cyclic side chains of peptides and proteins. No specific definition of a molecular sandwich exists, however. I suggest the following: stereospecific intercalation of a cyclic molecule between a pair of neighboring cyclic residues of a protein, polypeptide, or peptide. The intercalation may result in pi-pi stacking bonds if aromatic residues are involved, or in hydrophobic (van der Waals) interactions if the binding molecule or amino acid side chain is not aromatic. Specificity is conferred upon the molecular sandwich by the geometry and charge (if any) of the residues forming the sandwich and by the chemical affinities of the amino acid side chains immediately adjacent to the cyclic side chains. These adjacent residues will generally have one of two functions: 1) to form the stereospecific conformation of the sandwich; or 2) to stabilize the binding of an appropriate molecule into the sandwich by means of hydrogen or ionic bonds, van der Waals forces, or charge-transfer complexing with residues on the binding molecule. Theoretically, over a million distinct molecular sandwiches may exist for hexapeptide sequences. Many more sandwiches may be formed by secondary and tertiary protein conformations.

Molecular sandwiching has been reported: serotonin (5HT) binds to similar sequences on LHRH, ACTH, MSH, and myelin basic protein (Root-Bernstein and Westall, Brain Res Bull 12(4) (1984) 425); norepinephrine (NE) and dopamine (DA) bind to the enkephalins and morphiceptin (Root-Bernstein, Brain Res Bull 18(4) (1987)); the tricyclic antidepressants bind to aromatic residues on calmodulin (Reid, J Theor Biol 105 (1983) 63); and various interferons bind differentially to agarose—bound aromatic amino acids and dipeptides (e.g., L-Trp-L-Trp or L-Trp-L-Tyr) (Sulkowski, et al., J Biol Chem 251 (1976) 5381). The concept of molecular sandwiching may be useful for elucidating binding sites for neurotransmitters and drugs on peptides and proteins; for explaining co-transmission of particular peptides with monoamines; for understanding drug activity; and for designing peptides and drugs with specific binding activity.

CHARACTERIZATION OF CHOLINERGIC RECEPTORS I

197.1 ALTERATION IN THE EXPRESSION OF ACETYLCHOLINE RECEPTORS AS A FUNCTION OF THE CELL CYCLE. S. Bursztajn and Y. J. Jong. Neurology and cell Biology Department, Program in Neuroscience, Houston, Texas 77030.

The formation of myotubes is the result of a number of divisions by myoblasts with the final alignment and fusion into multinucleated cells. Previous studies indicate that once myoblasts fuse they withdraw from the cell cycle. We were interested in determining whether all myotube nuclei are post-mitotic, and how the expression of acetylcholine receptors (AChRs) changes when cells are grown in the presence or absence of mitotic inhibitors or stimulators. Studies were carried out on chick myotube cultured cells exposed to cytosine arabinoside (ARAC) or to phorbol 12-myristate 13-acetate (PMA), which we have previously shown to cause dispersal of AChR clusters and the suppression of new AChR synthesis. The number of surface AChRs assayed five days after plating taken at various time periods within 24 hours stayed constant in ARAC treated cells, whereas cells not exposed to ARAC increased their surface AChR number by 23% ± 3%. Cells exposed to ARAC and phorbol esters showed 66% ± 5% decrease in AChR number as compared to ARAC treated controls and a 74% ± 6% decrease when compared non-ARAC treated controls. Removal of phorbol esters from culture media resulted in the return of AChRs to control values. Measurement of total DNA revealed a significant increase in total µg of DNA per dish in phorbol ester treated cells. Cells treated with phorbol, pulsed for one hour with ^{3}H-thymidine and counted in a liquid scintillation counter incorporated 33% ± 4% more ^{3}H-thymidine than controls. In order to differentiate between the dividing cells which are myoblast and the ones which are fibroblasts, double label experiments were carried out. Cells were labelled with ^{3}H-thymidine and a monoclonal antibody to myosin, and subjected to autoradiography. By interchanging filters, both the silver grains over the nuclei and the myosin staining of myotubes were readily visualized. The number of nuclei per myotube segment that incorporated ^{3}H-thymidine was quantitated in the presence and absence of phorbol esters. Cells treated with phorbol esters showed a four fold increase in the number of nuclei per myotube labelled as compared to non-treated controls. Those myotube nuclei which incorporated ^{3}H-thymidine may have arisen either by immediate fusion with a myotube during the division cycle, or by division that occurred within the myotube. Our studies indicate that in a mature myotube culture, the dividing myoblasts contribute toward the increased expression of AChRs, but a continuous proliferative state results in dedifferentiation and suppression of AChR synthesis. Supported by NIH (NS24377) and NSF-(8515025) grants.

197.2 PHOSPHORYLATION OF ASSEMBLED AND UNASSEMBLED ACETYLCHOLINE RECEPTOR SUBUNITS IN CULTURED CHICK MUSCLE CELLS. A. Ross, J. Schmidt, and J.M. Prives (Spon. B. Walcott) Departments of Anatomical Sciences and Biochemistry, State University of New York, Stony Brook, NY 11794.

The four subunits of the nicotinic acetylcholine receptor (AChR) assemble posttranslationally to form the functional ligand gated ion channel that is expressed on the muscle cell surface. The molecular basis for dynamic events in subunit assembly, such as subunit-subunit recognition and conformational rearrangements associated with channel formation is not understood, but may well involve posttranslational modification of AChR subunits (J.P. Merlie, Cell 36;573-575, 1984).

To investigate the potential contribution of phosphorylation-dephosphorylation to the regulation of AChR subunit assembly, we have developed procedures that utilize immunoprecipitation of nonassembled and assembled AChR subunits from extracts of muscle cells labeled with (35S) methionine or with 32Pi. Noncrossreactive antibodies directed against specific subunits, as well as both polyclonal and monoclonal antibodies to AChR are used for immuno-precipitations, sucrose density gradients are utilized to separate unassembled subunits from assembled oligomeric AChR, and these subunits are resolved by SDS-PAGE.

We have found that two AChR subunits, the gamma subunit (Mr 50kDa) and the delta subunit (Mr 55kDa) are phosphorylated in intact chick muscle cells. When cell extracts were fractionated on sucrose gradients and the fractions immunoprecipitated with an antisera which recognized both assembled and unassembled delta subunit, it was found that this subunit is phosphorylated in both the assembled and the unassembled state. A quantitative immunoblotting technique was utilized to show that the pool size of assembled delta subunit greatly exceeded that of the unassembled delta subunit, and by comparing the phosphate content of the delta subunit in the assembled and unassembled pools it was deduced that the unassembled delta subunit is more highly phosphorylated than the assembled delta subunit. These results indicate that phosphorylation of the delta subunit precedes assembly into the oligomer, and that a dephosphorylation of this subunit occurs concomitantly with subunit assembly. These findings suggest that phosphorylation/dephosphorylation may play a role in the regulation of AChR assembly. Our current experiments are aimed at further testing this possibility.

Supported by grants from NSF and AHA.

197.3 INTERNALIZATION OF THE ACETYLCHOLINE RECEPTOR: CHARACTERIZATION OF ACIDIFIED COMPARTMENTS. J.S. Park* and S. Bursztajn (SPON: Y. Tomozawa). Department of Neurology, Program in the Neurosciences. Baylor College of Medicine, Houston, Texas 77030.

We have found that when the nicotinic acetylcholine receptor (AChR) is internalized, it passes through endosome-like organelles before reaching the lysosomes. Because low pH has been shown to play an important role in endosome and lysosome function, we are interested in determining what organelles in the AChR internalization pathway have low pH. AChR endocytosis in cultured chick myotubes was initially studied by incubating the cells with a colloidal gold probe (Mab 35-gold, 12nm) made with a monoclonal Ab against the AChR (Tzartos, et al., J.Biol. Chem., 256:8635, 1981) and tracing the intracellular distribution of Mab 35-gold vs. time by electron microscopy. At time 0, 80% of the Mab 35-gold was found at the outer cell surface and 20% was found in the invaginations of the cell surface. From 5 to 60 min, the Mab 35-gold could be found in endosome-like vesicles, and by 2h, >90% of the gold could be found in structures identifiable as lysosomes by acid phosphatase. In order to label low pH compartments, we incubated chick myotubes for 1 h at 37 C with the acidotropic compound DAMP, 3-(2,4-dinitroanilino)-3´ amino-N-methyldipropylamine, which accumulates in low pH compartments (e.g. lysosomes, endosomes, and trans-Golgi cisternae) and is retained after fixation. (Anderson, et.al., PNAS, 81:4838, 1984). The myotubes were then incubated with Mab 35-gold, and gold internalization over time was followed by electron microscopy as before. After fixation, the myotubes were embedded in Lowicryl, and the DAMP-containing organelles were identified in thin sections by indirect protein A-gold (5nm) labeling where anti-DNP was the first Ab. Although the Mab 35-gold colocalized with small amounts of DAMP-specific gold during early time points of internalization (e.g. 5 min.), the Mab 35-gold was not found in organelles with high DAMP gold density (multivesicular bodies and dense lysosomes) until 30 min. Further work is in progress to characterize the DAMP-rich organelles in the AChR internalization pathway and to determine what role acidification might play in the early stages of AChR internalization.

197.4 CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST A SYNTHETIC PEPTIDE CORRESPONDING TO A BUNGAROTOXIN BINDING REGION ON THE α-SUBUNIT OF THE ACETYLCHOLINE RECEPTOR. P.E. Preston* and E. Hawrot. Dept. of Pharmacology, Yale Univ. Sch. Med. New Haven, CT 06510.

Monoclonal antibodies (mAbs) have been generated against a 32 amino acid synthetic peptide corresponding to amino acids 173-204, from the α-subunit of the acetylcholine receptor (AChR) of Torpedo californica. This peptide has been shown to specifically bind the ligand α-bungarotoxin (PNAS 82:8790, 1985) and has been found to induce experimental myasthenia gravis in mice (Soc. Neurosci. Abstract 338.2, 1986).

All of the mAbs are IgMs and recognize the 32-mer in solid phase ELISA assay. Many of the mAbs also bind to Torpedo electric organ membranes as measured by ELISA. Some of the mAbs also bind to the membranes of intact BC3H1 cells, a mouse muscle cell line that expresses a high level of nicotinic AChR on its surface. The binding of the mAbs to cells was determined using two different assays. In the first assay, mAbs are incubated with intact BC3H1 cells followed by an incubation with ^{125}I-labelled Goat anti-Mouse Ig. The second assay involves metabolically labelling the mAbs themselves and monitoring the binding of the ^{35}S-labelled antibodies to the intact cells. None of the mAbs that bound BC3H1 cells bound to rat PC12 cells, a cell line which expresses a neuronal, rather than a muscle, type of nicotinic AChR.

Despite the fact that a majority of the mAbs recognize AChR in either Torpedo membranes or on BC3H1 cells or both, none of the mAbs recognize detergent solubilized Torpedo AChR. The mAbs failed to bind to purified Torpedo AChR in ELISA and failed to precipitate ^{125}I-Btx labelled Torpedo or BC3H1 AChR in an immunoprecipitation assay. In addition, we have failed to demonstrate binding to SDS denatured AChR subunits on Western blots. Thus these mAbs, although raised against small synthetic peptides, may recognize conformational determinants that are sensitive to detergent treatments. Furthermore, in two different assay systems, an ELISA assay and a ^{35}S-mAb binding assay, we have found that pre-incubation with ligands such as carbachol or curare, is required for mAb binding to purified Torpedo AChR (in ELISA) and increases binding of labelled mAb to Torpedo membranes. These findings suggest that a conformational change in the AChR following ligand binding may be required for optimal binding of these mAbs.

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197.5 α-BUNGAROTOXIN BINDING TO SYNTHETIC PEPTIDES CORRESPONDING TO SEQUENCES OF THE α SUBUNIT OF THE ACETYLCHOLINE RECEPTOR. P.T. Wilson*, E. Hawrot and T.L. Lentz. Depts. of Cell Biol. and Pharm., Yale Univ. Sch. Medicine, New Haven, CT 06510.

We previously reported that a synthetic peptide of 32 amino acid residues corresponding to residues 173-204 of the α subunit of Torpedo acetylcholine receptor (T32mer) bound α-bungarotoxin (BTX) (PNAS 82:8790 1985). We report here the results of further studies involving synthetic peptides corresponding to sequences in the region of 173-204. [125]I-BTX binding to the T32mer was competed with a variety of cholinergic antagonists and agonists in a solid phase assay. Affinity for binding to the T32mer was measured by the concentration of the ligand resulting in a 50% reduction in BTX binding (IC50). Cholinergic antagonists competed [125]I-BTX binding with IC50's of 42 nM for unlabeled BTX, 440 nM for α-cobratoxin, and 86 μM for d-tubocurarine (dTC). In contrast, cholinergic agonists competed BTX binding with IC50's in the 1-10 mM range and NaCl with an IC50 of 16 mM. These IC50 values are nearly equal to the IC50 values reported for binding to the intact isolated α subunit. We conclude that the T32mer expresses most or all of the determinants of BTX binding expressed on the intact isolated α subunit. Shorter peptides with sequences coresponding to portions of the 32mer sequence were also tested for their ability to bind BTX in solid phase assays. An 18mer, 181-198, bound BTX with an IC50 of 21 μM. Similarly, two 12mers, 185-196 and 193-204, bound BTX with IC50's of 24 μM. The lower affinities of these peptides as measured by IC50 indicates that they do not contain all of the determinants of BTX binding expressed on the 32mer. The affinity of BTX binding to 32mers corresponding to 173-204 of the human (H32mer) and calf (C32mer) α subunits was also determined from competition studies. For the C32mer, the IC50 for unlabeled BTX was 640 nM and for dTC 250 μM. For the H32mer, the IC50 for unlabeled BTX was 6400 nM and for dTC 48 μM. Similar affinities for BTX binding were obtained from equilibrium dissociation constants. For T32mer, Kd = 63 nM; for C32mer, Kd = 223 nM; for H32mer, Kd = 1035 nM. The presence of 0.01% SDS in the binding buffer increased the affinity of BTX binding to all three 32mers, but the effect was greatest for the T32mer. For T32mer, Kd = 7.2 nM; for C32mer, Kd = 49 nM; for H32mer, Kd = 361 nM. Under both conditions the affinity of BTX was greatest for T32mer and least for H32mer. Of the five amino acid differences that occur between the T32mer and H32mer, three are nonconservative and involve the substituion of aromatic residues in the Torpedo sequence for polar residues in the human. We therefore conclude that aromatic residues may be important in the higher affinity of binding of BTX to the T32mer. Supported by NIH NS 21896 and GM 32629.

197.6 GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO PRESELECTED DOMAINS OF THE DELTA SUBUNIT OF THE MOUSE NICOTINIC ACETYLCHOLINE RECEPTOR. Susan B. Edelstein* and Edward Hawrot (SPON: P. Forscher). Dept. of Pharmacology, Yale Univ. Med. Sch., New Haven, CT 06510.

We have used a synthetic peptide approach to produce a new panel of mouse-specific monoclonal antibodies (mABs) directed against the ectodomain of the delta subunit of the nicotinic acetylcholine receptor (AChR). We chose the mouse sequence SRLQWDANDFGNIT (residues 65-78, #411) and the sequence LSLKQEEENNRS (residues 160-171, #20) for the production of mABs since these regions have diverged considerably from the corresponding *Torpedo* sequence, are rich in charged residues, precede several proline residues and contain a potential site for N-linked glycosylation suggesting that these domains lie on the extracellular surface of the AChR.

Hybridoma supernatants were screened against unconjugated peptide using a solid phase enzyme-linked immunosorbent assay (ELISA). Eight hybridoma clones against peptide 20 and six clones against peptide 411 were chosen for further characterization. All the mABs were found to be IgMs when supernatants were screened for Ig subtype.

To determine if the mABs bound to the ectodomain of the AChR, a modified ELISA was used. BC3H-1 mouse cells, which express high levels of surface AChRs in response to low serum, were grown for 7-10 days in low serum in 96 well plates then lightly fixed prior to ELISA assay under conditions which did not permeabilize the cells. All of the mABs showed significant binding to wells containing fixed BC3H-1 cells compared to similarly processed wells containing no cells or mouse fibroblast LMTK cells.

The mABs also bound to partially purified *Torpedo* AChR in an ELISA assay. Interestingly, the mABs against peptide 20 showed greater cross-reactivity to the *Torpedo* AChR than the mABs against peptide 411 even though peptide 20 has much lower homology to the *Torpedo* sequence than does peptide 411. Regardless of the supernatant assayed, the mABs bound to *Torpedo* at a much reduced sensitivity compared to that seen with BC3H-1 cells. Whereas levels of mABs bound to *Torpedo* AChR and BC3H-1 cells appeared similar, the concentration of *Torpedo* AChR in the assay was 100-1000 times higher than the amount of BC3H-1 receptor present.

To determine if the mABs bound to the AChR, a modified immune precipitation-ELISA assay was used. NP40 extracts of *Torpedo* homogenate, BC3H-1 cells and LMTK cells were incubated with bungarotoxin-Sepharose to specifically bind the AChR. The beads containing bound AChR were then incubated with the hybridoma supernatants and assayed for mAB binding by an ELISA assay. Significant binding of the mABs could be detected in the *Torpedo* and BC3H-1 extracts but not in the LMTK and no cell extracts.

To determine whether the mABs bound specifically to the delta subunit, purified *Torpedo* AChR was used in series of protein blotting experiments. A band at 65kD corresponding to the delta subunit was darkly stained. In addition, some of the mABs appeared to cross-react with the gamma and beta subunits. As with the ELISA assays, the supernatants against peptide 20 showed higher cross-reactivity with the *Torpedo* receptor than did the mABs against peptide 411.

In summary, these studies are the first description of mABs that have been found to bind to the ectodomain of the delta sununit of the AChR, and as such, these mABs will be useful for probing the role this region plays in the functioning of the AChR.

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197.7 EPITOPE LOCALIZATION ON THE THREE-DIMENSIONAL AND PRIMARY STRUCTURE OF ACETYLCHOLINE RECEPTOR. S.J. Tzartos, A. Kordossi*, S.L. Walgrave*, A. Kokla* and B.M. Conti-Tronconi*. Hellenic Pasteur Inst., Athens 115 21, Greece, and Dep. Biochemistry, Univ. Minnesota, St. Paul, MN 55108.

The binding sites of several monoclonal antibodies (mAbs) on the acetylcholine receptor (AChR) were localized by two different approaches. The first approach involved competition experiments among mAbs for binding to intact Torpedo AChR. The mAbs used bind to known sites on the amino acid sequences 339-378 and 336-469 of the AChR α and β-subunits respectively, as determined by synthetic peptides[1]. mAbs to sequential epitopes separated by about seven residues did not compete whereas mAbs to more closely neighbouring epitopes mutually excluded each other. Our experiments strongly suggested that these mAbs bind to intact AChR at the sequences already determined by synthetic peptides and not at irrelevant discontinuous epitopes. This finding has important implications for current models of the AChR transmembrane structure.

The second approach involved mAb mapping by the use of 26 synthetic peptides corresponding to 83% of the sequence of the human α-subunit. Several mAbs of the above studied group were mapped at the expected[1] or at neighbouring amino acid sequences. This technique also allowed the fine localization of the main immunogenic region (MIR) of the AChR. The MIR to which the majority of the anti-AChR antibodies are directed had been localized earlier within residues 6-85 of the AChR α-subunit[2]. With the present experiments we localized the MIR in an 18-mer of the α6-85 region.

1. Ratnam et al (1986) Biochemistry 25:2633
2. Barkas, T. et al (1987) Science 235:77

197.8 COMPUTER MODELING OF BRAIN MUSCARINIC ACETYLCHOLINE RECEPTOR TOPOGRAPHY. L. Antonian and A. S. Lippa*, Matrix Research Labs, City College of New York, New York, NY, 10031, USA.

A few theoretical models for the muscarinic acetylcholine receptor (mAchR) have been suggested based on structural requirements of acetylcholine and certain of its analogues. More recent pharmacological classification of Ach agonists into full (class A) and partial (class B) agonists has made it possible to test the validity of these models and to further refine the receptor topography.

By using computer molecular modeling programs, we have compared the structures of full and partial agonists to their known binding properties and physiological activities of phosphoinositide hydrolysis and receptor desensitization at the hippocampal pyramidal cells. The full agonists examined were Ach, carbachol, S-metacholine, muscarine, and oxotremorine-M. The partial agonists studied were arecoline, pilocarpine, oxotremorine, and S-bethanechol. From such comparisons we propose a topographical model for the muscarinic acetylcholine receptor which enables the distinction between class A and B agonists.

The energetic and conformational studies were accomplished by using MacroModel, via quantitative and visual examinations of computerized molecular models. MacroModel is a molecular modeling program, especially designed for the purpose of structurally comparing small molecules. Relaxed geometries were determined using molecular mechanics MM2 force field and the conformational energies were then evaluated by energy minimizations. Quantitative data were obtained on relevant atom distances. Atomic charges were determined for all agonists by using ChemGraph, a different molecular modeling program.

A model for the agonist fit at the receptor, suggesting a two-step binding process, will be presented.

197.9 IRREVERSIBLE INHIBITION OF MUSCARINIC CHOLINERGIC RECEPTOR BINDING BY TYROSINE-DIRECTED ALKYLATING AGENTS IN RAT CEREBRAL CORTEX AND HEART.

J.-X. Wang", H.I.Yamamura, W.Wang" and W.R. Roeske. Department of Pharmacology and Internal Medicine. The University of Arizona, Tucson, AZ 85724.

In order to find the composition of amino acid residues in the ligand binding sites of M_1 and M_2 receptor subtypes, two tyrosine-directed alkylating reagents, p-nitrobenzensulfonyl fluride (pNSF) and flurosulfonylnapthonic acid (FSNA) were used to modify muscarinic receptor proteins from rat forebrain and heart. The ligand binding properties of these modified receptors were then studied.

The chemical reaction was carried out by incubation of rat forebrain and heart homogenates (5% in 10 mM Na/K phospnate buffer supplemented with 25 mM $MgCl_2$) with concentrations of either of tnese two agents at room temperature for 15 min. Tne reaction was stopped by immersing the samples in an ice-water bath followed by immediate centrifugation at 48,000 X g at 4°C for 10 min. The precipitants were washed 3 times with ice-cold buffer by centrifugation at the same conditions to remove unreacted chemicals. The final pellets were resuspended in the same buffer for receptor binding assay. Both reagents caused an irreversible dose-dependent inhibition of specific binding of [^{3}H](-)QNB with an ED_{50} of about 1 mM for FSNA and 2.5 mM for pNSF in both tissues. Saturation experiments performed in the homogenates with or without chemical modification using 0.8 mM or 1.6 mM FSNA indicated that alkylation of tyrosine residue resulted in a decrease of receptor density (B_{max}) without a siginificant alteration of the affinity (K_d) of remaining receptors. The effect of FSNA (1.5 mM) was blocked by incubation of the homogenates with muscarinic agents prior to the addition of FSNA. The relative potency of muscarinic antagonists (at a concentration of 1 uM) in protecting of muscarinic receptors was atropine > pirenzepine > AF-DX 116 in both tissues. The agonist carbachol was about 100,000 times less potent than atropine in protecting muscarinic receptors. Including 100 uM of Gpp(NH)p in the tissue preparation did not reverse the protection of muscarinic receptors by carbachol. Our results suggest that there is a tyrosine residue(s) in the ligand binding area of both M_1 and M_2 receptors in rat forebrain and heart, respectively. This amino acid residue does not seem to be responsible for the heterogeneity of ligand binding affinity for the muscarinic receptors in these two tissues.

This work was supported by NIH grants and an AHA grant from the Arizona Affiliate.

197.10 Partial Purification of Muscarinic Receptors with Antibodies to Propylbenzilylcholine Mustard-BSA Conjugates. Priscilla F. Strang and Donna D. Flynn, Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Propylbenzilylcholine mustard (PBCM) is an affinity alkylating agent for the cholinergic muscarinic receptor (MR). Polyclonal antibodies (PAb) were produced in mice and rabbits immunized with PBCM-coupled to bovine serum albumin (BSA) (Biochem Pharmacol. 35: 1209, 1986). PAb's to PBCM, BSA, and the PBCM-BSA conjugates were present in the serum as shown by immunodots. IgG fractions were purified by DEAE ion exchange chromatography. PAb's to PBCM also crossreacted and bound with high affinity to ^{3}H-Quinuclidinyl benzilate (QNB) as evidenced by ammonium sulfate precipitation of PAb-^{3}H-QNB complexes. These data suggest that these PAbs share some structural features with the binding site for PBCM and QNB on the muscarinic receptor. Antibodies to these PAb's may recognize directly the ligand binding site on the MR. To test this possibility MR were solubilized from rat brain in 10 mM CHAPS/50mM $NaPO_4$ buffer (pH 7.4) containing Na_3EDTA. Antibodies were reacted with solubilized receptor and the antibody complexes were precipitated with Pansorbin. These pelleted complexes were dissociated with 2.5m $MgCl_2$. Soluble supernatants containing the dissociated antigen were run on SDS-PAGE. An enrichment in a peptide band at ~70,000-80,000 daltons was observed. This peptide has the identical mobility as the PBCM-affinity alkylated MR.

These results suggest that anti-anti-PBCM PAb's may be useful in the selective purification of muscarinic cholinergic receptors.

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197.11 MOLECULAR DYNAMICS OF CHOLINERGIC LIGANDS BOUND TO THE NICOTINIC ACETYLCHOLINE RECEPTOR. K.A. McGroddy *, G.L. Millhauser, Z.Lieberman* and R.E. Oswald, Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., 14853.

The connection between the molecular details of ligand binding to the nicotinic acetylcholine receptor (AChR) and the complex behavior of its ion channel is not yet understood. The hypothesis that specific structural fluctuations of the protein are related to the gating properties of the ion channel has been difficult to prove experimentally. We have used ^{19}F Nuclear Magnetic Resonance (NMR) spectroscopy of fluorinated cholinergic ligands to investigate the static and time-dependent properties of ligand binding to the AChR. Our ultimate goal is to correlate these results with single channel recording measurements in order to understand the relationship between the dynamics of agonist binding and channel activation.

^{19}F Nuclear Magnetic Resonance spectroscopy was used for several reasons. The fluorine nucleus is very sensitive both to local motion and to its chemical environment. In addition, fluorine is rarely present in the biological systems of interest so there is little background signal. A trifluorinated system was desirable in order to increase the strength of the NMR signal. Several ligands were synthesized and tested in order to determine their cholinergic activity. Patch clamp experiments showed that a recently reported trifluorinated agonist, 1,1-dimethyl-4-trifluoroacetyl-piperazinium iodide (F-PIP), bound with high affinity and induced considerable single channel activity.

The NMR experiments were performed using AChR from *Torpedo californica* electroplaque and F-PIP as the agonist. The resulting spectrum consisted of two signals: one corresponding to unbound F-PIP and the other to the receptor-bound ligand. The bound signal was characteristic of a molecule undergoing highly anisotropic reorientation. This motion could be either intramolecular ligand motion, movement of the protein-agonist complex in the membrane, or rotation of the entire bound molecule about a preferred axis in the binding site.

Intramolecular ligand motion was ruled out after a careful study of the unbound agonist using ^{1}H NMR spectroscopy. Although the free molecule does experience rotation about internal bonds, this rotation is several orders of magnitude slower than the observed bound state rotation. Motion of the receptor-agonist complex was also an unsatisfactory explanation for our results since the time scale of protein motion in the membrane does not correspond to the time scale of the observed motion. We conclude that the bound cholinergic ligand is able to rotate about an axis through its length as it sits in the AChR binding site. This is the first step toward determining the molecular dynamics of an agonist molecule in its binding site.

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197.12 NICOTINE: INTERACTIONS OF ITS STEREOISOMERS WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR. R. Rozental*, Y. Aracava*, K.L. Swanson, E.X. Albuquerque. Dept. of Pharmacol. and Exp. Therap., Univ. Maryland Sch. Medicine, Baltimore, MD 21201.

The multiple actions of nicotine stereoisomers were evaluated at the peripheral nicotinic acetylcholine receptor (nAChR). The agonist potencies of (-)-nicotine, (+)-nicotine (both ditartrate salts) and carbamylcholine were compared by assay of rectus abdominis (*Rana pipiens*) contracture. In order to minimize desensitization, low drug concentrations were selected. Compared to carbamylcholine, complete recovery from nicotine-elicited contractures, (-) or (+), was prolonged (30 min) and a relatively reduced tension was observed upon subsequent application of agonist after a wash phase. The order of potency was carbamylcholine ≈ (-)-nicotine > (+)-nicotine with 15, 23, and 130 μM, respectively, being necessary to produce 20% of maximal contractures. The (-) isomer was 8 times more potent than the (+) isomer.

Antagonistic effects of both nicotine isomers (0.5 - 5.0 μM) were illustrated by concentration-dependent depression of peak amplitude of the endplate current (EPC) in voltage-clamp studies of the frog sartorius muscle. For instance, 1 μM of either (-) or (+)-nicotine halved the peak amplitude of the EPC. Neither isomer modified the decay time constant of the EPC significantly. Trains of EPCs were elicited to test if desensitization could account for the depression of the peak EPC amplitude. There was no significant effect of either nicotine isomer from 0.5 to 5.0 μM on the relative amplitude of the last EPC versus the first EPC evoked by tetanic stimulations (25 Hz for 2 sec), although concentration-dependent reduction of the initial peak EPC amplitude was observed.

Single channel currents, recorded from perijunctional regions of single fibers of the frog interosseal muscle, showed the agonist property of nicotine isomers in the concentration range of 1-50 μM. The (-) isomer was more potent than its enantiomer, concurring with the results of the contracture assay. Both nicotine isomers activated single channel currents which were interrupted by a greater number of short closures than were the ACh-activated currents. Single channel conductance was similar to that reported for ACh (≈30 pS, 10°C). In the presence of a stimulating concentration of ACh (0.4 μM), lower concentrations of either nicotine isomer (0.1 to 10 μM) induced bursting-type channel activity. This effect, which was not significantly stereospecific, may be correlated with the decreased peak amplitude of the macroscopic EPC. These actions of nicotine suggest competitive and noncompetitive interactions with the acetylcholine receptor at the agonist recognition site and an allosteric site, possibly associated with the ion channel and/or coupled with desensitization. (Support: U.S. Army Med. Res. & Dev. Comm. Cont. DAMD17-84-C-4219.)

197.13 BISPYRIDINIUM COMPOUNDS SAD-128 AND HI-6 MODULATE ENDPLATE CURRENTS OF FROG SARTORIUS MUSCLE. M. Alkondon* and E.X. Albuquerque. (Spon: D. Burt) Dept. Pharmacol. & Exper. Ther. University of Maryland Sch. Medicine, Baltimore, MD 21201.

Pyridinium compounds with oxime groups such as, 2-PAM and HI-6, have earlier been shown in our laboratory to have a modulatory effect on the ionic currents occurring at the frog endplate region. In the present study another bispyridinium compound named 1,1'-oxybis(methylene)bis4-(1,1,dimethylethyl) pyridinium dichloride (SAD-128) which lacks the oxime group in its structure has been tested on the endplate currents (EPCs) measured at the frog neuromuscular junction and its effects compared with that of the closely related compound HI-6. SAD-128 at concentrations below 10 μM did not significantly alter the EPC parameters. At concentrations between 100-500 μM, SAD-128 produced a depression of EPC peak amplitude which was greater at negative holding potentials. The current-voltage (I-V) relationship was linear in the range between +50 mV to -70 mV. However, between -100 and -150 mV, especially at high concentrations (500 μM) of SAD-128, there was a curvature and negative slope observed in the I-V plots. On the other hand, the decay phase of the EPC appears to be prolonged at concentrations between 50 and 500 μM. At holding potentials between -100 and -150 mV, two phases of EPC decay were noticed especially at concentrations between 50 and 100 μM. At these doses, analysis of the two phases revealed the following points: When the holding potential was increased from -100 to -150 mV, the time constant of the fast phase decreased, slow phase increased whereas an inverse relation of the percentage current in each of these phases was noticed. At higher concentrations (500 μM), the slow phase became predominant and a clear distinction between the two phases could not be discernible. SAD-128 also produced similar biphasic decays in the miniature EPCs at concentrations between 10 to 100 μM at holding potentials between -100 to -140 mV. HI-6, on the other hand, produced single exponential decays of EPCs even though it produced similar nonlinear depression in I-V plots. Moreover, HI-6 in most of the doses, appear to fasten the EPC decays especially at negative potentials. Acetylcholine (ACh)-induced channel openings (open times) were shortened by HI-6 in a voltage-dependent manner as revealed by patch clamp studies. The ACh-receptor channel-blocking effect of these pyridinium compounds (as revealed by the above results) may remain as one of the mechanisms by which they produce antidotal effects against organophosphate agents. (Support: U.S. Army Med. Res. & Devel. Com. Contr. DAMD17-84-C-4219).

197.14 INTERACTION OF AMANTADINE WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR MACROMOLECULE OF FROG SKELETAL MUSCLE. L.M. Dumbill* and E.X. Albuquerque. Dept. of Pharmacol. and Exp. Ther., Univ. of Maryland Sch. Med., Baltimore, MD 21201.

Amantadine hydrochloride (1-adamantanamine hydrochloride) is a prophylactic drug used in the treatment of A_2 influenza (Asian flu) and is also effective in the treatment of human Parkinsonism. Earlier binding and voltage-clamp studies showed a depression of the peak endplate current amplitude and a shortening of the half-decay time, suggesting a non-competitive ion channel blockade (*Mol. Pharmacol.* 14:787, 1978). However, at positive potentials amantadine induced a lengthening of the half-decay time. To discern the mechanisms underlying these alterations, it was decided to study the micromolecular effect of the drug. The effect of amantadine upon the nicotinic acetylcholine receptor (nAChR) macromolecule was studied using patch clamp technique. Single channel recordings were made on interosseal muscle fibers of the frog, *Rana pipiens*.

Amantadine exhibited an agonistic effect on the nAChR complex at high concentrations (1 - 50 μM). At 50 μM, the frequency of amantadine-induced channels was comparable to that observed in ACh controls (300 nM). In contrast to ACh, at holding potentials between -60 and -160 mV, amantadine activated channel currents with a significantly increased level of open channel noise. The opening events caused by amantadine appeared as bursts interrupted by many rapid channel closures. Single channel conductance, however, remained similar to that reported for ACh-activated channels, i.e. ≈30 pS at 10°C. At depolarized membrane potentials (+80 to +120 mV), channel lifetimes appeared to be very brief (≤ 0.8 msec) with a decreased frequency of openings. This decrease in detected frequency may result from the exclusion of brief events by the instrument filtering bandwidth (3 kHz).

In an admixture of ACh (300 nM) and various concentrations of amantadine, channel openings with increased flickering during bursts were recorded. Single channel conductance was unaffected. Although further analysis of the kinetics of amantadine action on the nAChR of the neuromuscular junction and other cholinergic synapses is required, our results indicate that the agent has agonistic and open channel blocking properties. (Support: U.S. Army Med. Res. & Develop. Comm. Contract DAMD17-84-C-4219).

198.1 AUTORADIOGRAPHIC LOCALIZATION OF FLOW OF TRACER SUBSTANCE IN EXTRACELLULAR FLUID COMPARTMENTS OF RAT CNS B. Hutto*, L. Brady, and M. Herkenham Unit on Functional Neuroanatomy, NIMH, Bethesda, MD 20892

The cerebrospinal fluid (CSF) is rich in many of the brain's informational substances as well as their metabolites. CSF flows through two continuous compartments: the large cavities (ventricles and subarachnoid spaces) and the extracellular spaces surrounding all brain cells. The CSF may thus serve as a conduit for the parasynaptic transport of informational substances to receptors dispersed throughout the central nervous system. The possibility of endocrine forms of intercellular communication in the central nervous system is predicated on the existence of ample intercellular space for the movement of the messenger molecules.

To visualize these intercellular channels and to demonstrate access to them from the CSF, [^{14}C]inulin was intracisternally (20 µl) or intraventricularly (5 µl) injected into 200-280 g male Sprague Dawley rats. Inulin is an inert carbohydrate, similar in MW (~5000) to several of the larger neuropeptides (e g : CRH, GRF, ß-endorphin), but it remains in the extracellular space. Intracisternal injections were made via cannulae implanted 3 days earlier. The rats were sacrificed 5, 10, 30, 60, or 120 min after injection, and the brains were frozen. Thin sections were cut, rapidly dried, and apposed to LKB Ultrofilm.

The films showed that after cisternal injections most of the inward spread and the movement rostrally was from the subarachnoid space along the ventral and lateral surfaces of the brainstem, although the choroid plexus and ependyma of the lateral, 3rd, and 4th ventricles were eventually labeled lightly. At 5 and at 10 min inulin penetrated into the brainstem, cerebellum, entorhinal cortex, cerebral peduncle, ventral hypothalamus, mammillary nuclei, alveus, and the olfactory and optic tracts. By 1 h the label entered the hippocampus and retrosplenial cortex. Elsewhere penetration was greatest in the brainstem, ventral diencephalon, and ventral and medial forebrain, attaining a depth of 2-3 mm. By 2 h the label reached the thalamus and septum, and the brainstem and spinal cord were rather homogeneously labeled. The dorsolateral cerebral cortex remained free of label. At no time did the median eminence or the pituitary have appreciable label.

Inulin injected into the lateral ventricle followed a caudal course at the early time points and by 30 min had spread from the lateral, 3rd, and 4th ventricles into adjacent structures such as the septum, caudate, hypothalamus, periaqueductal gray matter, dorsal medulla, and ventral cerebellum. At 1 h the ipsilateral hippocampus and deep layers of somatomotor cortex were labeled, and penetration in the aforementioned areas was deeper. Penetration from the contralateral lateral ventricle did not occur.

Comparing ventricular with cisternal injections, it appears that site of injection determines structures initially labeled, though at longer time points similar structures along the ventral and medial surfaces are labeled, suggesting a similar route of clearance. Taken together the data show a rapid movement of inulin along CSF flow routes throughout the major cavities and a slower penetration into interstitial spaces adjacent to these cavities.

198.2 **QUANTITATIVE AUTORADIOGRAPHY: COMPARISON OF LABELING IN CEREBRAL CORTEX OF RHESUS MONKEY WITH [^{3}H]- [^{125}I]- AND [^{14}C]-COMPOUNDS.** M.S. Lidow, D.W. Gallager, P.S. Goldman-Rakic and P. Rakic, Yale University, School of Medicine, Section of Neuroanatomy, New Haven, CT 06510

Film autoradiography is widely utilized for localization of radiolabeled probes in nervous tissue. However the technique suffers from certain limitations. The most commonly used isotope, [^{3}H], emits low energy ß-particles which are absorbed more by myelinated axons than by myelin-free cell bodies. The resultant quenching interferes with estimates of the concentration of labeled compounds in myelin-rich areas. To avoid this problem, the use of [^{125}I] or [^{14}C] is often recommended. In the present study, a possible advantage of using [^{125}I] and [^{14}C] versus [^{3}H], was evaluated by labeling adjacent cryostat sectioned cortex of rhesus monkey with pairs of similar compounds, one of which labeled with [^{3}H] and another - with [^{125}I] or [^{14}C]. These included: [^{3}H]spiperone and [^{125}I]iodospiperone; [^{3}H]formaldehyde and [^{14}C]formaldehyde; and others. Sections were pressed against [^{3}H]-sensitive Ultrofilm. Computer-aided image analysis was used to measure the optical densities (OD) of film images corresponding to different layers of the cortex. In most cases, [^{3}H]-labeled compounds produced finer patterns of dark and light bands within the cortex than their [^{125}I]- or [^{14}C]-labeled counterparts. The dark bands were usually wider on images of [^{125}I]- and [^{14}C]-labeled sections than on images of [^{3}H]-labeled sections and images of sections labeled with [^{125}I] or [^{14}C] tended to have fewer light bands. The light bands that were present usually corresponded to light bands on images of [^{3}H]-labeled sections, though the former were often narrower and less pronounced. The light bands on [^{3}H] generated images that had no corresponding [^{125}I]- or [^{14}C]-generated images were usually ≤ 0.1mm wide and/or were not very prominent. As the position of such bands sometimes corresponded to cortical layers poor in myelin, their presence could not easily be explained by greater absorbtion of ß-particles emitted by [^{3}H]. Rather the differences in labeling with [^{3}H] and [^{125}I] or [^{14}C] could be explained by scattering of emissions from [^{125}I] and [^{14}C], which easily penetrate tissue and travel for relatively long distances in all directions, thereby exposing the film not only directly above the labeled compound (as in the case of [^{3}H]) but also at some distance from it. The result is that small areas with a low density of label are obscured and the size of areas with a high density of label is artificially increased. In support of this conclusion we found a significant increase in OD as far as 0.7mm from the images produced by [^{125}I] and [^{14}C] standards. Our findings indicate that substitution of [^{3}H] by [^{125}I] and [^{14}C] may address the quenching problem but results in a loss of resolution for fine detail such as laminar patterns in cerebral cortex.

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198.3 THE USE OF HIGH ACTIVITY ^{3}[H] STANDARDS TO QUANTITATE ^{125}[I] FILM AUTORADIOGRAPHY. R.P. Artymyshyn and B. B. Wolfe Dept. of Pharmacol., U. of PA, Phila., PA 19104

Film autoradiography using ^{125}[I] as the radionuclide is attractive because of the relatively short exposure times required. Quantitating the autoradiographs requires the generation of tissue mash standards with known amounts of radioactivity/mg protein. To determine the amount of radioactivity present in a given tissue, the density (darkness) of autoradiographs produced by labelled tissue sections is compared to the density of the autoradiographs produced by sectioned tissue mash standards. The production of tissue mash standards is time consuming and must be done relatively often because of the short half life of ^{125}[I]. To avoid the repeated production of standards and to decrease interexperiment variability we calibrated a set of high activity ^{3}[H] standards for use with ^{125}[I] autoradiography.

Standards with a range of 7390 to 0.0 µCi of ^{3}[H]/g of plastic were obtained from American Radiolabeled Chemicals, Inc. (#123A) St. Louis, MO. It was found that these standards would produce autoradiographs of suitable density at times of 4 to more than 72 hours.

Tissue mash standards were prepared in a conventional manner (Unnerstall et al, '82, J. Neurosci. Meth) in a concentration range of 470 to 4 pCi/ug protein. These standards were frozen and sectioned at varying thicknesses, and the sections thaw mounted onto slides. To check for possible variations in the amount of label within each standard, the radionuclide concentrations/ug protein were measured at regular intervals throughout the block.

Because the relationships between film response, tissue quenching and section thickness are not linear, the calibration of the ^{3}[H] standards was done separately for each time point and thickness. Brain mash standards at 12 concentrations of ^{125}[I] were produced and sectioned at thicknesses of 6, 10, 14, 16, 20, 24, 32, 40, and 60 microns. Each set of tissue standards was placed in x-ray cassettes along with slides bearing the plastic ^{3}[H] standards, apposed to LKB Ultrofilm for times of 2 hours to 1 week. autoradiographs were analyzed using a DUMAS image analysis system. A standard curve was generated for each set of tissue standards and this was correlated with a ^{3}[H] standard. This accurate calibration of the ^{3}[H] standards gives us long lived standards for ^{125}[I] autoradiography useful over a wide range of radionuclide concentrations, tissue thicknesses and exposure times. Supported by MH09499 and NS22040.

198.4 **A NEW DENSITOMETRIC PROCEDURE TO MEASURE PROTEIN LEVELS IN TISSUE SLICES USED IN QUANTITATIVE AUTORADIOGRAPHY.** J.A. Miller*, P. Curella* and N.R. Zahniser (SPON: M.K. Ahlijanian) Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Receptor densities obtained from quantitative autoradiography (QAR) analysis do not always agree with those obtained from scintillation counting of tissue sections which have been scraped from the slide. Disparate protein measurements may contribute to these differences. Using QAR it has been possible to normalize receptor binding data only by expressing the molar quantities of receptor bound as a function of wet weight of tissue or amount of protein in tissue paste standards. We have developed a new quantitative staining technique using the dye Coomassie Brilliant Blue G 250 (2.5% in isotonic buffer, pH 7.5) using a 180 min incubation and a 10 min wash in buffer at room temperature. This is a densitometric procedure which uses commercially-available hardware and software employed in the quantitation of receptor autoradiographs. A nonlinear curve-fitting procedure to a logistic function was employed for the quantitative densitometric analysis of both protein and receptors. Using the densitometric assay for protein to correct for regional variations in protein levels, as opposed to using the average protein concentration in the radiolabeled tissue paste standards, results in approximately a two-fold decrease in the density of ß-adrenergic receptors measured in several brain areas (Table 1). Also, better agreement is seen with Bmax values determined with membrane binding assays and normalized for protein with a spectrophotometric assay (Bradford, M.M., Anal. Biochem. 72: 248, 1976; Table 1). Additionally, the process of staining tissue with Coomassie Blue for protein is reversible; allowing further histological analysis after protein measurement. This technique is a more reliable method for normalizing receptor densities of tissue protein levels and allows for more accurately comparing results obtained with QAR and membrane binding techniques.

Table 1. Estimation of density of ß-adrenergic receptors from Scatchard analysis of IPIN binding (n = 4 to 6).

Bmax values (fmol/mg protein)

	A	B	C
Binding Assay:	QAR	QAR	Membrane
Protein Assay:	Spectrophotometric	Densitometric	Spectrophotometric
Source of Protein:	Brain Paste	Brain Sections	Membranes
Brain Region			
Cerebellum	66 ± 6.7 *	41 ± 6.0	36 ± 3.6
Cortex	59 ± 10 *	34 ± 6.3	38 ± 2.7
Brainstem	41 ± 5.4 *	24 ± 2.0	18 ± 1.0
Hippocampus	53 ± 4.2 *	30 ± 1.9	33 ± 2.4
Thalamus	43 ± 5.3 *	29 ± 7.2	16 ± 2.5

* Significantly different from both B and C

This work was supported by USPHS AG 04418.

198.5 **NEUROCHEMICAL BASIS OF IN VIVO IMAGING OF BRAIN DOPAMINE NEURONS BY RADIOACTIVE LABELED L-DIHYDROXYPHENYLALANINE (DOPA).**
P.M. Douillet*, R.M. COHEN* and C.C. CHIUEH, (SPON: N. Ostrowski), NIMH, Bethesda, MD 20892

The goal of the present study was to provide a neurochemical basis for developing labeled L-DOPA derivatives to be used in positron emission tomography (PET) imaging studies of dopamine (DA) neurons. The metabolic pattern of ^{3}H-L-DOPA (1 nCi/kg i.v., 90 min) in striatum (ST) and cerebellum (CB) of Swiss mice pretreated with carbidopa (25 mg/kg, i.p., -30 min) was determined by HPLC after various pharmacological pretreatments.

The background activity was due mainly to 3-O-methyl-DOPA and to a sulfate conjugate. These background peaks were found equally in both ST and CB and their sum consists of more than 90 % of the background activity in the ST. However, the total background activity in CB was 20 % higher than in ST because of the slower decarboxylation of L-DOPA and the higher norepinephrine content. The ratio of total activities in ST and CB was 2.1 ± 0.1 (N=7). Inhibition of the DOPA-decarboxylase by NSD-1015 decreased completely the decarboxylated labeled DOPA metabolites, i.e. those related to DA activity (DA, DOPAC, HVA) in ST and thus decreased the ratio ST/CB to 1.1 ± 0.1 (N=7). The background activities in ST and CB were correlated with the total plasma activity but were not affected by GBR-12909 (DA uptake inhibitor) or haloperidol pretreatments. GBR-12909 did not alter the DA activities. Haloperidol increased the DA turnover of both endogenous and labeled DA and decreased the total DA activity in ST by 20 % (ST/CB = 1.7 ± 0.1, N=7).

The current neurochemical results indicate that the imaging of DA neurons by radioactive labeled L-DOPA is due mainly to the intraneuronal formation and storage of newly synthesized DA. The ratio of activities in ST and CB at 90 min could be a biased but sensitive index of labeled DA activity in ST which would allow to adjust for labelled L-DOPA input. The ratio ST/CB could be improved when 6-^{18}F-L-DOPA is used for imaging of brain DA since it is less methoxylated than L-DOPA. Our and other studies show that 2-^{18}F and 5-^{18}F-L-DOPA are not good imaging tracer since they are O-methylated extensively in vivo. Therefore, it is necessary to purify the radioactive labeled L-DOPA for brain imaging procedures, i.e. PET and autoradiography. Deficit in intraneuronal DOPA-decarboxylase activity or DA storage capacity as observed in parkinsonian subjects could be quantified. Change in the turnover rate of DA neurons following acute neuroleptic challenge could be measured in vivo in neuropsychiatric patients by using radioactive labeled L-DOPA.

198.6 LOCALIZATION OF DOPAMINE UPTAKE SITES AND DOPAMINE D1 AND D2 RECEPTORS USING AXOPLASMIC TRANSPORT AUTORADIOGRAPHY.
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The cellular localization of striatal dopamine (DA) receptors and high-affinity DA uptake sites as presynaptic on the afferent DA neurons or postsynaptic on striatal cells has proven to be challenging. Localization by direct methods is technically difficult and most indirect methods are based on the analysis of changes in ligand binding in the striatum seen after lesions to striatal afferents. An alternative approach is based on the transport of neural components from the soma to the nerve terminals, which can be interrupted either by knife cuts, electrocoagulations, or injections of 6-hydroxydopamine (6-OHDA) or colchicine. Such lesions have been shown to produce buildups of neurotransmitter, enzymes and receptors proximal to the injury. In this study we disrupted axonal transport to investigate the localization of D1 and D2 receptors and DA uptake sites in the nigrostriatal and striatonigral fibers.

Unilateral lesions of the ascending nigrostriatal DA neurons were produced by injection of 6-OHDA (8 ug/4 ul) in the lateral hypothalamic area at the level of the entopeduncular nucleus. Unilateral lesions of the descending striatonigral pathway were produced by electrocoagulation (25 mV, 2 mA, 100 kHz for 30 sec) in the internal capsule. DA binding sites were localized by autoradiography following in vitro binding of either [^{3}H]SCH 23390 (D1 receptors), [^{3}H]spiroperidol or [^{3}H]sulpiride (D2 receptors), or [^{3}H]mazindol (high-affinity DA uptake sites). Adjacent tissue sections were processed for acetylcholinesterase (AChE) histochemistry by a modified Karnovsky-Roots procedure. Disruption of the nigrostriatal pathway produced a buildup of [^{3}H]mazindol binding sites caudal to the lesion, in the vicinity of the ipsilateral substantia nigra. There was also a buildup of D2 binding sites and AChE near the SN which corresponded with the mazindol buildup. We found no evidence of a buildup of D1 receptors. These data suggest that at least some striatal D2 receptors, DA uptake sites, and AChE are located presynaptically on nigrostriatal dopaminergic fibers. There appear to be no striatal D1 receptors transported in these neurons.

In contrast, electrocoagulations along the striatonigral tract produced a buildup of D1 receptors near the entopeduncular nucleus, rostral to the site of the lesion. No corresponding buildup of D2 receptors, DA uptake sites, or AChE was seen. This suggests that at least some of the nigral D1 receptors are located presynaptically on striatonigral terminals.

198.7 D_1 DOPAMINE RECEPTORS IN HUMAN CHOROID PLEXUS. S.J. Boyson and L. O'Keefe*. Depts. of Neurology & Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

A high density (2800 ± 180 fmol/mg protein) of D_1 receptors in the choroid plexus of rat brain was noted in quantitative autoradiographic studies of rat brain (Boyson et al., J. Neurosci.6:3177-3188, 1986). Dopamine has been shown to increase, and haloperidol to decrease, choroid plexus blood flow in sheep (Townsend et al., Brain Res. 290:165-169, 1984).

Human choroid plexus was collected within 24 hours of death and stored at -70°C until use. Membranes were prepared by homogenizing tissue in buffer (50 mM Tris, 10 mM $MgSO_4$, 2 mM EDTA, 154 mM NaCl, pH 7.4) and centrifuging at 20,000g for 20 min. 3H-SCH-23390 was used to label receptors, and alpha-flupenthixol was used to define nonspecific binding in Scatchard analysis of saturation binding. The density of D_1 receptors was 470 ± 230 fmol/mg protein (n=3). The K_d was 3.0 ± 0.5 nM.

The presence of dopamine receptors in the choroid plexus and the modulation of blood flow in this region by dopamine and haloperidol suggests, by analogy with the kidney, where production of urine is proportional to blood flow, that dopaminergic agents might be useful therapeutically to modulate cerebrospinal fluid production. Disorders in which this could be useful include pseudotumor cerebri and acute elevations of intracranial pressure.

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198.8 COMPARATIVE DISTRIBUTION OF DOPAMINE D-1 AND D-2 RECEPTORS IN THE CEREBRAL CORTEX OF RATS, CATS AND MONKEYS
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The laminar and regional distribution of dopamine D-1 and D-2 receptors were studied in adult rats, cats and monkeys. Adjacent cryostat sections were processed for D-1 and D-2 dopamine receptors and cresyl violet staining. D-1 receptors were labelled using [^{3}H]-SCH 23390 and D-2 receptors were labelled using [^{3}H]-spiroperidol in the presence of unlabelled mianserin to block 5HT-2 binding. Both ligands were used at a concentration equal to their respective receptor K_D values to allow direct comparison of receptor densities. Receptor density histograms were determined in a variety of cerebral cortical regions for all three species. Nissl stained sections were used to determine the depth of each lamina in the different regions.

The cerebral cortex of all three species contained both D-1 and D-2 receptors. The density of D-1 receptors exceeded the number of D-2 receptors in most laminae and regions. The D-1 receptor had a more heterogeneous laminar pattern than did the D-2 receptor. Both D-1 and D-2 receptors were seen in homotypic, heterotypic and mesotypic cortex.

The rat cortex displayed regional differences in the laminar pattern of the D-1 receptor, with lamina V and VI generally denser than other laminae. The D-2 receptor was more homogeneous in it's laminar pattern, with lamina I being denser than other laminae.

The cat and monkey displayed a strikingly different pattern of D-1 receptors in the cortex compared to the rat. The density was much higher in the cat and monkey than in the rat. The cat and monkey also displayed more heterogeneous laminar and regional patterns of D-1 receptors than did the rat. Generally, layers I, II, V and VI were highest. The D-2 receptor was more homogeneous. The D-1 receptor had different regional patterns of distribution that varied in a longitudinal pattern. These results have some similarities to the pattern of tyrosine hydroxylase-immunoreactive fibers seen in primate neocortex (Lewis et al., J. Neurosci. 1:279-290, 1987).

These results support the notion that the dopamine system has a more complex role in cerebral cortical function than was previously suspected based on flouresence and other histochemical techniques. The role of dopamine in cortical function may also differ among mammalian species.

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198.9 AUTORADIOGRAPHIC EVIDENCE FOR THE MECHANISM OF MPTP-INDUCED TOXICITY IN THE DOPAMINE SYSTEM OF MONKEYS.
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MPTP administered intravenously to primates produces a parkinson-like effect characterized by extreme rigidity which can be reversed by L-DOPA. Histological examination of treated brains shows selective destruction of midbrain dopamine neurons in the substantia nigra pars compacta. The mechanism of toxicity which leads to selective destruction of subpopulations of dopamine neurons is largely unknown. We addressed this question by examining the anatomical distribution of $^{14}C_6$-phenyl-MPTP or ^{14}C-methyl-MPTP (200 - 500 µCi) given intravenously to monkeys (rhesus and cynomolgus) which were either normal or had prior unilateral destruction of the dopamine system induced by a single carotid artery injection of unlabeled MPTP 1 year prior to the injection. Survival times after the injections were 1, 3, or 10 days. The animals were then anesthetized, and their brains were removed fresh, hemisected, and frozen for cryostat sectioning. Sections 25 µm thick were melted onto slides, quickly dried on a hot plate, exposed to LKB film with C-14 standards for several weeks, and subsequently fixed, defatted and Nissl-stained. The films were developed and examined for localization of radioactivity with respect to underlying cytoarchitecture. Sections not used for autoradiography were homogenized and analyzed by HPLC, which confirmed that 98% of the radioactivity was MPP+. After 1 day survival in normal monkeys, radiolabeled MPP+ was heterogeneously distributed throughout the brain, as previously described (Markey et al., *Nature*, 1984, **311:** 464). After 3 and 10 days, MPP+ was selectively and highly retained in the striatum relative to cerebral cortex, although the locus coeruleus, parabrachial area, hypothalmus and intralaminar nuclei also retained label. ***At no survival time was there significant MPP+ labeling in the substantia nigra pars compacta.*** In monkeys which had received prior unilateral destruction of the nigrostiatal pathway (as evidenced by absence of dopamine markers in the striatum on the side of the prior carotid artery MPTP injection and by examination of the Nissl-stained sections), there was no significant radioactivity in the denervated caudate and putamen, though the caudal nucleus accumbens retained label bilaterally. Since the caudate-putamen on the lesion side appeared normal in other respects, we interpret the absence of label to indicate that MPP+ in the normal striatum resides in dopamine terminals. In another set of animals, in which [^{14}C]MPTP was given at the same time as a toxic dose, there was tremendous accumulation of radioactivity in the location of the degenerating nigrostriatal pathway after 3 and 10 days survival, but again, there was no accumulation in the nigra itself. Taken together, these data suggest that MPP+ kills dopamine neurons by a process of retrograde degeneration after it has been incorporated into and poisoned dopaminergic terminals and fibers.

198.10 **AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-CYANOIMIPRAMINE BINDING SITES IN RAT BRAIN.**
G.B. Kovachich, C.E. Aronson*, J. Ryu*, G.A. Ordway*, A. Frazer and D.J. Brunswick. Veterans Administration Medical Center and Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Cyanoimipramine (CN-IMI) is a potent antidepressant which has been shown to bind with high affinity to sites associated with serotonin uptake. We have demonstrated that this ligand binds to slide-mounted tissue sections of rat brain with pharmacologic specificity consistent with binding to sites associated with serotonin uptake (Kovachich and Brunswick, Neurosci. Abstr. 12:1079, 1986).

We have investigated the localization of binding sites for (^{3}H)-CN-IMI in rat brain using quantitative autoradiography. Slide mounted tissue sections (20 u thick) were incubated with varying concentrations of (^{3}H)-CN-IMI (0.01 to 2.0 nM) for 24 hrs at 4° in buffer (pH 7.4) containing sodium chloride (150 mM) and Tris (50 mM). Saturation curves were performed for 3 brain regions: olfactory tubercle, frontal cortex (agranular insular cortex) and caudate putamen. Analysis of the saturation curves using the Prophet computer system indicated that binding was to a single class of high affinity sites. The Kd (nM) values were similar for the three regions (mean ± s.d.), 0.09±0.05, 0.08±0.03 and 0.12±0.01, respectively. The Bmax (fmoles/ mg protein) values were 1624±297, 835±75.4 and 483±70.0, respectively. Based on these data, a concentration of 0.5 nM (^{3}H)-CN-IMI was chosen for analysis of the distribution of its binding sites in various brain areas. Highest densities (1000 to 4000 fmole/mg protein) were found in the dorsal raphe nucleus, interpeduncular nucleus, locus coeruleus, central gray areas, paraventricular thalamic nucleus and olfactory tubercle. Moderate levels of binding (500 to 1000 fmole/mg protein) were detected in the superficial layer of the superior colliculus, suprachiasmatic nucleus, reuniens thalamic nucleus, parietal cortex area 1, and lateral hypothalamic area. Low levels (less than 500 fmole/mg protein) were found in globus pallidus, caudate putamen, ventral thalamic areas, and throughout the cerebellum. Nineteen days after bilateral intraventricular injection of a total of 200 ug 5,7-dihydroxytryptamine the binding of (^{3}H)-CN-IMI was reduced to less than 100 fmole/mg in all areas tested, except in the dorsal raphe and interpeduncular nuclei (where an approximate 65% reduction in binding occurred). This distribution of binding sites is similar to that seen previously with (^{3}H)indalpine (Savaki et al., J. Neurochem., 45:521, 1985). The results indicate that (^{3}H)-CN-IMI is a useful ligand for autoradiographic analysis of the serotonin transporter. **(Supported by research funds from the Veterans Administration and from NIMH (MH 36761).**

198.11 AUTORADIOGRAPHIC LOCALIZATON OF ^{3}H-PAROXETINE-LABELED SEROTONIN UPTAKE SITES IN RAT BRAIN.
E.B. De Souza and B.L. Kuyatt*. Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224

Inactivation of brain serotonin is achieved primarily by high affinity uptake into synaptic nerve terminals. Since serotonin uptake sites are highly concentrated on serotonin-containing nerve terminals, we can directly localize and quantify the distribution of serotonergic nerve terminals in brain using autoradiographic procedures following the labeling of binding sites associated with the uptake of serotonin. Paroxetine is a potent and selective inhibitor of serotonin uptake into neurons. In the present study, serotonin uptake sites have been identified, localized and quantified in rat brain by autoradiography with ^{3}H-paroxetine. ^{3}H-Paroxetine binding in slide-mounted sections of rat forebrain was of high affinity (K_D = 10 pM) and the inhibition affinity constant (K_i) values of various drugs in competing for ^{3}H-paroxetine binding significantly correlated with their reported potencies in inhibiting synaptosomal serotonin uptake. ^{3}H-Paroxetine-labeled serotonin uptake sites were highly concentrated in the dorsal and median raphe nuclei, central gray, superficial layer of the superior colliculus, lateral septal nucleus, paraventricular nucleus of the thalamus, and the islands of Calleja. High concentrations of ^{3}H-paroxetine binding sites were found in brain stem areas containing dopamine (substantia nigra and ventral tegmental area) and norepinephrine (locus coeruleus) cell bodies. Moderate concentrations of ^{3}H-paroxetine binding sites were present in laminae I and IV of the frontal parietal cortex, primary olfactory cortex, olfactory tubercle, regions of the basal ganglia, septum, amygdala, thalamus, hypothalamus, hippocampus and some brain stem areas including the interpeduncular, trigeminal and parabrachial nuclei. Low densities of ^{3}H-paroxetine binding sites were found in other regions of the neocortex and very low to nonsignificant levels of binding were present in white matter tracts and in the cerebellum. Lesioning of serotonin neurons with 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine caused large decreases in ^{3}H-paroxetine binding. The results of the present study demonstrating an excellent correlation between drug specificity for ^{3}H-paroxetine binding and for synaptosomal serotonin uptake, and an autoradiographic pattern of ^{3}H-paroxetine binding which is virtually identical to the reported distribution of serotonin-containing nerve terminals and cell bodies, indicate the feasibility of using this approach for mapping serotonin uptake sites and serotonin terminals in rat brain.

198.12 A COMPARISON OF THE IN VIVO DISTRIBUTION OF ^{3}H-GBR 12935 IN NAIVE RATS AND RATS WITH UNILATERAL NIGRAL LESIONS.
N. Touchet* J.M. Trugman, G.F. Wooten. Department of Neurology, University of Virginia, Charlottesville, VA 22908.

The in vivo distribution of ^{3}H-GBR 12935, a potent neuronal dopamine uptake inhibitor, was studied in naive rats (male Sprague-Dawley) and in rats with unilateral 6-hydroxydopamine lesions of substantia nigra (SN). ^{3}H-GBR 12935 (10 µCi/0.5 ml saline) was administered by tail vein injection. The animals were killed by decapitation at 5, 10, 20, 30, 60, 90, or 120 min after administration of the drug. Brains were removed and dissected over ice into 5 regions: left and right samples of sensory-motor cortex, striatum, olfactory tubercle-nucleus accumbens (OA), midbrain, and cerebellum. Tissue samples were weighed, sonicated in 0.5 ml of H_2O, and tritium content determined by liquid scintillation spectroscopy. Data were expressed as a ratio of regional tritium content (DPM/mg) to tritium content in the cerebellum of each animal (region/cerebellum ratio).

In naive animals, region to cerebellum ratios increased with time in striatum and the OA and remained the same in sensory-motor cortex and midbrain (N=3 rats for each time point). For striatum the ratio increased from 1.09±.03 at 10 min to 1.76±.48 at 90 min, and 1.75±.16 at 120 min. The OA ratios were 1.02±.11 at 10 min, 1.33±.16 at 90 min, and 1.40±.17 at 120 min; sensory-motor cortex was 1.08±.04 at 10 min, 1.09±.17 at 90 min, and 1.15±.4 at 120 min; and midbrain ratios were .74±.36 at 10 min, .98±.09 at 90 min, and 1.01±.07 at 120 min. The animals with a unilateral SN lesion showed a lower ratio in the striatum ipsilateral to the lesion. Lesioned rats (N=6) killed 120 min after administration of the drug had striatal/cerebellar ratios of 1.21±.21 ipsilateral to the lesion and 1.77±.17 contralateral to the lesion. The striatal/cerebellar ratio in the striatum ipsilateral to the lesion was different from the 120 min naive group ($p<0.01$). In the SN lesioned group, the ratio of region/cerebellum tritium content for OA, sensory-motor cortex, and midbrain did not differ from controls.

In summary, ^{3}H-GBR 12935 selectively localizes in the striatum in normal rats after in vivo administration. In rats with unilateral SN lesions there is a decrease in the striatum:cerebellum ratio of drug ipsilateral to the lesion. The tissue ratios for the OA, sensory-motor cortex, and midbrain regions in lesioned rats did not differ from controls. These results are consistent with prior studies that show ^{3}H-GBR 12935 to be a potent and selective ligand at neuronal dopamine uptake sites and suggest that ^{3}H-GBR 12935 may also be used as an in vivo marker to label the neuronal dopamine uptake system.

199.1 FRONT-END CONTROL LAWS FOR HUMAN ARM MOVEMENT. B.J. McFadyen. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Due to the purposeful nature of the human being, movement control must have a predominant anticipatory component which will accommodate goals and account for internal and environmental constraints. Although discussed within the motor behavioural and neurophysiological literature, most simulations of motor control do not provide details of the use of anticipation. Simulations rely, for the most part, on servo and reflexive mechanisms. Good results may be obtained from elaborate non-linear feedback models; however, more attention and detail should be given to representing control at the front end of the model where operations are performed on both system inputs and feedback information together to provide anticipation.

Simulating a second order, single actuator system, data from perturbed forearm movements were used. Feedforward configurations (transfer functions operating on system inputs) provided a base on which to introduce front-end control and further expand and speculate on general anticipatory control strategies. Different feedforward configurations produced good results (no more than 0.3 degrees deviation from desired trajectory for unperturbed movement) compensating for both system loads and environmental disturbances. It is recognized that single joint motion is not the best representation of natural human movement; however, these configurations would also account for the interaction torques in multi-limb movement.

A first approximation to an internal, predictive model allowed the combination of information from the environment, internal inputs, as well as feedback to form an anticipatory control system. The use of an internal model accommodates the interactions between the various system elements, and introduces a simulated tie between neurophysiological mechanisms and cognitive processing. Although feedback is always necessary for accurate limb movement, control at the "front end" allows for the inclusion of anticipatory strategies. Physiological significance and multi-segmental control are discussed.

199.2 EMG ACTIVITY DURING HUMAN ARM MOVEMENTS MADE AT CONSTANT VELOCITY. J.D. Cooke, Dept. of Physiology, University of Western Ontario, London, Ontario, Canada.

"Fast"movements are normally associated with phasic muscle activation whereas "slow" movements are associated with gradual or tonic EMG changes. We have had subjects perform movements which display both phasic and tonic EMG components during the movements. Subjects performed visually guided tracking movements about the elbow. The target template was a ramp position change (constant velocity) presented as a phase plane (velocity vs position).

Movements were initiated by phasic agonist/antagonist activity (AG1/ANT1). This was followed by tonic EMG activity during the constant velocity phase. Movements were terminated by phasic antagonist/agonist activity (ANT2/AG2). As movement duration was decreased, the two sets of phasic activation formed an EMG pattern typical of that seen in "fast" step tracking movements.

During the constant velocity phase EMG activity of the individual muscles was non-linearly related to mean velocity during the constant velocity phase. However, relative EMG activity (biceps - triceps) during this phase of the movement varied linearly with the mean velocity. This relation was the same for flexions and extensions and was not dependent on whether velocity was varied by changing movement duration or movement amplitude. Mean relative EMG activity during the constant velocity phase also varied linearly with the magnitude of externally applied viscous loads.

The data show that, in the same way that length-dependent muscle non-linearities are linearized in the whole limb, so too are velocity-dependent non-linearities. Although the EMG - viscous force relation may be non-linear for the individual muscles, the relations is linear when the forces produced by the opposing muscles are considered. The data also shows that acceleration and deceleration of the limb are produced by paired or linked phasic activation of agonist and antagonist muscles. The data suggests that these paired activations underly the "triphasic" pattern seen in many "fast" movements.

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199.3 ACQUISITION OF ARM MOVEMENT SKILL AND MOVEMENT ADAPTATION IN HUMANS WITH CEREBELLAR DYSMETRIA. J.N. Sanes, B. Dimitrov* and M. Hallett. Human Motor Control Section, Medical Neurology Branch, NINCDS-NIH, Bethesda, MD 20892.

The cerebellum has been implicated in the acquisition and retention of motor skills. It has been reported that eye movement processes of cerebellar patients adapt slower when distorting prisms are worn. There have also been numerous reports indicating that classical conditioning is disrupted by cerebellar lesions and by lesions of cerebellar input-output pathways. To further the understanding of the cerebellar role in motor learning, we examined repetitive arm movements performed under different visual conditions.

Patients (n = 8) with spino-cerebellar degeneration or diffuse cerebellar atrophy were evaluated. The patients were selected for the presence of arm dysmetria and for the absence of intention tremor. Two-dimensional arm movements were evaluated. These movements consisted primarily of elbow and shoulder joint rotations, performed by sliding a hand-held electric stylus, across the surface of a digitizing tablet. The patients made movements so as to trace a closed lined pattern with 4 or 5 vertices that was placed on the digitizing tablet. The patients were required to perform the movements as fast and as accurate as possible, to come as close as possible to each of the vertices in the pattern, and to make the movement continuous without intermediate stops. First, patients made 50 movements about a pattern with 5 vertices with normal vision. Second, patients made 50 movements about a pattern with 4 vertices under mirror-reversed vision without being able to view their hand. Elapsed movement time and movement accuracy were the primary measures for data analysis.

For the task in which movements were performed repetitively with normal vision, movement time decreased and movement accuracy improved across the series of movements. When patients performed movements with mirror-reversed vision, several abnormalities of performance were observed. Several patients had long delays in movement initiation and completion for the first several movements. For these patients, the duration of the first mirror-reversed movement could be well in excess of 30 sec, even with encouragement by the examiner. The first movement performed by normal subjects were never that long. This movement initiation and elapsed time deficit rarely persisted beyond 5-10 movements. The accumulated time to complete the movement decreased with successive movement repetition for all cerebellar patients, but continued improvements were typically not observed beyond repetitions 10-20. Indeed, it was often the case that additional repetitions beyond the first 20 movements resulted in a deterioration of performance.

These data indicate that cerebellar patients can improve performance of arm movements made under different visual conditions, but that there are limitations to visuomotor adaptation due to cerebellar dysfunction. Cerebellar patients improve upon both the elapsed time and accuracy of movements performed with normal vision. Although the total time taken by patients to complete movements made with mirror-reversed vision decreased, this improvement was not nearly as great as normal subjects. It is possible that improvement of movements requiring complex visuomotor coordination is limited by cerebellar dysfunction.

199.4 ARM MOVEMENT TRAJECTORIES AND MOVEMENT CONTROL STRATEGIES OF EXPERT AND NON-EXPERT DART THROWERS. J.L. Leavitt*, R.G. Marteniuk, and H. Carnahan*. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

We addressed three questions concerning the three-dimensional trajectories of dart throwing. First, our previous work on Fitts' Law suggested that for aiming movements terminating on impact with the target, unique movement trajectories (resultant velocity profiles) were produced for targets of different sizes; however, for different movement amplitudes the trajectories scaled to a common profile. We wanted to see if similar results could be obtained for a throwing task where throwing distance and accuracy were manipulated. Second, we wanted to verify the claim by expert dartmen that regardless of the target size, the release of the dart always occurs at the peak vertical height of the displacement trajectory. Third, we wanted to determine the extent to which expert dart throwers are bound to the context of actual competition. We did this by contrasting dart throwing in a regulation environment with a reduced context situation (non-regulation throwing distance and a non-dart board target).

While manipulations of throwing distance and target size influenced movement outcome (landing accuracy of the darts), neither the time from the start of the forward movement to dart release nor the time from dart release to peak vertical displacment were affected. Overall, the results indicated that expert dart throwers vary the force with which the dart is released by varying the distance over which the arm travels while keeping time of contact with the dart constant. This type of strategy is consistent with a pulse-height movement generation model where a constant movement trajectory duration is maintained while trajectory slopes are varied to accomplish different impulses required by the task demands.

Other results suggested that expert dart throwers do not release the dart when they think they do. The dart is released an average of 58 ms prior to the peak vertical displacement of the hand. Finally, while there were qualitative differences between the movement trajectories of expert and non-expert performers, neither group was affected by the reduction of task context.

199.5 JOINT VELOCITY PROFILES DURING VERTICAL ARM MOTION. J. Hollerbach and D. Bennett* (SPON. C. Atkeson). Dept. Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Previously, we have inferred from experimental arm movements that a trajectory between targets is planned at the joint level with a staggered joint interpolation stategy (*Motor Control,* ed. G.N. Gantchev et al., Plenum, 1987, pp. 197-208). Stagger is seen in many motions and appears to be invariant with the direction of movement, arguing against it being an artifact caused by gravity or dynamical interactions. The stagger is hypothesized to be set in order to achieve relatively straight-line motions without calculating inverse kinematics.

We present further evidence for this notion based on simulations and experiments, focusing particularly on the time profiles. Unlike strict joint interpolation, the specific velocity profile in staggered joint interpolation definitely affects the path. A question then arises whether one velocity profile is generally best at yielding straight-line paths. Simulation shows that a gaussian velocity profile achieves a straighter line than do other profiles such as minimum jerk in almost all of the work space.

A second question concerns the tangential velocity profile, which was previously found to be invariant across a variety of experimental conditions (*J. Neuroscience, 5*(1986): 2318-2330). Strictly speaking, this invariance is not possible under staggered joint interpolation, yet simulations show that tangential velocity profiles are generally unimodal and vary little in shape. This indicates that tangential velocity profile shape is not a good indicator of coordination strategy, lacking sensitivity.

A third question is how well the experimental joint velocity profiles agree with the prediction of shape invariance, save for a delay and scaling. The data bear out this prediction reasonably well, although the exact velocity profile shape cannot be inferred directly because of the variance in the data. Gaussian velocity profiles do qualitatively capture the tails in the data better than do minimum jerk profiles. Simulations with experimentally determined stagger and scaling produce good agreement with the data.

Although subjects make relatively direct movements between targets, they have great difficulty in producing a perfectly straight vertical line, where substantial elbow joint reversal is required. This led us to perform some visual studies which seem to indicate that our perception of straightness in the saggital plane is biased in a characteristic manner that corresponds to the curvature in the motor movements. This research is supported by NIH Grant AM26710.

199.6 VARIATION IN HUMAN POSTURAL ACTIVITY PREPARATORY TO WELL-PRACTICED RAPID ARM MOVEMENT. C.S. Layne and L.D. Abraham. Dept. of Physical Education and Institute for Neurological Sciences Research, The University of Texas, Austin, TX 78712.

Patterns of postural activity preceding rapid voluntary arm movements have been described by several investigators (e.g. Belen'kii et al., Biofizika, 12:135, 1967; Lee, J. Mtr. Beh., 12:185, 1980) consisting of a relatively stable proximal-to-distal pattern of leg muscle activation, often preceded by silent periods in postural muscles. Such data have been collected primarily using a reaction time (RT) paradigm. We examined these early postural patterns (EPP) in a similar task which required coincidence anticipation (CA) of (rather than reaction to) a visual stimulus. The results suggested that in CA tasks inter-individual variation in EPPs exceeds that reported in RT tasks.

Five normal right-handed adult males were given extensive practice in very rapid bilateral reaching to acquire a ball located at shoulder height in front. The movement was cued by a visual stimulus and was to begin coincident with a criterion (visual) stimulus, which followed the first by 1000 ms. Prior to data collection, each subject practiced reaching for 100 trials on each of three days, ultimately displaying consistent coincident movement onset. Surface EMG was used to monitor the activity of the following muscles of the right side: soleus and gastrocnemius (TS), biceps femoris (BF), and biceps brachii (BB). The subjects stood on a platform capable of producing small backward shifts. These postural perturbations, and the addition of 3 lb wrist weights, were used to probe the consistency of EPPs over a range of mechanical postural constraints.

Data were analyzed from blocks of ten trials for each experimental condition. Six different patterns of muscle onsets were identified, with each subject showing little or no variation in pattern. Only one pattern involved an initial silent period; two of the patterns contained no consistent leg muscle activity prior to BB onset. In four of the patterns the TS were the first leg muscles to become active. All but one of the subjects used the same EPP with the addition of wrist weights as without, and each subject's EPP was largely unaffected by unexpected backward perturbations during the EPP. Perturbations just prior to the EPP abolished most of the EPPs. These data suggest that the EPP patterns described previously may have been significantly affected by the temporal constraints of an RT task. Since our well-practiced CA task allowed more time to prepare for rapid movement onset, each subject was able to prepare to move in his preferred way, which was fairly resistant to mechanical task modification. (Supported in part by a BRSG grant through the University Research Institute.)

199.7 TRUNK CONTROL DURING ARM MOVEMENTS - STUDIED WITH THE VICON SYSTEM. A.M. Iannone, M. Hallett, V. Reese and S. Stanhope. Department of Neurology, Medical College of Ohio, Toledo, Ohio 43699, and NINCDS, Bethesda, Maryland 20892.

The Vicon system was used to study arm movements in the human. Our objective was to develop a standard set of movements from which we could compare the effects of neurologic disease. 7 normal subjects were studied. Movements studied were Finger-to-Nose (F-N), Reach for paper cup and lift (R), and Catch a ball (C). Subjects were seated on a backless stool, arm was placed in an identical position, from which (F-N), (R), or (C) could be done. Movements were carried out over three distances:

a. The length of the subject's arm (shoulder joint to finger tip).
b. 80% of normal arm length.
c. 120% of normal arm length.

There were two sets of commands used: the first was to make the move as accurately as possible, without regard to speed; the second to make the move as rapidly as possible without regard to accuracy.

Each different movement utilized different strategies for arm placement; for normal (F-N) the hand was put into the pointing position at the beginning of the movement and the bulk of the moving was done at shoulder and elbow joints; for short (F-N) the elbow was left in greater flexion and there was backward movement of the trunk at the same time; for long (F-N) full extension of the elbow and simultaneous forward movement of the trunk occurred during the reaching.

(R) movements required different strategies in which hand and finger placement at the necessary angles to grasp the cup placed different constraints on the subjects.

Strategies to counteract arm momentum while reaching, and to correct for the gravitational effects of the extended arm, are used in controlling the muscles of the shoulder and spine. The motor program from its beginning must contain these elements.

199.8 REVERSAL OF RECRUITMENT ORDER DURING VOLUNTARY LENGTHENING CONTRACTION OF TRICEPS SURAE MUSCLES IN MAN. M. Schieppati*, C. Romanò* and A. Nardone*. (SPON: F. Clementi). Istituto di Fisiologia Umana II, Via Mangiagalli 32, I-20133 Milano, Italy.

Recruitment and derecruitment characteristics of soleus (Sol), gastrocnemius lateralis (GL), medialis (GM), peroneus (Per) were studied by means of surface EMG recording, during voluntary movements implying muscle shortening or lengthening contractions, or during isometric contractions (ICs). The subjects had their foot fixed to a mould allowing rotation along an axis centered on the tibio-tarsal joint and performed the following tasks under visual control of position or force signal: plantar flexions (PFs) against a constant load, dorsal flexions (DFs) starting from a maintained foot flexion and yielding to the load by muscle relaxation, augmenting or decreasing plantar torques (ICs). The tasks were performed at various angular velocities or rates of force change. In all the tasks, the tibialis anterior was silent.

In all the subjects the muscles gradually increased or decreased their activity roughly in parallel, during ICs and PFs. During DFs, gradual derecruitment was present in Per; the behavior of the triceps muscles, instead, varied between two extremes. 1) Sol activity decreased prior to onset of movement, GL (or GM, depending on subject or knee angle) activity increased to well above the level of the tonic contraction and remained high through out DF. Amplitude and rate of rise of GL EMG were larger, and Sol derecruitment more rapid, the higher the velocity of DF. GL EMGs of similar extent were reached only during ballistic PF. 2) Sol EMG decreased prior to DF onset, but displayed during DF bursts of large amplitude, superimposed on low-level background activity. GL EMG showed only minor increases during DF. In the various subjects the extent of GL recruitment during DF was positively correlated with the duration of the Sol twitch relaxation. Irrespective of the activation patterns of Sol and GL, the excitability of their motoneurones tested by the H reflex was depressed to a similar extent during DFs. This gives further evidence that the increases in GL or Sol EMG are sustained by fast motor units, since H reflex amplitude depends mainly on activation of slow motor units.

We suggest that substitution of GL for Sol, or of fast for slow motor units within Sol, allows better force gradation during DF lengthening contraction, owing to shorter duration of relaxation times in fast muscles or motor units.

199.9 CHARACTERISTICS OF SYNERGIC RELATIONSHIPS IN HUMAN ELBOW JOINT MUSCLES DURING STATIC THREE DEGREE-OF-FREEDOM JOINT TORQUES. T.S. Buchanan* and W.Z. Rymer (SPON: W.T. Rainey). Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Physiology Department, Northwestern University Medical School, Chicago, IL 60611.

Previous studies on the synergic relationships of human elbow joint muscles during flexion/extension (f/e) and varus/valgus (vr/vl) isometric tasks have demonstrated that (1) patterns of muscle activity are independent of joint torque magnitude and (2) intermuscular relationships can vary as a function of load direction, even among "synergists" (Buchanan et al., *J. Neurophys.* 5:1225-1241, 1986). The current study uses myoelectrical information to study the central command involved in muscle coordination and joint stabilization during supination/pronation (s/p) torques and to study the effects that such torques have on f/e and vr/vl tasks.

The myoelectrical activities in the biceps brachii, brachioradialis, brachialis, and triceps brachii have been simultaneously recorded with both surface and bipolar, fine-wire intramuscular electrodes. These recordings were made while the subject was in a fixed position with the elbow at 90° flexion, the shoulder at 0° flexion, and the wrist at a mid-supinated position. The subject was instructed to match a visually-displayed, three degree-of-freedom force target by isometrically contracting against a 3-d.o.f. load cell apparatus fixed to the wrist via a short forearm cast. The displayed targets scanned a ranged from 10% to 30% of maximum vr/vl torque. The amplified, bandpass-filtered EMGs were digitally recorded together with the three torques: f/e, vr/vl, and s/p. The data was later reduced off-line to determine the average rectified EMG for all trials under each load condition.

The EMGs were analyzed separately for each of 5 s/p torques. From these data sets, patterns of activity were obatined that could be described in terms of f/e and vr/vl torques. These patterns showed distinct relationships that were simply scaled up and down with f/e and vr/vl torque magnitude. Most significantly, the shapes of these patterns were found to be independent of s/p loads. That is, supination and pronation forces did not influence the relative EMG activity observed between f/e and vr/vl torques. The magnitude of the EMGs were relatively constant for different s/p torques for some muscles (e.g. brachialis), but were observed to scale with supination and pronation in others (e.g. biceps brachii).

These patterns of activity imply that flexion/extension and varus/valgus elbow torques are controlled independent of supination/pronation torques. This type of control scheme — treating these loads as two independent variables — could be advantageous from a neural control perspective. For example, separate interneuronal pools could be responsible for supination/pronation torques while flexion/extension and varus/valgus torques would be much more closely coupled.

This work supported by NIH grant 2-R01-NS19331.

199.10 ASYNCHRONOUS INITIATION OF COORDINATED ROTATIONS ABOUT THE SHOULDER AND ELBOW. Z. Hasan and G. M. Karst. Department of Physiology, University of Arizona, Tucson, AZ 85724.

In the study of target-directed limb movements involving rotations about several joints it is often assumed that the nervous system prescribes the trajectory of the distal point along an approximately straight-line path, and subsequently computes the joint torques necessary to obtain that trajectory. From a computer simulation of this strategy we were struck by the precision needed, even for the initial torques, in order to aim the initial acceleration toward the target. We explored experimentally the possibility that a less stringent strategy may be employed for the initiation of movement.

During arm movements to different targets in the horizontal plane the shoulder and elbow angles were monitored by means of potentiometers. We recorded the EMG activity of the clavicular portion of pectoralis major and of the posterior deltoid (horizontal shoulder adductor and abductor muscles, respectively) in addition to that of the biceps and triceps brachii.

Our main experimental finding was that the rotations about the shoulder and elbow joints were not, in general, initiated simultaneously. The order of shoulder and elbow rotations depended in a consistent manner upon the placement of the target. Simultaneity was seen when the two rotations were comparable in magnitude, and especially when the two were in opposite directions (e.g., shoulder adduction and elbow extension), confirming earlier reports. But when, for instance, the target was so placed that the shoulder adducted by 26 deg and the elbow flexed by 7 deg, the initiation of elbow rotation occurred approximately 80 ms after the initiation of shoulder rotation. A similar delay was seen when a comparably small elbow rotation occurred in the extension direction. In either case, elbow flexor activity was seen during the delay interval, presumably to prevent elbow extension that would have arisen from inertial effects of the moving upper arm.

Conversely, when the target position required a large elbow rotation but small shoulder rotation, the former was initiated earlier than the latter. During the delay interval, which could exceed 100 ms, activity of the appropriate shoulder muscle prevented rotation about the shoulder despite the inertial coupling with the forearm. (The order of muscular activation was always proximal to distal, even when elbow rotation preceded shoulder rotation.) Staggering of the rotations according to magnitude may be a simplifying aspect of the strategy for movement initiation.

199.11 INVARIANT CHARACTERISTICS AND INVOLUNTARY RESPONSES. G.L.Gottlieb, A.S.Tanavde* and G.C. Agarwal,Department of Physiology, Rush Medical College, Chicago, IL 60612.

We have previously reported plastic behavior of human elbow compliance when subjected to sudden torque perturbations[1,2]. Recent questions have been raised [3] concerning the possible contamination of our results by involuntary responses of the subjects, particularly when subjected to loading, as compared to unloading perturbations. This report summarizes our findings using three methods to characterize the compliant response of the elbow and two sets of instructions to the subjects.

Step torque perturbations were applied to the elbows of normal human subjects who were instructed either a) do not react to the perturbation or b) resist the perturbation as rapidly as you can. Compliance was measured either as *i.* the ratio of elbow displacement to perturbing torque when the elbow reached a stable equilibrium, *ii.* the same ratio measured at the peak velocity of the displacement and *iii.* the elastic component of a linear, second order differential equation, fit to the first 100 ms of the perturbation trajectory.

When the subject does not react, qualitatively similar results are found with all three methods. Those are that for both loading and unloading perturbations, the effective stiffness of the joint is greatest for the smallest perturbations. When the subject resist the perturbation however, the first method gives radically different results while the other two methods are unaffected.

We conclude that the S-shaped compliance curve we have previously reported is significant for both loading and unloading. Furthermore, if measurements can be made within about 100 ms of the perturbation's onset, the results are unaffected by reactions by the subject.

SUPPORTED IN PART BY NIH GRANT AR-33189. [1] Gottlieb,G.L. and Agarwal,G.C, Soc. Neurosci. Abst. 9:632, 1983, [2] Gottlieb, G.L. and Agarwal,G.C., J.Neurophysiol (in press), [3] Berkinblit, M.B, Feldman, A.G. and Fukson, O.I. Beh.&Brain Sci.9, 1986

199.12 PERCENT OF MAXIMAL VOLUNTARY ISOMETRIC CONTRACTION (MVIC) ON QUADRICEPS FEMORIS MUSCLE (QF) INDUCED BY NEUROMUSCULAR ELECTRICA STIMULATION (NMES) OR TRANSCUTANEOUS ELECTRICAL STIMULATION (TENS). L.E. Tremblay*, D. Dufour*, Y. Lessard* and V. Poulin (SPON: A.Y. Bélanger). Physical Therapy Dept., Fac. of Medicine, Laval University, Québec, Canada, G1K 7P4

Neuromuscular electrical stimulation and transcutaneous electrical nerve stimulation are known to strengthen and reduce nociceptive perception, respectively. Both of these in adjunct to a management rehabilitation program have been proved very useful. Since these modalities (NMES) and (TENS) aim different goals, two kinds of apparatus were proposed for each treatment strategies. This projet was designed to compare the capacity of each apparatus to induce a torque in the (QF). Seventeen healthy male volunteers ($\bar{X}$ = 23.1 ± 4 years old) were involved in this study. All subjects were tested in standardized protocol for dynamic knee extension with cybex II+ at 30° sec in order to determine the angle at which the QF's peak torque is observed on the isokinetic curve. Thereafter each MVIC and the torque of the evoked contraction was measured at this angle. Two commercial stimulators (medtronic$_r$), respond II (NMES) and selectra (TENS) were used with quite similar electrical parameters (waveform, skin impedance, pulse duration, rate (50 HZ), electrode width). Two experimental strategies were used for each stimulator; bipolar method (surface electrode over the QF muscle) and monopolar (cathode just over the femoral nerve). The following parameters were measured; % of MVIC, maximal intensity tolerated (I, in mA), confortability (visual analog scale) (V.A.S.).

	Bipolar Method			Monopolar Method		
	% MVIC	I	V.A.S.	% MVIC	I	V.A.S.
NMES:	41.2±12.4	99.7±1.2	7.1±2.3	67.1±16.3	83.1±12.3	9.1± .9
TENS:	6.5± 9.8	100	4.7±2.3	81.3±15.4	94.2± 9.1	7.4±1.2

The selectra was found more comfortable ($P < 0.01$) and more effecient ($P < 0.01$) in the monopolar fashion to produce a QF torque. The TENS was probably more successful because of its antinociceptive properties. Strangely the NMES stimulator (designed to increase muscular strength) was less effective in evoking a large torque in the QF.

199.13 DIFFERENCES BETWEEN MONKEY TIBIALIS ANTERIOR AND GASTROCNEMIUS EMG BEHAVIOR DURING PERFORMANCE OF COMPARABLE TASKS-S. A. Sahrmann, M. H. Clare, E. B. Montgomery, and Wm. M. Landau, Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

Limb postures associated with central nervous system lesions suggest qualitative differences in motor control of flexor versus extensor motoneurons. This report provides quantitative information regarding the differences between tibialis anterior (TA), and gastrocnemius (G) during performance of a conditioned controlled task.

A macaque nemestrina was trained to perform two mirror image isometric ankle tasks triggered and guided by light signals. One from rest (0 force) to large plantarflexion (LP, 650 gms), hold for a random period (0.5 - 1.5 sec), rapidly reverse into large dorsiflexion (LDr, 450 gms) and return to rest and the other the mirror image task (LD with LPr). Surface electrodes were used to record TA and G emg. Force and rectified and integrated emg were converted to digital signals and recorded on-line by a VAX 11 computer. Trial by trial and averaged records were reconstructed by the computer and used for data analysis.

Averages of 12 repetitions from 20 recordings over a 5 month period were used for data analysis. The onset and offset of the TA was slower than the G. The time to peak for the TA for LD was 323 msec while that for the G during LP was 42 msec. The emg duration of the TA for LDr was 326 msec while that of the G for LPr was 132 msec. The reciprocal relationships of these antagonist muscles also differed. G emg had ceased for 40 msec before onset of TA emg for LDr. TA emg was still subsiding during the onset of G activity for LPr and after a brief period of inhibition had an afterdischarge which occurred during the return to dorsiflexion. No afterdischarge was present in the G during the mirror image task LPr. Force behavior followed the EMG for all but the sustained hold. TA emg was maintained at 87% of peak during the last 500 msec of the hold, while the G dropped to only 65% of peak even though the force was maintained at a constant level.

Analysis of fiber types in a non-exercised monkey indicated that the distribution of type I was 16% in the TA and 27% in the G, while type II was 84% in the TA and 73% in the G. The differences of movement behavior cannot be attributed to muscle fiber types.

These results indicate that even though the animal performed comparable tasks in dorsal and plantar flexion with similar time and force requirements, there were different central control patterns of reciprocal contraction and timing.

199.14 THE EFFECTS OF INSTRUCTIONS ON THE SPEED AND ACCURACY OF VISUALLY GUIDED LIMB MOVEMENTS. J.D. Fisk and M.A. Goodale, Camp Hill Hospital, Halifax and University of Western Ontario, London, Canada.

Visually directed movements of the eyes and limbs are coordinated to provide the maximal amount of information for the accurate completion of the movement. Many studies have examined the environmental determinants of movement speed and accuracy but in the present study the environmental conditions remained constant, while the demands for speed and accuracy varied according to the subject's instructions. Thus it was the volitional set of the subject that determined the changes in their response characteristics.

Sixteen subjects participated in the study. Each subject was seated, with his head supported in a head rest, facing a black screen at 50 cm viewing distance. The targets were 4 LEDs at 10° and 20° to the left and right of a central point. The subject's eye position was monitored by infrared reflection while the position of the limb in 3-dimensional space was determined by a computer-assisted analysis of the images provided by 2 rotary-shutter video cameras. All subjects performed 3 blocks of trials in which each target was illuminated 8 times in a random sequence. The speed and accuracy demands of the blocks of trials were varied by asking the subject to point to the target "as quickly as you can" (Speed condition); "as accurately as you can" (Accuracy condition); or both "quickly and accurately" (Speed/Accuracy condition). The order of the blocks was randomized across subjects. The eye and hand latency were analysed as were numerous kinematic measure of the limb movement and the accuracy of the finger position.

The Speed/Accuracy condition was considered as the baseline condition since speed and accuracy were equally emphasized in the instructions. Eye movement latency was reduced by increasing either the speed or accuracy demands of the task while hand movement latency was reduced only in the Speed condition. The increased discrepancy between eye and hand movement latency in the Accuracy condition would have allowed the eyes to attain the target position before the arm movements were initiated. Kinematic measures indicated no difference in the acceleration phase of the movement for the Accuracy or Speed/Accuracy conditions. In the Speed condition however, the subjects reached a higher maximal velocity. The deceleration phase of the movement differed systematically according to the instructions. Deceleration was most rapid in the Speed condition and was slowest in the Accuracy condition, providing more opportunity for visually based modifications of the movement trajectory. Constant and variable error were largest in the Speed condition. The results indicate the subtle, changes in the programming and execution of visually guided movements can occur if instructions alter the volitional set of the subject.

Supported by an MRC Canada grant to M.A. Goodale.

199.15 PATTERNS OF AGONIST MUSCLE ACTIVITY IN MONKEYS TRAINED TO PERFORM WRIST MOVEMENTS IN DIFFERENT DIRECTIONS. D.S. Hoffman and P.L. Strick. V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-HSC at Syracuse, Syracuse, NY 13210.

We have examined the patterns of muscle activity in monkeys trained to perform step-tracking movements of the wrist joint. The movements required eight different combinations of radial-ulnar deviation and flexion-extension and differed from one another by 45 deg. Pairs of fine wire electrodes were inserted percutaneously into 10 different finger and wrist muscles. Each wire was stimulated to determine the direction of wrist movement evoked by muscle contraction. Stimulation also confirmed that both electrode wires were placed in the same head of each muscle.

Each forearm muscle examined produced a prominent **agonist** burst of muscle activity for more than one direction of movement. The agonist burst began in all forearm muscles at approximately the same time and preceded movement onset by approximately 50 msec. No clear differences were observed between wrist and finger muscles either in the timing or pattern of agonist bursts during movement. Four muscles had substantial agonist bursts for 4 or more directions of movement. The activity in these muscles was considered to be "broadly tuned" for movement direction. Three muscles had substantial agonist bursts for only 2 directions of movement. The activity in these muscles was considered to be "narrowly tuned." The tuning in the remaining 3 muscles was intermediate; these muscles had substantial agonist bursts for 3 directions of movement.

A **best agonist direction**, i.e., the direction of movement for which the agonist burst was greatest, was determined for each muscle. The amplitude of agonist bursts was reduced for directions of movement which differed from the best agonist direction. The reduction in amplitude, however, could be as little as 18% for movements which differed from the best agonist direction by as much as 75 deg. There was a close correspondence between the best agonist direction and the direction of movement evoked by electrical stimulation for 4 wrist muscles. However, in 3 wrist muscles, the best agonist direction differed from the direction of movement evoked by electrical stimulation of the muscle by as much as 60 deg.

Our observations indicate that each direction of wrist movement was produced by adjusting the amplitude of agonist bursts in more than one muscle. In fact, most directions of movement (n=6) were produced by agonist bursts in 4-5 muscles. These results do not support the concept that the direction of wrist movement is determined by a limited number of muscle synergies.

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199.16 GOAL-DIRECTED POINTING MOVEMENTS FOLLOWING CHEMICAL MICROLESIONS WITHIN THE POSTERIOR PARIETAL CORTEX OF TRAINED MONKEYS. O. Bock*, R. Eckmiller, and R. Andersen (SPON: W.J. Daunicht) Division of Biocybernetics, University of Düsseldorf, FRG.

Two monkeys (Macaca fascicularis) had been trained to point at visual targets, presented on a cylindrical screen 15 cm in front of their eyes. The pointing arm grasped a manipulandum which restricted hand movement to a horizontal arc just underneath the target display. Manipulandum position was visible to the animal via an LED.

Following baseline data acquisition, chemical microlesions (injections of 3x1 µl 15% Ibotenic acid) were administered to the left parietal cortex. Subsequent histology verified lesion sites at the border between Areas 7a and 7b.

Results

1. Several hours after the lesion deficits gradually developed and persisted throughout the examination period of more than one week.
2. Some deficits were side-specific relative to the lesion, whereas others depended on the movement direction (ipsilateral, i.e. right arm to the right and left arm to the left, versus contralateral) of either arm in the entire pointing space.
3. Dynamic overshoot in the movement trajectories increased markedly in incidence and size for ipsilaterally directed pointing movements within the entire pointing space. In contrast, the overshoots for contralaterally directed movements were considerably smaller.
4. Latency increased markedly for contralaterally but not for ipsilaterally directed pointing movements within the entire pointing space.
5. Dynamic overshoot and latency were not correlated on a trial-to-trial or a day-to-day basis.
6. When visual feedback on manipulandum position was turned off, static pointing accuracy was significantly reduced only in the right hemispace (i.e. contralateral to the lesion), independent of the pointing arm.

Conclusions

A. Dynamic overshoot and latency deficits strongly suggest an involvement of the posterior parietal cortex in two independent and direction-selective mechanisms of pointing movement control.
B. An additional hemispace-specific mechanism is indicated by the accuracy deficit in the absence of visual feedback.

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199.17 BLOCKING THE ANTAGONIST NERVE REDUCES THE AMPLITUDE OF THE SHORT-LATENCY STRETCH REFLEX RESPONSE IN TRICEPS SURAE MUSCLES OF CATS. T. Sinkjaer* and J.A. Hoffer. Dept. of Clinical Neurosciences, Univ. of Calgary, Alberta T2N 4N1, Canada.

The objective of this study was to quantify the contribution from afferents in antagonist muscles to the short-latency reflex response measured in ankle extensors of freely standing cats.

Cats were trained to stand unaided on four pedestals and produce a desired force with the left hindlimb (J Neurosci Meth, in press). Once trained, they were chronically implanted with EMG, force, length and temperature transducers and nerve recording, stimulating and blocking cuffs in the left hindlimb (Soc Neurosci Abstr 9:470, 1983 and 10:330, 1984). Conduction in the common peroneal (CP) nerve was reversibly blocked by infusing 2% lidocaine into a CP nerve cuff. The completeness of the block was demonstrated by the disappearance of the compound action potential recorded by a sciatic nerve cuff when the CP nerve was stimulated distal to the block. Reflex responses in freely standing cats were elicited by displacing the left rear pedestal randomly up- or downward with a servo-controlled motor as the cat was maintaining a given force.

The peak amplitude and area of the short-latency EMG burst (8-12 ms after stretch onset) were measured in the soleus (S) and lateral gastrocnemius (LG) muscles. Results obtained before and after CP nerve block were compared for matched values of background muscle force, length and temperature. The experiment was repeated after decerebration. Results are shown in the table with arbitrary units:

	Normal				Decerebrated			
	S EMG		LG EMG		S EMG		LG EMG	
	Peak	Area	Peak	Area	Peak	Area	Peak	Area
before CP block	1.56	1.00	2.50	1.37	4.24	2.01	12.60	7.08
after CP block	1.14	0.74	1.48	0.90	2.54	1.44	5.24	3.00
percent change	-27	-26	-41	-34	-40	-28	-58	-58

In the normal cat, the background triceps force and soleus muscle length were 25 N and 7.8 cm before block and 27 N and 7.8 cm after block. After decerebration the values were 49 N and 8.3 cm before block and 47 N and 7.9 cm after block.

The reduced short-latency reflex in stretched ankle extensors after blocking the CP nerve demonstrates that ankle flexor afferent input can have powerful influence on short-latency autogenic and heterogenic reflexes to ankle extensor muscles in freely standing normal as well as decerebrated cats. This observation is consistent with the "antagonogenic" reflex action described by Nichols (Soc Neurosci Abstr 11:213, 1985) for decerebrated cats.

Funded by the Alberta Heritage Foundation for Medical Research, MRC of Canada and Technical Research Council of Denmark. Present address of TS: Inst. of Electronic Systems, Aalborg Univ., Denmark.

199.18 EFFECT OF ANTAGONIST MUSCLE VIBRATION ON POSITION SENSE DURING VOLUNTARY MOVEMENTS. J.T. Inglis* and J.S. Frank (SPON: E. Cafarelli). Dept. of Anatomy, Queen's University, Kingston, Ontario and Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

During voluntary movement, muscle spindles of both the agonist and antagonist muscles potentially can supply information about position of the limb. The following 3 experiments attempted to examine these contributions by separately vibrating over the triceps and biceps muscles during forearm positioning. Muscle vibration is known to increase muscle spindle discharge (Burke et al., J. Physiol., 261:673, 1976) and cause systematic distortions of limb position sense in humans (Goodwin et al., Brain, 95:705, 1972). In the first experiment, subjects (n=8) performed a horizontal flexion of the right arm to a mechanical stop randomly positioned at 20, 40 or 60°. Vision was occluded and vibration was applied to the right arm. The perceived position of the right limb was assessed by instructing subjects to simultaneously match the right arm position with the left limb. Vibration of the biceps muscle had no effect on limb matching accuracy. However, triceps vibration resulted in a significant overestimation of the vibrated limb position by 6-13° ($p < .01$). The procedures for the second experiment were similar to the first, except that movements of the right limb were self-terminated. A screen was mounted over the arms and subjects were instructed to move the right arm until it was positioned beneath a marker on the screen. Vibration of the biceps muscle had no effect on either the positioning accuracy of the right limb or matching accuracy of the left limb. However, triceps vibration resulted in significantly shorter movements (6-10°, $p < .05$) by the right limb and an overestimation of right limb position by the left, matching limb. In a final experiment vibration was applied to the stationary left arm, while subjects attempted to position the right arm beneath a marker on the screen covering the arms. Positioning accuracy of the right arm was not effected by this vibration, suggesting that the mismatch between left and right limb performance reported in experiments 1 and 2 is due to a perceptual and not a motor bias. These findings further support the hypothesis that muscle spindle afferent information from the lengthening antagonist muscle contribute to limb position sense during voluntary movement (Capaday and Cook, Exp. Brain Res., 42:228, 1981).

199.19 KINEMATICS OF BODY MOVEMENTS ASSOCIATED WITH POSTURAL ADJUSTMENTS. M.W. Woollacott, C. von Hofsten* and B. Roseblad*. (SPON: Peter M. O'Day). Psychology Dept., University of Umea, Umea, Sweden.

Studies examining the participation of both leg and upper body muscles in postural control have shown that, in response to support surface translations in the anterior or posterior direction, responses are activated first in the ankle muscles at about 90 ms and radiate upward to the upper leg and trunk muscles. In addition to these responses, there are very early responses in the neck muscles on the opposite aspect to the body, activated at latencies close to those seen in the stretched ankle muscles. These responses could be activated by stimulation of the vestibular system, or neck proprioceptive system, if head movements were induced very early with respect to platform movement onset. Alternatively, if head movements occurred later in time, the ankle proprioceptive inputs themselves could be hypothesized to trigger the neck muscle responses as part of a preprogrammed synergy encompassing agonist and antagonist muscles of the entire body.

This study examined the movements of the individual body segments of a group of 10 standing adults during anterior and posterior platform displacements and compared body movements to neck and ankle muscle response onset times. A Selspot optoelectronic system was used to measure the displacement of the ankle, knee, hip, hand, shoulder and head. Though anterior/posterior neck and head displacements were late (145 and 197 ms respectively for 3 cm posterior platform movements), in comparison to neck flexor muscle response onset (121 ms average), vertical movements of the shoulder and head occurred early (40 and 67 ms after platform movement onset). This head movement could activate neck proprioceptors and/or the vestibular system to cause neck muscle responses.

In order to determine if neck proprioceptors were primarily responsible for response activation, we repeated the experiment using a neck stabilization device, on one of our subjects. In this condition, with minimal neck muscle stretch but normal vestibular and ankle joint somatosensory inputs, we found normal neck muscle response latencies. This supports the hypothesis that the ankle joint somatosensory and/or vestibular systems are the primary contributors to the early neck muscle responses seen during horizontal support surface displacements.

199.20 THE EFFECT OF VISUAL FIELD, EXTRACORPOREAL SPACE AND LIMB ON LOCALIZATION OF VISUAL TARGETS. B. Sivak, C.L. MacKenzie, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, N2L 3G1 and D. Elliott*, School of Physical Education, McMaster University, Hamilton, Ontario, L8S 4L8.

The precision with which subjects can localize a target in space can be influenced by a number of factors. Assessment of the spatial relationship between limb, body and extracorporeal space in relation to the target are important in the generation and execution of appropriate movement commands. This study represents an effort to examine the relationship among visual space, responding limb and body space on the spatial control of pointing accuracy.

A specially designed contact lens system was used to isolate specific visual fields (Sivak, Sivak and MacKenzie, 1985). The left visual field of the left eye and the right visual field of the right eye were used in this study. Because testing was monocular, visual information was thus lateralized to either the left or the right hemisphere. Monocular no lens control conditions were included. Eight right-handed subjects made slow positioning movements with either the left or the right arm (without vision of the arm) to target lights located on a planar surface to the left,right or in front of the body midline. The target lights remained on for the duration of the trial. Using an X-Y digitizer, the subject's produced position was compared to the X-Y coordinates of the target light on each trial. Directional, amplitude and radial error measures were computed.

Results revealed a tendency to overshoot the targets in all conditions as determined by amplitude error. Subjects had smaller directional error when vision was restricted to the left visual field of the left eye than when vision was restricted to the right visual field of the right eye. Localization of the target was more accurate with the right than the left hand; localization with the left hand showed a rightward bias in directional error. Surprisingly, the lens conditions were no less accurate than the monocular control conditions on any error measure even though the lens conditions eliminated a whole visual field as well as much of foveal vision. It appears that for this type of slow positioning movement in which vision of the limb is not available, peripheral vision provides adequate information for localization of visual targets.

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200.1 CALCIUM SPIKE DURATION: PROLONGATION IN HIPPOCAMPAL NEURONS OF AGED RATS. P.W. Landfield and T.A. Pitler, Dept. of Physiol. Pharmacol. Bowman Gray Sch. Med., Winston-Salem, NC 27103.

There is increasing evidence that altered Ca homeostasis is a concomitant of neuronal aging. However, it is uncertain whether initial changes in Ca function depend on altered Ca buffering, extrusion, effectiveness, or influx, and whether these changes result in a net increase or decrease in internal Ca (cf. reviews in Khachaturian, 1984, Handbook of Studies on Psychiatry and Old Age, Elsevier; and Gibson and Peterson, 1987, Neurobiol. Aging).

Several studies from this laboratory have indicated that high Mg can counteract some of the effects of aging on hippocampal neurophysiology and behavior and, therefore, that some of the aging changes may be due to an increased action of Ca (e.g., Landfield, 1981, Soc. Neurosci. Abstr., Landfield and Morgan, 1984; Brain Res.; Landfield et al, 1986; J. Neurophysiol.). In addition, we have found that Ca-dependent, K-mediated afterhyperpolarizations (AHPs) are prolonged in brain cells of aged rats (Landfield and Pitler, 1984, Science).

To determine whether this apparent age-related increase in Ca-dependent actions results from reduced buffering/extrusion, increased effectiveness of Ca, or increased Ca influx, we studied Ca-mediated potentials in CA1 neurons from hippocampal slice preparations. Neurons were impaled with CsCl-filled micropipettes, to block Ca-dependent K conductance, and were then treated with tetrodotoxin, to block active Na conductance. Only cells in which the AHP's were fully blocked, and in which input resistance was greater than 30 MΩ were used in these analyses.

Under these conditions, Ca spikes could be elicited readily by intracellular depolarizing constant current pulses (70 ms). Spikes were generated with pulses at 150% of spike threshold, and the duration of the spike (from peak of the fast component to the return to baseline) was measured.

Cells from aged rat slices exhibited significantly prolonged Ca spikes (mean $\pm$ SE (ms) = 276.2 $\pm$ 19.2) in comparison to cells from young rat slices (mean $\pm$ SE (ms) = 216.7 $\pm$ 11.6).

These results suggest that an increased Ca current occurs in hippocampal neurons during aging and raise the possibility that increased membrane Ca conductance plays an important role in age-related alterations of Ca homeostasis. Although a number of factors could be responsible for the prolongation, apparent Ca-mediated inactivation of Ca conductance is found in hippocampal cells (Pitler and Landfield, 1987, Brain Res.). Therefore, age changes in the Ca inactivation process could be a factor in prolonged Ca conductance. (Supported by AG-04542 and AG-04207).

200.2 SYNAPTIC CHANGES IN THE DENTATE MOLECULAR LAYER OF AGED RATS. E. Fifkova and K. Cullen-Dockstader*. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Previously we have shown that there is a significant age-related increase of cytoplasmic Ca^{2+} in dendritic spines with age (Fifkova and Cullen-Dockstader, Brain Res., 376:351, 1986) and a significant decrease in the density of synaptic vesicles at the active zone of axon terminals (Fifkova and Cullen-Dockstader, Neurosci. Abstr., 11:894, 1986). Both changes were observed in the dentate molecular layer (DML), and they suggest that synaptic transmission may be impaired. In order to find out whether other synaptic parameters also suffer with age, we have undertaken the recent studies in the DML and in the stratum oriens (SO) of CA_1, in male rats (Fischer 344) ages 3, 9, 24 and 30 months (5 animals per age group). Since in both layers the majority of synaptic contacts are on dendritic spines, the parameters examined were: cross-sectional area and perimeter of spines and axon terminals, length and width of the spine stalk, length of synaptic apposition and synaptic density. In the SO no morphometric changes were detected as a function of age. In the DML the results were computed separately in three zones which are determined by individual inputs to this layer. The perimeter and cross sectional area of spines and the length of synaptic apposition were increased across ages and thirds, with the differences significant in the proximal third of the DML. The dimensions of axon terminals were stable in the proximal and middle thirds, however, in the distal third they were significantly larger. Measurements of the width and length of the spine stalks were taken when the entire stalk profile was in the plane of section. In random sections, only 5% of the spine population meets this criterion, therefore, the measures were pooled for the entire DML A significant increase of the stalk width was observed by month 24. Since it is the spine stalk which determines the strength of synaptic transmission in an axospinous synapse, this change may have important implications regarding neuronal plasticity in senescent animals. The previously observed age-related increase of cytoplasmic Ca^{2+} in spines suggests a decrease of Ca^{2+} signaling function. This function requires a large Ca^{2+} concentration gradient which, given the high extracellular Ca^{2+}, can be achieved only if the intracellular Ca^{2+} is very low. Therefore, a persistently high intraspinous Ca^{2+} in aged rats may impair synaptic transmission. The decreased density of synaptic vesicles at the active zone in aged rats will contribute to this impairment. The observed age-related enlargement of synaptic parameters, which in young animals would be associated with increased synaptic transmission, may in old animals compensate for the observed defects at the pre- and postsynaptic site. The enlargement of the spine stalk is of particular interest, since this change is induced in young animals by increased synaptic activity and is thought to be involved in the mechanism of synaptic plasticity in the DML. Permanently enlarged spine stalks may have no more capacity for further changes, which may explain an age-related decline in the plasticity of the system. Supported by NIA grant AG04806.

200.3 THE EFFECTS OF AGING ON BASAL LAMINA AND NEUROVASCULAR APPOSITIONS IN THE RAT DENTATE FASCIA. A. Topple, E. Fifkova and K. Cullen-Dockstader*. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Aging cerebral blood vessels in humans, primates and rats show a number of morphological alterations, including increased thickness of the basal lamina (Hicks et al., Neurosci. Abstr., 7, 183, 1981), reduced capillary wall thickness and numbers of endothelial mitochondria per capillary (Burns et al., J. Gerontol., 34, 642, 1979), and an increased capillary lumen (Hunziker et al., J. Gerontol., 34, 345, 1979). The present study reports similar basal lamina findings in the rat dentate fascia and extends the analysis to include an examination of the effects of aging on neurovascular appositions.

Fifteen male Fischer 344 rats 3-, 9-, 24- and 30-months old were perfused via the descending aorta with 3% glutaraldehyde and prepared for electron microscopy. The tissue was stained with 5% uranyl acetate followed by four component or Reynold's lead stain. All measurements were made from micrographs enlarged to 70,200 X. Increasing age is associated with progressively increasing total thickness of the basal lamina compared to the 3 month animals: a 22% increase at 9 months, a 44% increase at 24 months ($p < 0.05$), and a 69.8% increase at 30 months ($p < 0.01$). The number of individual layers of basal lamina did not change with age.

Aging is not associated with a significant change in the number of neuronal appositions per vessel. The number of axon terminals, of small axons, of myelinated axons and dendrites were analyzed. There was a noticeable trend towards reduced numbers with age for the terminals, dendrites and small axons which however did not reach significance levels. These neural processes rarely contact the vessel basal lamina directly. Rather, they are usually separated from it by a narrow space containing a thin glial process and what appears to be extracellular space. The width of this space, which does not change with age, is 26 nm. No presynaptic densities, characteristic of synaptic contacts, were observed. Further, no postsynaptic densities similar to those observed at the neuromuscular junction were seen. These results replicate earlier findings on age-related changes in vascular basal lamina in the hippocampus and extend them to include vessels in the dentate fascia. Additionally, this study reports no age-related alterations in neurovascular appositions in the dentate fascia. Supported by AG04804.

200.4 INCREASED SENSITIVITY TO KAINIC ACID INDUCED SEIZURES IN AGED RATS. R. Dawson, Jr. and D.R. Wallace, Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610

Kainic acid is a potent excitatory and neurotoxic analogue of glutamic acid. Systemic administration of kainic acid produces convulsions and subsequent widespread neuronal loss. While it is known that neonatal rodents are much more susceptible than adults to the convulsive and neurotoxic action of excitatory amino acids, few studies have examined the sensitivity of the aged brain to excitotoxic amino acids. The aim of the present study was to compare the seizure susceptibility of aged rats to that of young adults using kainic acid to provoke seizures via an excitotoxic mechanism.

Aged, female (Long-Evans) rats (30 months) and young adult (6 months) females were injected i.p. with 15 mg/kg kainic acid and observed for 2 hours. Preconvulsive and convulsive behaviors were noted and the latency to the first clonic-tonic seizure recorded, as well as, the number of clonic-tonic seizures. A second group of 6 month old female rats were injected i.p. with 30 mg/kg kainic acid and their behaviors were recorded.

The data ($\bar{X} \pm$ SE) are presented below:

Group	Seizure Incidence	Latency(min)	No. of Seizures
30 month (15mg/kg)	7/7*	45 $\pm$ 8*	9.7 $\pm$ 2.6*
6 month (15mg/kg)	0/9	---	---
6 month (30mg/kg)	4/6	83 $\pm$ 13	1.8 $\pm$ 0.5

*$p < 0.05$ old vs. 6 month (15mg/kg) or 6 month (30mg/kg).

These data suggest an increased sensitivity of the aged brain to the convulsive actions of systemically administered kainic acid. An increase in the sensitivity of the aged brain to excitotoxic amino acids may be of importance in age-related neuronal loss and neurodegenerative diseases. Supported by a grant from The American Federation for Aging Research.

200.5 SYNAPTOGENESIS IN AGED RATS ASSOCIATED WITH LONG TERM POTENTIATION. F.-L.F. Chang, K.R. Isaacs, D. Treacy*, & W.T. Greenough. Neural & Behav. Biology Program and Department of Psychology & Anatomical Sciences, University of Illinois, Champaign, IL 61820.

We and other laboratories have demonstrated that in adult rat hippocampal CA1, there is an increase in the number of shaft synapses associated with the expression of long term potentiation. Furthermore, we noted an increase in the number of sessile spine synapses which is consistent with a process of synaptogenesis through a sequence of shaft to sessile spine to well formed spine synapse. In the current study, we examined whether the same phenomena occur in normal healthy aged rats.

We used 7 Sprague-Dawley rats aged from 24 to 27 months. 400 micron transverse hippocampal slices were preincubated for one and half an hours before the start of stimulation and recording. Long term potentiation was produced by 6 trains of 100 Hz, 1 sec duration stimulations (stimulation intensity was adjusted to produce half maximal postsynaptic response), while activity control slices received 600 1 Hz stimulations of comparable intensity over a period of 10 min. Slices were then immersion fixed in 1% paraformaldehyde, 2.5% glutaraldehyde in phosphate buffer and processed for conventional electron microscopic examination.

LTP slices showed an average 73% increase in spike amplitude, while the control slices showed a decrease of 6%. This electrophysiological expression of LTP is comparable to those we obtained from young adult rats aged from 3 to 4 months (LTP: 80% increase, control: no change). For morphological study, an area adjacent to the recording electrode in the stratum radiatum 100 to 150 microns from the s. pyramidale / s. radiatum border was examined. We found an increase in the number of both sessile and shaft synapses (for sessile: 1.09 ± 0.17 per 100 square microns for LTP and 0.51 ± 0.19 for activity control, $t = 3.47$, $p < .01$; while for shaft synapses: 1.59 ± 0.20 per 100 square microns for LTP and 1.13 ± 0.19 for activity control, $t = 2.52$, $p < .025$) The 41% increase in the number of shaft synapses and 114% increase in the number of sessile spine synapses are lower than the effects we have observed in young adult rats (95%, 174% respectively). However, the equally robust electrophysiological expression of LTP suggests that there are potentially other mechanisms in addition to the synaptogenesis that may contribute to longer lasting components of LTP.

The decreased magnitude of synapse effects may be attributed to aging, but the still sizeable increase in synapse number in addition to the normal physiological expression of LTP suggests that the process of LTP is not impaired in normal aging.

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200.6 LESION-INDUCED SYNAPTOGENESIS IN THE LATERAL SEPTAL AREA OF AGED AND YOUNG ADULT F344 RATS. S. Scott, D. Price*, S.T. DeKosky and S.W. Scheff. Depts. of Anatomy and Neurology, Lexington VA and Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, KY 40536.

Aged rodent hippocampus has been reported to manifest a defect in lesion-induced axon sprouting and synapse replacement, requiring a significantly longer time course to attain pre-injury levels of synaptic density. The universality of this finding is currently being tested. Since the septal area was the first CNS structure to show lesion-induced synaptic plasticity and a limbic structure, we tested the capacity of this aged brain structure to cope with extensive denervation.

Young adult (2-3 mos) and aged (24-28 mos) Fischer 344 rats were subjected to a unilateral aspiration of the fimbria. This lesion results in massive denervation of both septum and hippocampus. Subjects were allowed to survive 4,10,15,30 and 60 days after the injury and were perfused with 1% glutaraldehyde and 4% paraformaldehyde in phosphate buffer. Both septum and hippocampus were prepared for ultrastructural analysis; only the ipsilateral lateral septal area was evaluated for changes in the synaptic density as a function of time post fimbrial lesion.

Both young and aged animals show a precipitous drop in synaptic density at 4 days following the injury. By 10 days both age groups have begun to show substantial increases in synaptic density and by 15 days post lesion both age groups show an over production of synaptic contacts. This over production is greater than the normal synaptic density for this area. By 30 days after the lesion the young adult animals begin to "shed" some of the synapses and return to prelesion density by 60 days. Aged animals manifest the same increased density in synaptic contacts but fail to shed synapses by the 30 days. By 60 days the aged rats return to prelesion levels. This failure of the aged group to "shed" extra synaptic contacts in a timely manner may represent a different type of defect in plasticty. The over-production of synaptic contacts in the lateral septal area following massive denervation has not been reported for other limbic structures indicating that the lateral septal area responds very differently than the hippocampus following damage. Finally, the overproduction of synaptic contacts and the eventual "shedding" of the contacts supports the contention that lesion-induced synaptogenesis closely mimics developmental synaptogenesis in significant ways. (Supported by NIH grant NS21541 and the VA Medical Research Service)

200.7 **NGF RECEPTOR IMMUNOREACTIVITY IN AGED RAT BRAIN.** F. Gómez-Pinilla, C.W. Cotman and M. Nieto-Sampedro. Department of Psychobiology, University of California, Irvine, CA 92717.

Brain NGF receptors (NGFR) appear to be associated to cholinergic structures. There is increasing evidence that in several cholinergic areas the system NGF/NGFR may play a role in neuronal plasticity. Since the potentiality for plasticity is different in young vs. old animals we decided to study the distribution of NGFR in some selected brain areas of aged rats (24-27 month-old) and compare it to the distribution of NGFR in young adults (2-5 month-old). In order to study the distribution of NGFR we used affinity-purified monoclonal anti NGFR (IgG 192, kindly provided by Dr. E.M. Johnson,Jr.) and peroxidase-anti peroxidase immunohistochemistry.

In young-adult rats, NGFR immunoreactivity was found in the olfactory bulb (OB) (glomerular layer), medial septum (MS), diagonal band of Broca (DB), nucleus basalis of Meynert (BM) and hippocampus (H). Parallel immunohistochemical staining of aged rat brain showed the following. In the olfactory bulb, the immunostaining was at least as strong as young-adult rats. In the MS, DB and BM, NGFR immunoreactivity was mainly located in cell bodies. Immunostaining in dendrites was weak and occurred only in the proximity of the soma. In contrast, NGFR immunoreactivity in young-adult rats was distributed in numerous neuronal processes and subprocesses which extended long distances from the soma. The number of NGFR-positive neuronal processes per unit of cross-section area in aged rats was about one third of the number seen in young animals. In the hippocampus NGFR immunoreactivity was practically non-existent These findings show that there are local differences in contents of NGFR (i.e. OB: high, MS-DB-BM-H: low). In addition, the low levels of NGFR in the septal-hippocampal system of normal aged rats may account for the slower sprouting response observed in this system after entorhinal ablation. These results suggest that a local loss of NGFR may be a potential cause for the decline in plastic capabilities observed in aged brain cholinergic systems.

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200.8 NERVE TERMINAL CURRENTS IN YOUNG AND OLD CBF-1 MOUSE NEUROMUSCULAR JUNCTION. N. Anis* and N. Robbins (SPON: R. W. Hardy). Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

Neuromuscular transmitter output and turnover is altered in muscles of old CBF-1 mouse, but the mechanism of these alterations is poorly understood. Morphological studies from our laboratory showed that this change in transmitter output in aged animals is not due to a change in the number of release sites or nerve terminal contact area. Other explanations include altered Ca buffering capacity in aged terminals, or changes in nerve terminal ionic channel properties. We examined the latter hypothesis by inserting microelectrodes close to the nerve terminals inside the perineurial space and recording external current along the superficial nerve bundles (Mallart, J. Physiol. 368:565, 1985) in sternocleidomastoid muscle of mature (8-12 mo.) and old (24-30 mo.) CBF-1 mice. Signals recorded in curarized preparations consisted of double negative peaks. The first peak is generated by local inward Na current (blocked by tetrodotoxin) during propagation of nerve action potentials. The second negative deflection is generated by nerve terminal K current (blocked by tetraethylammonium; TEA or 3,4-diaminopyridine; 3,4-DAP). Blocking of K currents with TEA and 3,4-DAP gave rise to a positive-going Ca current (blocked by Cd++, Co++ and Mn++) with slow and fast components. In aged muscles, the rise and half-decay times of Na, K and fast Ca currents and the timing of the K and fast Ca currents were similar to those in young muscles. The relative current amplitudes of both K and Ca (relative to Na current) were also unchanged in aged animals.

These results indicate that the presynaptic channel kinetics of young and old SCM muscles are similar. However, the half-decay time of the slow Ca current in old muscle was at least twice as long as in young muscle. The physiological role of this slow Ca current remains to be elucidated, but to the extent that may be present in unblocked preparations, it could transiently elevate intracellular Ca, perhaps producing long-term effects. (Supported by NIH AG00795)

200.9 LITHIUM EFFECTS ON NEURONAL MEMBRANES IN SENESCENT RATS. I.J. Wajda, M. Banay-Schwartz*, T. De Guzman*, I. Manigault*, and A. Lajtha. Center for Neurochemistry, N.S. Kline Institute, Ward's Island, New York, NY 10035.

Chronic treatment with lithium affects the density of a number of receptors, influences the levels of several amino acids, and sometimes produces changes in the ionic content of the brain. These changes indicate that lithium has an effect on neuronal membranes. In the previous studies we used adult and 6-week-old Wistar rats. Since senescence is characterized by many morphological and biochemical alterations in the brain tissue, and since lithium is used in the treatment of affective disorders in geriatric patients, it was of interest to study the effects of lithium on senescent brain. We used two groups of female Fisher-344 rats, one 6 months old and one 20 months old. They were placed on lithium diet for 3 weeks.

Serotonergic binding sites were assayed in cerebral cortex with [^{3}H]ketanserin. We found an increase in K_d in the older group kept on lithium, indicating a decrease in the affinity of the serotonergic receptors. The density of those sites was not affected by lithium. The 6-month-old rats showed a decrease in serotonergic binding sites and no change in the affinity of the receptors. Dopaminergic binding sites were examined in the caudate nuclei with [^{3}H]domperidone, and there was no change in either the density or the affinity of dopaminergic binding sites in either the old or the young rats. In comparing the two control groups of the older and young animals, we noticed a decrease of the serotonergic binding sites in the old rats but little change in the dopaminergic system.

The lithium levels in the brain and plasma of lithium-treated rats were close to 1.0 mEq/kg or L. Free amino acid levels were estimated in five parts of the brain: cerebral cortex, midbrain, pons, medulla, and cerebellum. Amino acids were extracted from the tissue, and derivatized products were resolved by HPLC and detected fluorometrically. Twenty amino acids were estimated. GABA, glycine, alanine, aspartic acid, and glutamic acid increased in several parts of the brain. These increased in old rats were brought down by the lithium treatment. Glutamine, on the other hand, showed a decrease in older animals and was not affected by lithium treatment.

It is of interest to remember that GABA, glycine, and glutamic acid belong to the group of putative neurotransmitters, and that both glutamine and alanine are involved in the metabolism of ammonia in the brain. It is possible that the alteration produced by lithium treatment are connected to the therapeutic action of lithium.

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200.10 REGULATION OF FREE INTRACELLULAR CA^{2+} LEVELS IN SYNAPTOSOMES FROM AGED RATS. M.L. Michaelis, C. Foster* and E.W. Nunley.* Dept. of Pharmacology and Toxicology and Center for Biomedical Research, University of Kansas, Lawrence, KS 66045.

The possibility that alterations in neuronal Ca^{2+} regulation occur with aging has led to a number of studies on Ca^{2+} homeostasis as a function of brain aging. Our work has focused on characterization of synaptic plasma membrane systems responsible for transporting Ca^{2+} out of the neurons, i.e., ATP-dependent Ca^{2+} transport and the Na^+ - Ca^{2+} antiporter. We reported earlier that the kinetic characteristics of the antiporter and the (Ca^{2+} + Mg) ATPase are altered in synaptic membranes from aged Fisher 344 rats, and the changes suggest that Ca^{2+} extrusion may be less efficient in nerve terminals from aged animals. More recently we examined the antiporter activity in 27-28 month old Fisher 344 rats and found the V_{max} for Ca^{2+} transport in these animals to be substantially lower than that for adult (6-8 month) rats. Detailed examination of the ATP-dependent transport of Ca^{2+} in synaptic membranes from 7, 22, and 27 month old animals has also revealed decreases in the V_{max} for transport activity.

Since a decrease in Ca^{2+} extrusion may lead to elevated free $[Ca^{2+}]_i$ levels, studies were undertaken to determine the free $[Ca]_i$ in synaptosomes from aged and adult animals under a variety of conditions. We used the fluorescent Ca^{2+} indicator dye Fura-2 to monitor $[Ca^{2+}]_i$ in synaptosomes under basal conditions, following depolarization by KCl or veratridine, and following activation of receptors or membrane events that might lead to elevations in $[Ca^{2+}]_i$. The resting free $[Ca^{2+}]_i$ levels in nerve terminals from the 23-24 month old rats were higher than those in 7-8 month-old animals (158 ± 10 nm for the adult versus 203 ± 12 nm for the aged animals). In addition, depolarization of the synaptosomes with elevated KCl or veratridine led to substantial increases in free $[Ca^{2+}]_i$ and the aged animals consistently showed greater increases in the free Ca^{2+} signals. The release of Ca^{2+} from internal sites by activation of receptors linked to inositol phosphate turnover was also detected by the Fura-2, but the aged animals differed only slightly from the controls in their response to this type of activation. Nevertheless, the data obtained with the Fura-2 support the notion that the Ca^{2+} buffering capacity of aged nerve terminals is less effective than that of terminals from younger animals. It is possible that the decreases in Ca^{2+} extrusion activities may contribute to the elevated free $[Ca^{2+}]_i$ levels in the nerve terminals of aged animals (Supported by PHS grant AG 04762, AA 04732, and Ctr. Biomed. Res., U. of Kansas).

200.11 AGE RELATED ALTERATION IN CENTRAL RECEPTORS FOR CALCIUM CHANALES BLOCKERS. A. Golik* and D. Modai* (SPON: N. Allon). Dept of Medicine, Assaf Harofeh Med. Ctr. - Tel Aviv Univ. Israel.

Calcium antagonists such as nifedipine, verapamil and diltiazen, modulate the activity of calcium channels via specific receptors. These calcium channel blockers are increasingly used in elderly patients. Since age related decrease in the density of various receptors has been reported, it is of interest to examine age related changes in the distribution of calcium channel receptors. We used ^{3}H-labelled PN-200-210 and studied its binding to brain of CW1 mice at different age groups (3,6,9,12,15,18 and 32 months). In vitro analysis were based on both whole brain autoradiography and on binding studies. The autoradiograms enabled the evaluation of binding to specific brain regions using a computerized image analyzer. Kd and Bmax values were obtained by Scatchard analysis of the binding data. No significant age related changes could be found with either of the two methods. The highest density of binding sites was seen in the hippocampal formation independent of age group. Binding affinity and receptor density were similar in all age groups studied (Kd=60pM, Bmax=190 fmole/mg protein). Since peripheral binding sites were not included in this study, further research is required in order to fully evaluate the clinical implication of the above data for geriatric pharmacology.

200.12 **REMOVAL OF LIPOFUSCIN GRANULES FROM CELL BODIES OF AGED MICE BY COLCHICINE.** M. C. Bundman, V. J. Roberts and C. Gorenstein. Dept. Pharmacology, University of California, Irvine. Irvine, CA 92717.

One of the most significant and reliable changes which can be correlated with aging is the progressive accumulation, in postmitotic cells, of pigmented organelles known as lipofuscin granules. Although the accumulation of lipofuscin is linear with age in many areas of the brain, the actual role of lipofuscin in the aging process is unclear. A number of studies have demonstrated a correlation between the accumulation of lipofuscin and interference with normal metabolic activity, loss of dendrites, decrease in total cellular RNA and cell death. Prolonged treatment with centrophenoxine has been shown to reduce the amount of neuronal lipofuscin and to improve performance in certain learning tasks.

Previously, we showed that a single i.c.v. injection of colchicine results in a redistribution of lysosomes from the soma of neurons to the dendrites. In light of the close relationship between lysosomes and lipofuscin granules we asked whether colchicine treatment could also induce a redistribution of neuronal lipofuscin granules.

Male, CFW mice, 28 months of age, were injected i.c.v. with 40 µg colchicine and sacrificed 24 hours later. At the light microscope level we observed a redistribution of dipeptidyl aminopeptidase II (Dpp II), a lysosomal and lipofuscin granule marker enzyme, from the soma of neurons to the dendrites. All neuronal populations containing Dpp II displayed this response to colchicine except neurons in the mesencephalic nucleus of the trigeminal nerve. Quantitation of this phenomenon at the electron microscope level demonstrated that, in the facial nucleus, colchicine induced a rapid and significant redistribution of both lysosomes and lipofuscin granules. In cell bodies there was a 44 % reduction in the density of lysosomes and a 63% reduction in the density of lipofuscin granules. In the dendrites, we observed a five-fold increase in lysosome density and a ten-fold increase in the density of lipofuscin granules. Under these conditions we did not observe a redistribution of mitochondria from the cell bodies to the dendrites.

These results indicate that colchicine may prove useful as a tool to study the role of lipofuscin in the aging process.

200.13 TIME COURSE OF ASTROCYTE RESPONSE TO NEURAL TRAUMA IN YOUNG AND AGED RATS. B.T. Faddis* and K.S. Topp* (SPON: A. Gabor). Dept. of Human Anatomy. School of Medicine. University of California. Davis, CA 95616.

Neuroglia appear to play an important role in repair of damaged neural tissue. It is not presently known whether this glial function is modified during aging. We evaluated numerical changes and hypertrophy in astrocytes in young and aged rats following neural trauma. Male Fischer 344 rats of 3 and 16-19 months of age received unilateral needle wounds through the cortex (CO) and underlying hippocampus (HC). Animals were allowed to survive for 2-5 days following injury and were sacrificed by vascular perfusion with 1.5% paraformaldehyde, 1.25% glutaraldehyde. Vibratome slices through the lesion were stained immunohistochemically for glial fibrillary acidic protein (GFAP), flat-embedded in methacrylate, sectioned at 1.0 um and counterstained with toluidine blue. Differential cell counts from oil-immersion subfields adjacent to the lesion in the CO and HC were averaged for each animal. The mean nuclear size of astrocytes in the CO and HC was determined for each animal and astrocyte counts were adjusted to reflect the size changes observed following injury.

In young animals, HC astrocyte counts increased on postlesion day 2, while CO astrocyte counts did not significantly increase until day 3. There was no further increase in astrocyte numbers and the counts remained steady through days 3, 4 and 5. HC astrocyte hypertrophy was significant on postlesion day 2 and maintained on days 3 and 4, but diminished on day 5. Astrocytes in the CO were hypertrophied on day 2 and remained enlarged through days 3, 4 and 5.

In aged animals, HC astrocyte counts showed a significant increase on postlesion day 3, while CO astrocyte counts did not increase until day 4. There was no further increase in astrocyte numbers and the counts remained steady through days 4 and 5. HC astrocyte hypertrophy was significant on postlesion day 2, but gradually decreased thereafter revealing no significant size differences between days 3, 4 and 5 and controls. Astrocytes in the CO were hypertrophied on day 2 and the enlargement was maintained through days 3, 4 and 5.

Our observations show that, in both young and aged rats, trauma-induced hypertrophy of astrocytes in the HC and CO increased on postlesion day 2. Similarly, in both animal populations the numerical density of astrocytes increased in the CO one day later than the response in the HC. Despite these parallels, in the aged rats there appears to be a delay in the numerical increase in astrocytes in the HC and CO following neural trauma.

This work was supported by the American Foundation for Aging Research, the National Institute on Aging, the Sigma Xi Foundation and the University of California.

200.14 DEVELOPMENTAL CHARACTERISTICS OF ELECTRICAL PROPERTIES IN CULTURED RAT BRAIN NEURONS. G.W.Lu and H.W.Wang. Department of Neurobiology, Capital Institute of Medicine, Beijing, China

The rat brain neurons taken from 15-17 day embryo were grown in dissociated cell culture and maintained in vitro for 3 weeks. The developmental process of the neurons was observed from early stage of development in terms of passive and active electrical properties and their changes under perfusion of channel blockers.

The neurons development could be morphologically divided into stages I(from plating time to 6 days), II(7-11 days), III(12-21 days), and IV(21-22 days). The relative value of resting membrane potentials (RMP) for stages I, II, III and IV was 1, 3.46, 4.07 and 6.54, respectively; the correponding value of input resistance (Rin) was 1, 1.48, 2.53 and 4.56, respectively. The current-voltage curve in stages II and III was exponential in the direction of depolarization and the Rins change with time was nearly linear; occurrence rate of spontaneous activity increased with prolongation of culture and the amplitude of evoked action potentials reached maximum during stages III and IV. The RMP and Rin were markedly decreased by perfusion of TEA-containing solution, while no effect was seen during TTX perfusion.

The results indicate that potassium ion channels develop slower in stages I and II, faster in stages III and IV, and nature by the end of stage IV.

200.15 EFFECTS OF THE OSMOLAL CONCENTRATION CHANGE OF EXTERNAL SOLUTION ON TISSUE CULTURED NERVE CELLS FROM A FETAL STAGE TO AN AGED STAGE. H.Horie,S.Ikuta*,T.Takenaka & S.Ito*.Dept of Physiol. Sch. of Med. Yokohama City Univ., Yokohama,236,Japan.

Membrane fluidity, sensitivity to fibronectin and fibronectin receptor density of neurons change with aging. These results indicate that one of the basic functions of nerve cell membranes, respose to the osmolal concentration change of external solutions , might change with aging . In this paper we measured the membrane function by using tissue cultured dorsal root ganglion neurons dissected from 3 age groups of mice (fetal, 3-month-old and 27-month-old). Neurons were cultured on glass coverslips, 40x50mm, and the coverslips were fixed with a thin tape on the bottom of a 0.6mm thick stainless steel plate(50x80mm) with an elliptical hole : 30x50mm. The plate was placed on the inverted Zeiss Axiomat microscope and neurons were observed by the AVEC-differential interference contrast video microscopy. (Hamamatsu Photonic, Japan)

When an external control solution (67mM NaCl and 134mM sucrose containing Tyrode solution) was replaced with a sucrose free control solution whose osmolality was about a half of the control , diameters(Do) of neurons rapidly icreased to a maximum value(Dm) (1st stage) and then decreased slowly to the original sizes(2nd stage). After this stage cell sizes were constant and decreased rapidly by perfusing the control solution(3rd stage). The relative value Dm/Do was 1.10 in a half osmolal concentration solution of the control and 1.05 in a three quarters osmolal concentration solution. The time (Tm) for diameters to reach maximum values was changing with aging, 74 sec in 18-day-old fetus, 183 sec in 3-month-old and 312 sec in 27-month-old. Dm/Dt was 1.10 and this value was common in 3 age groups. When two times higher osmolal solution was applied to neurons, the diameters rapidly decreased and the relative value of diameters to the initial ones reaced 0.87. This value was constant under the treatment. This change was reversible. It is plausible from these results that the cell membrane expansion in the first stage was caused by entrance of water. After cell sizes reach to the maximum value, channeles in the membranes might open by this membrane expansion and intercellular solutes might pass through the open channels to an external solution. This decrease of intracellular osmolal concentration can explain the decrease of cell size in the second stage. This explanation was supported by the fact that at the third stage cell sizes rapidly decreased by the application of the control solution. The increase of Tm with aging indicates that rate of elastic response of neurons to osmolal change might decreases and the cell membrane might become rigid with aging.

200.16 EFFECTS OF AGE ON THE RESPONSE TO PARTIAL DENERVATION IN MOUSE MUSCLE: MORPHOLOGICAL CORRELATES. J.M. Jacob* and N. Robbins. (SPON: R.A. Ratcheson). Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

After transection of the L5 spinal root in CBF-1 mouse, soleus motor neurons expand their field of innervation 2-3 fold. This expansion of the motor unit size can be used as a test of the relative capacity of young and old motor neurons to increase or redistribute cellular metabolites to a larger nerve terminal field. Both young (5-8 mo.) and old (25-30 mo.) mouse soleus muscles have the same number of motor units and muscle fibers. Twitch tension in both young and old muscle largely recovered by 60 d after operation, but in old muscles, transitter release was reduced in both original and sprouted terminals, and latency from nerve stimulus to epp was prolonged (see Jacob and Robbins, Neurosci. Abst., 1986). Therefore, we sought morphologic correlates of these findings.

Two methods were used: zinc iodide osmium staining of individual or whole mount muscle fibers or whole mounts of muscle using fluorophores coupled to tetanus toxin c-fragment to stain nerve terminals and FITC-coupled alphabungarotoxin to stain post-synaptic receptors (see Robbins and Polak, this volume). Terminals in muscle partially denervated for 60 d showed junctions of near normal size, close overlap of nerve terminals and receptors and innervation by myelinated nodal sprouts. At 60 d in the old operated muscle, there were four classes of nerve muscle junctions: I) denervated, i.e. receptors but no nerve terminals (22%); II) fine nerve terminals over receptors (28%); III) nerve terminals covering 50-85% of the post-junctional receptors (20%) and IV) normal nerve muscle junctions (30%). The innervation of class II and III nerve terminals was primarily by unmyelinated or lightly myelinated nodal sprouts. Studies are currently in progress to determine the approximate axon calibers of the sprouted axons but preliminary data indicate that the relative lack of myelination resulted from failure to increase axonal size.

In summary, after partial denervation, functional maturation of nerve terminals appears complete by 60 d in young but not in old mice. In the latter, some denervated fibers do not obtain long-term innervation, and in others, terminal reinnervation by sprouts tends to be sparse or incomplete. These last findings may account for the reduced transmitter release. In addition, failure of adequate myelination of the sprouted nerve fibers readily explains the longer latency. (Supported by NIH grants AG00795 and T32-AG-00105).

200.17 DYNAMIC MORPHOLOGICAL REMODELLING OF SOLEUS AND EDL NEUROMUSCULAR JUNCTIONS OF C57BL/6J MICE WITH AGE. M.H. Andonian, D. Weese and M.A. Fahim. USC Andrus Gerontology Center, Los Angeles, CA 90089-0191.

The mammalian neuromuscular junction (NMJ) appears to undergo a dynamic remodelling process with advancing age. Previous studies suggest that the aging NMJ becomes progressively larger and more complex, and that this phenomena occurs to a greater degree in the EDL than the Soleus (Robbins & Fahim, J. Neurocytol, 14, 1019-1036, 1985). In order to better understand this process, quantitative morphometry was conducted on SOL and EDL nerve terminals of C57BL/6J mice aged 4, 8, 10, 12, 18, 22, 24, and 32 months. Each age group consisted of five animals.

The muscle were dissected from the mice under Metofane anestnesia, and stained with the zinc-iodide-osmium (ZIO) technique. Camera lucida drawings were made of between 5 and 15 acceptable NMJs (showing >90% of area) in each muscle in each animal. The drawings were digitized into the computer using the planar morphometry system of Southern Micro Instruments (Atlanta, Georgia), and the area, perimeter, longitudinal extent length and fiber diameter determined.

Repeated-measures ANOVA of the data revealed significant ($P < .05$) differences between many of the ages in both the SOL and EDL nerve terminal morphometry. The levels of significance were greater when the fiber diameters were covaried. When the seperate age groups were treated as a continuous variable, regression analysis of the data showed significant ($P < .05$) positive age trends for area, perimeter, and length for the EDL, with similar trends in the SOL perimeter.

The morphology of the NMJ underwent a dynamic remodelling in both muscles with advancing age. Older NMJs were progressively more complex, highly branched and regionalized. The magnitude of the changes was greater in the EDL than the SOL. These results suggest that remodelling is an ongoing process which may be subject to age-related changes in the animal, or its environment.

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200.18 AGE-RELATED CHANGES IN THE REGIONAL DISTRIBUTION OF HORMONES IN THE ANTERIOR PITUITARY OF THE MALE RAT. J.M. Goldman*, R.L. Cooper*, G. Rehnberg*, J.F. Hein* and W.K. McElroy*. (SPON: C. Lau). Reprod. Toxicol. Br., Hlth. Effects Res. Lab., U.S. Environ. Protect. Agency, Res. Tri. Pk., NC 27711.

In mammals, there is a differential distribution of hormones in the anterior pituitary (AP) (e.g., Schafer, S.J. and McShan, W.H. Proc. Soc. Exp. Biol. Med. 146:546, 1974), which corresponds to the localization of particular types of hormone-containing cells. Since age-related changes in AP hormonal concentration (conc) have been reported (e.g., Bethea, C.L. and Walker, R.F. J. Gerontol. 34:21, 1979; Emanuele, N.V. et al., Dev. Brain Res. 20:179, 1985), the present study was intended to explore the effects of age on these patterns of distribution. Four and twenty-four month-old male Long-Evans rats were quickly decapitated. Trunk blood was collected and AP free of micro- or macroadenomas were each cut on ice into 8 fragments of approximately equal weight [2 rostral (left/right), 3 central (left/medial/right), and 3 caudal (left/medial/right)]. Weighed fragments were sonicated in medium 199/0.3% bovine serum albumin (Hepes-buffered, pH 7.4) and aliquots taken for analyses of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (Prl) and thyroid-stimulating hormone (TSH). The patterns of distribution of LH and FSH were similar, with a decrease in conc seen in the more caudal fragments. Age-related declines in gonadotropin conc were seen in both rostral regions and in the medial fragments of the central and caudal regions. TSH conc increased along the rostral-caudal axis in the 4-mo. rats, but no statistically significant age differences were present for any region. While Prl was evenly distributed among all regions, there was a general decline in regional conc. Since the gonadotropin- and TSH-containing cells are apparently clustered regionally, comparisons were also made between ages using measures of total hormonal content per fragment, thus circumventing any influence of age-related alterations in AP (and fragment) size upon the obtained values. The principal effect with this comparison was a decrease in LH and FSH within the medial area of the caudal strip. The regional hormone relationships were as before. The results indicate that, while in general there is no decline with age in the content of LH, FSH, Prl or TSH in the rat AP, there could be a selective loss of gonadotropes from the medio-caudal area, either through cell loss or a decline in cell number due to a shift of the gonadotropes out of the region. Given the regional and hormonal specificity, it is unlikely that the observed effects represent changes in the amounts of LH and FSH within a stable cellular population.

200.19 ALTERATIONS IN BRAIN ANGIOTENSIN II BINDING IN THE AGED RAT. B.W. Massey*, L.M. Plunkett, R.H. Kennedy* and E. Seifen. Dept. of Pharmacology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

Recent studies have localized angiotensin II (ANG) binding sites and immunoreactivity to brain areas associated with sympathetic nervous system activity and cardiovascular function. The peptide has been proposed to act as a neuromodulator in these brain areas. In the present study, ANG binding was studied in senescent and adult Fischer 344 rats using quantitative autoradiographic techniques. Aging in the Fischer rat is accompanied by changes in sympathetic nervous system and cardiovascular function. This study was designed to examine ANG binding in specific brain areas associated with sympathetic activity and peripheral cardiovascular control.

ANG binding was determined in discrete brain nuclei of 5 and 25 month old male Fischer rats. Quantitation was performed by incubation of 16 um thick tissue sections with 2 nm ^{125}I-(Sar^{1})ANG followed by autoradiography, computerized densitometry, and comparison to ^{125}I-standards. The areas examined included the nucleus tractus solitarius (NTS) and the organon subfornicalis (SFO). In both of these areas ANG injection produces changes in cardiovascular parameters. ANG binding was significantly increased in the SFO from 589 $\pm$ 79 to 1048 $\pm$ 47 fmol/mg protein in 5 versus 25 month old rats respectively. In the NTS, binding was significantly decreased from 1378 $\pm$ 91 to 806 $\pm$ 116 fmol/mg protein (5 versus 25). These results suggest that ANG receptor density is altered during aging and that these changes are associated with brain areas known to regulate the peripheral cardiovascular system.

200.20 BINDING OF PHORBOL ESTERS IN THE BRAIN OF YOUNG AND AGED RATS AFTER ACUTE AND CHRONIC ETHANOL TREATMENT. F.Battaini, R.Del Vesco*^, S.Govoni^, M.S.Magnoni*^ and M. Trabucchi. Chair of Toxicology II University of Roma and ^Inst. of Pharmacological Sciences,University of Milano, ITALY.

Increasing evidence indicates that protein kinase C (PKC) regulates neuronal excitability interfering with neurotransmitter release,K+ and Ca++ channels,receptor function.

Phorbol esters specifically bind and stimulate PKC and their tritiated derivatives provide a tool to investigate the involvement of this enzyme in brain functioning. In fact changes in phorbol ester binding have been correlated with modifications in PKC activity in various systems.

The physiologic activator of PKC is diacylglycerol produced after activation of the phosphoinositide (PI) cycle by a number of transmitters and conditions known to alter neuronal excitability.

Recently it has been demonstrated the ability of ethanol to interfere with PI metabolism in discrete brain areas and after specific stimuli.

We have used labeled phorbol 12,13,dibutyrate (3H-PDBu) to investigate PKC function in rats of different ages acutely and chronically treated with ethanol (3 g/kg per os, 3 hrs and 6% v/v in the drinking water for 21 days respectively).

In adult rats (3 month old) the chronic ethanol treatment was able to specifically modify 3H-PDBu binding in cortical membranes decreasing Bmax values by 25%. The acute treatment decreased Bmax values and increased the affinity. No significant modifications were observed in hippocampus in both conditions. In aged animals (24 month old) 3H-PDBu binding in cortical membranes was not significantly different from adult values; the chronic and acute ethanol treatment down regulated Bmax values (-27% and -24% respectively).

The data indicate that ethanol specifically down regulates 3H-PDBu receptors in rat cortical membranes; this may be related to the reported ethanol induced increase in the sensitivity of brain PI turnover to selected agonists. The down regulation of 3H-PDBu receptors could be a compensatory process controlling brain cellular excitability modified by chronic ethanol.

The modification in cortical 3H-PDBu binding, observed in aged rats, indicate the capability of old animals to still react in a compensatory manner at selected stimuli.

200.21 **MORPHOLOGICAL CHANGES IN NEURONS IN AREA 17 OF AGING RHESUS MONKEYS.** S. Vincent* and A. Peters (SPON: D. L. Rosene). Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118 and Yerkes Regional Primate Research Center, Atlanta, GA 30322.

Neuronal populations in the primary visual cortex of 27 and 35 year old monkeys have been compared with those in 5 year old monkeys. On the basis of counts of neuronal profiles containing nuclei in 250 μm wide strips of 1 μm thick sections passing through the entire depth of the cortex, no obvious neuronal loss with age could be detected. The counts were made in a portion of area 17 on the lateral surface of the hemisphere just behind the lunate sulcus. This lack of obvious neuronal loss is compatible with our electron microscopic observations on this area of cortex, for in the older monkeys the neurons show little cytological alteration beyond the presence of a few lipofuscin granules. In contrast to the neuronal cell bodies, however, profiles of dendrites with large vacuoles in their cytoplasm are not uncommon in the old animals. Sometimes these vacuoles are enlarged so that only a thin rim of cytoplasm remains in the dendrite, and it is possible that this vacuolation leads to the formation of a not infrequent profile in old animals, one of a membrane bound hole. Such holes often contain remnants of mitochondria and other membranous debris. The dendritic origin of these holes is suggested because the membrane bounding them sometimes form synaptic junctions. In addition to these smaller diameter holes, there are other, much larger holes, or spaces in the neuropil of the old animals. These larger holes can be 10 μm or more in diameter, and some of them appear to be a late stage in the degeneration of myelinated axons, for they are bounded by a thin laminated sheath. Such holes are often empty, but the remnants of compressed axoplasm may be present along one side of the hole. By contrast, other large spaces often contain membranous debris and have a very attenuated rim of cytoplasm, with a disrupted membrane bounding them. Many of these spaces are the size of neurons, and we are examining additional material to try to determine if these spaces are the result of neuronal degeneration. Similar spaces have been previously encountered in the cerebral cortex of 48 month old, diet restricted rats, in which neuronal loss was significant (Peters, Harriman and West. Neurobiol. Aging 8:7-20, 1987). Supported by NIH grants AG 00001, T32 NS 07152, and RR-00165.

200.22 AGE RELATED ALTERATIONS IN PREDOMINANTLY FAST MUSCLE IN FISCHER 344 AND LONG EVANS RATS. T.J. Walters, H.L. Sweeney, and R.P. Farrar. Dept. of Kinesiology, Institute for Neurological Sciences Research. Univ. of Texas at Austin.

Aging has been reported to produce either declines in or no change in aerobic capacity of skeletal muscle. Some of the disparity in results has been attributed to strain related neuropathies in Sprague Dawley and Wistar rats. Two strains that have been reported to be free of disturbances in neuronal function are the Fischer 344 (F344) and Long Evans (LE). In this study we examined the plantaris muscle for changes in mass, muscle/body weight ratios, aerobic capacity, and shifts in the myosin light chain (MLC) pattern. Pathogen free rats were purchased from the Harlan Sprague Dawley facility and maintained in quarantine until sacrifice. Muscles were weighed fresh,and a portion saved at -80°C for both MLC ratios and citrate synthase (CS) activity. F344 rats were analyzed at 10-14 , 24 and 30 mos. of age while LE were analyzed at 10-14 and 30 mos. of age.

30 mo. old rats of both strains demonstrated a reduction in CS activity compared to strain matched 10-14 mo. old rats, however the decline was greater in the LE (21% decline in F344 versus a 32% decline in LE.) In the F344 strain the decline in CS activity was complete at 24 mos. without a further decrease between 24 and 30 mos.. An age related decline in muscle mass of 37% and 17% occured in the 30 mo. LE and F344 respectively. The decline in muscle mass in the F344 occured completely between 24 and 30 mos. When the muscle wt. to body wt ratio was examined in the 30 mo. rats the decline in muscle mass was significantly greater in the LE when compared to the F344 (46% decrease vs 19% decrease). Examination of the myosin LC ratios revealed a relative reduction in MLC_{3f} to MLC_{1f} in both strains of 30 mo. rats.

The results of this study demonstrates that although declines in aerobic capacity and mass of predominantly fast muscle occur in both LE and F344 rat strains, reductions are greater in the LE. Additionally both strains display a shift in MLC pattern with age.

RECEPTOR REGULATION II

201.1 CHRONIC (BUT NOT ACUTE) STRESS INCREASES HYPOTHALAMIC A_1 ADENOSINE RECEPTORS IN RATS. S.M. Anderson, J.R. Leu, G.J. Kant. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Previously, we presented data demonstrating a 15% increase in the binding of the A_1 adenosine receptor ligand, [^{3}H]cyclohexyladenosine ([^{3}H]CHA) (5-7 nM) to membranes prepared from the hypothalamus but not from the frontal cortex, striatum, or hippocampus of rats exposed to chronic stress for three days (Soc Neurosci Abstr 11:773, 1985; Pharmacol Biochem Behav 26:829, 1987). That behavioral model incorporates continual performance, avoidance/escape from footshock and disruption of sleep.

The multidimensional nature of our 'sustained performance procedure' prompted us to examine the effects of stress on hypothalamic A_1 adenosine receptors in rats exposed to several different types of stress. 1) [^{3}H]CHA (5-7 nM) binding to hypothalamic membrane preparations from individual rats chronically stressed for 14 days in our 'sustained performance procedure' was 13.5% greater than binding to membranes from controls. These data are consistent with our previously published results (using a three day stress trial) and demonstrate maintenance of this receptor up-regulation during a longer (14 day) stress period. 2) In other experiments, rats were stressed by restraining them in lucite cylinders (5.7 cm diameter) three hours per day for one day (acute) or ten days (chronic, recurrent). [^{3}H]CHA binding to hypothalamic membrane preparations was 13% higher in the chronically stressed rats vs. controls. The acutely restrained rats were not significantly different from control in amount of [^{3}H]CHA binding to hypothalamic membranes. 3) In an abbreviated variation (four day trial) on the classical 'activity-stress' paradigm stressed rats were fed ad lib for one hour each day and provided 24 hr access to running wheels. [^{3}H]CHA binding to hypothalamic membranes of rats stressed in this activity wheel experiment was 10% higher than binding to membranes from control animals. 4) There was no difference in the binding of [^{3}H]CHA (5-7 nM) to hypothalamic membranes between controls and animals given acute footshock (15 min trial, 1.6 mA electric shock, variable schedule with an average intershock interval of 30 sec and shock duration of 5 sec).

Data from saturation binding experiments (0.09-26 nM [^{3}H]CHA) using pooled membrane preparations from stressed or control rats (from the 14 day 'sustained performance procedure') indicate that the up-regulation of the A_1 adenosine receptors in the hypothalamus of chronically stressed rats is due to an increase in the apparent number of binding sites without change in the affinity of the receptor for [^{3}H]CHA. Similarly, data from saturation binding experiments using pooled hypothalamic membranes from chronically restrained rats demonstrate that the up-regulation of hypothalamic A_1 adenosine receptors in these animals vs. controls is due to a change in the number of receptors rather than receptor affinity.

Our present data demonstrate small but consistent increases in the number of hypothalamic A_1 adenosine receptors in rats stressed in several chronic stress models and no change in these receptors by acute stress.

201.2 TEMPORAL RELATIONSHIP OF THEOPHYLLINE-INDUCED UPREGULATION OF CENTRAL A_1 ADENOSINE RECEPTORS AND ALTERATIONS IN SEIZURE SUSCEPTIBILITY. R.C. Sanders, P. Szot and T.F. Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Chronic caffeine or theophylline (THEO) exposure elicits increases in rat cerebral cortical A_1 adenosine receptor (ADO-R) densities (upregulation) (B.B. Fredholm, Acta Physiol. Scand. Suppl., 508, 31, 1982, T.F. Murray, Eur. J. Pharmacol., 82, 113, 1982). However, the temporal requirements for A_1 ADO-R upregulation have not been established. The purpose of this study was to determine the magnitude of A_1 ADO-R upregulation associated with selected THEO exposure-periods and to compare this time-course with that for alterations in seizure threshold in similarly treated rats. Male Sprague-Dawley rats received twice daily injections of THEO (75 mg/kg) or 0.9% saline (SAL) for 1, 3, 7, 14, or 21 days. Forty-eight hours after the final dose rats were sacrificed and brain regions rapidly dissected and frozen for radioligand binding assays. A_1 ADO-R were selectively labeled in brain membrane preparations using N^6-cyclohexyl-[^{3}H]adenosine ([^{3}H]CHA) (New England Nuclear, spec. act. 9 Ci/mmol) as the radioligand. In cerebral cortical membranes significant increases of 13%, 27% and 19% in specific [^{3}H]CHA binding occurred at 7, 14 and 21 days, respectively. Cerebellar membranes demonstrated significant increases of 15% and 19% at 14 and 21 days, respectively. Previous reports have demonstrated these increases in [^{3}H]CHA binding following THEO exposure occur as a result of changes in receptor densities with no significant alterations in affinities. A_1 ADO-R upregulation has been suggested to play a role in reduced susceptibility to various chemoconvulsants (P. Szot, R.C. Sanders and T.F. Murray, Neuropharmacol., in press). To further define the role of A_1 ADO-R involvement in seizure susceptibility, bicuculline (BIC) seizure threshold determinations were performed in rats following the above chronic regimens. These experiments revealed a temporal pattern similar to A_1 ADO-R upregulation. THEO-exposed rats displayed significant increases in BIC seizure threshold of 17% and 25% following 1 and 2 weeks of exposure, respectively. These results lend further support to the contention that A_1 ADO-R upregulation is a consequence of chronic THEO exposure and that increased seizure thresholds following THEO exposure represents a functional correlate of the observed upregulation. (Supported by N.I.H. grant NS 23227)

201.3 DECREASED DENSITY OF PERIPHERAL BENZODIAZEPINE BINDING SITES IN CHRONIC, BUT NOT ABSTINENT ALCOHOLICS. F. Lafaille*, M. Dongier*, M. Dumas*, R. Quirion and B.E. Suranyi-Cadotte. (SPON: M. Ball). Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Quebec, H4H 1R3.

PK 11195 is an isoquinoline carboxamide derivative, which has been shown to bind with high affinity to peripheral benzodiazepine binding sites (PBR) in several tissues, including platelets (Benavides et al, Biochem. Pharmacol. 33; 2467, 1984). Although the functional role of PBR remains to be clarified, these sites may participate in the potent electrophysiological, convulsive and anxiogenic effects of peripheral benzodiazepine ligands (Mizoule et al, Life Sci. 36, 1059, 1985; Drugan et al, Pharmacol. Biochem. Behav., 24, 1673, 1986). Additionally, chronic alcohol administration in rats has been found to alter the density of PBR in brain (Schoemaker et al, Eur. J. Pharmacol. 120, 1248, 1983). To extend these findings, we studied platelet PK 11195 binding in 10 alcoholics (6 males, 4 females; mean age ± SEM = 43 ± 6 years), 10 recovered alcoholics (7 males, 3 females; mean age = 51 ± 8) and 11 healthy, non-alcoholic subjects (6 males, 5 females; mean age = 38 ± 4). All subjects were drug free for at least 4 weeks prior to platelet ^{3}H PK 11195 binding assays (Benavides et al, Biochem. Pharmacol. 33, 2467, 1984). The density (Bmax, fmol/mg protein) of ^{3}H PK 11195 binding on platelets of alcoholic subjects who continued to drink was significantly lower (mean Bmax ± SEM = 2115 ± 37; $p < 0.01$) than in healthy controls (3560 ± 54), with no significant difference in the affinity (Kd, nM) of ^{3}H PK 11195 for its binding site between the two groups (alcoholics, mean Kd ± SEM = 4.8 ± 1.7; controls = 5.1 ± 1.9). In contrast, mean Bmax values of platelet ^{3}H PK 1115 binding in alcoholics abstinent for at least 4 months was not significantly different from controls (mean Bmax = 3472 ± 46). Ethanol, in vitro (5 mM-43 mM) did not compete for ^{3}H PK 11195 binding sites on platelet membranes. These data suggest that a reduction in the density of platelet ^{3}H PK 11195 binding sites in chronic alcoholics is not due to a direct ethanol effect, and that a normalization of this variable may occur in abstinent alcoholics. Furthermore, these findings support the role of PBR in the physiological changes associated with alcoholism. Supported by the Douglas Hospital Alcoholism Research Program.

201.4 THE EFFECTS OF SWIM STRESS ON THE OCCUPANCY OF BRAIN BENZODIAZEPINE RECEPTORS MEASURED WITH $[^{3}H]$Ro-15-1788 IN MICE. S.I. Deutsch*, R. Weizman*, A. Weizman*, F.J. Vocci, Jr.*, K. Kook*, and S.M. Paul*. (SPON: P. Skolnick). Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

The GABA-benzodiazepine-receptor complex has been recently shown to be modified by environmental "stressors". There have, however, been conflicting results concerning the direction of these changes using classical in vitro techniques. Recently, an in vivo method for measuring benzodiazepine receptor binding using $[^{3}H]$Ro15-1788 was described that avoids many of the artifacts associated with the traditional in vitro approaches (Goeders and Kuhar, 1985; Deutsch et al., 1987). Using this method, we examined the effects of acute and chronic ambient temperature swim stress on the occupancy of benzodiazepine receptors measured with $[^{3}H]$Ro15-1788 in seven regions of mouse brain. In addition, we studied the effects of adrenalectomy on specific and nonspecific $[^{3}H]$Ro15-1788 binding in vivo in both stressed and unstressed animals.

Acute ambient-temperature swim stress (2 and 10 minutes) resulted in a significant increase in the in vivo binding of $[^{3}H]$Ro15-1788 in all brain regions when compared to nonhandled (naive) and sham stressed mice. Specific binding was unaltered 24 hours after a single swim-stress. Repeated swim stress (2 and 10 minutes) for seven consecutive days resulted in a significant decrease in specific $[^{3}H]$Ro15-1788 binding in some but not all brain regions. Adrenalectomy did not significantly alter basal levels of specific $[^{3}H]$Ro15-1788 binding in unstressed animals. However, adrenalectomized mice showed an even greater increase in specific $[^{3}H]$Ro15-1788 binding when measured 20 minutes after a single 10 minute ambient-temperature swim stress. Experiments are in progress to determine whether these changes are due to alterations in the distribution of $[^{3}H]$Ro15-4513 in the adrenalectomized/stress group. These data support a role for the involvement of central benzodiazepine/GABA receptors in the response to stress. Furthermore, glucocorticoids may play a role in the regulation of the sensitivity of the GABA receptor complex.

201.5 AVERSIVELY-MOTIVATED COPING BEHAVIOR AND LEARNING ALTER THE BENZODIAZEPINE/GABA CHLORIDE IONOPHORE RECEPTOR COMPLEX IN THE RAT: Robert C. Drugan[1,2], Steven M. Paul[1], Phil Skolnick[2] and Jacqueline N. Crawley[1], [1]Clinical Neuroscience Branch, NIMH, and [2]Laboratory of Neuroscience, NIDDK, NIH, Bethesda, MD, 20892

We have recently reported that rats exposed to escapable or inescapable shock who subsequently did not develop "learned helplessness" revealed significant changes in the binding of radioligands to the benzodiazepine/GABA receptor complex. Rats that did not develop learned helplessness (coping animals) had a significant increase in the binding of $[^{3}H]$muscimol to the $GABA_A$ receptor, and a significant decrease in the binding of $[^{35}S]$TBPS to the chloride channel in cerebral cortex, when compared to rats who developed learned helplessness 24 hours following inescapable tail shock. Moreover, 2 hours following exposure to a session of escapable shock a significant decrease in the binding of $[^{35}S]$TBPS in cerebral cortex when compared to inescapably shocked rats and naive controls was observed. In addition, exposure to an aversively-motivated learning task, (a two-way shuttlebox-escape from footshock task without prior stress exposure) resulted in a rapid (5 min. post-test) reduction in the binding of $[^{35}S]$TBPS to cerebral cortical membranes when compared to yoked shock and naive controls. These data suggest a rapid alteration in the benzodiazepine/GABA receptor complex in response to aversively-motivated coping behavior or to a simple learning task.

We are presently characterizing the binding kinetics, timecourse, and specificity of observed changes in $[^{3}H]$muscimol and $[^{35}S]$TBPS binding in several brain regions following exposure to escapable and inescapable "stress".

Preliminary results suggest that the changes in $[^{35}S]$TBPS (~20% decrease) measured in vitro from membranes prepared from escapably shocked rats are rapid and short-lived. In contrast, 26 hours following escapable shock we observed an increase in $[^{35}S]$TBPS binding to cerebral cortical membranes. Adrenalectomy does not appear to modify the escapable shock-induced decrease in $[^{35}S]$TBPS binding, suggesting that glucocorticoid feedback is not the primary mechanism mediating the effects of coping behavior on the benzodiazepine/GABA receptor complex.

201.6 COCAINE-INDUCED CHANGES IN BENZODIAZEPINE RECEPTORS IN DISCRETE AREAS OF THE RAT BRAIN. N.E. Goeders, M.A. McNulty*, S.M. Dworkin*, S.I. Dworkin and K.H. McAllister*. Departments of Pharmacology and Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

A variety of clinical and animal data suggest that the repeated administration of cocaine may be associated with numerous neurobiological alterations including changes in D_2 dopaminergic receptors in the striatum and nucleus accumbens (Goeders and Kuhar, Alcohol and Drug Res 7:207, 1987). Other investigations have demonstrated that a complex interrelationship exists between dopaminergic and GABAergic neuronal systems (Scheel-Kruger, Acta Neurol Scand 107:9, 1986). Since GABA is an integral component of the GABA/benzodiazepine receptor complex (Richards et al. Experientia 42:121, 1986) and since chlordiazepoxide affects intravenous cocaine self-administration in rats (McAllister et al., Soc Neurosci Abstr, 1987), this investigation was designed to investigate the effects of chronic cocaine administration on benzodiazepine receptors.

Adult male Fisher 344 rats were injected daily with cocaine HCl (20 mg/kg, ip) or an equivalent volume of saline (1 ml/kg) for 15 days. Twenty minutes following the final injection, the animals were sacrificed by decapitation and the brains were rapidly removed and dissected over ice. The dissected brain regions were stored at -70°C until assay. Saturation experiments were carried out with $[^{3}H]$ Ro 15-1788 to characterize changes in benzodiazepine receptors (i.e., K_d and B_{max} values) resulting from the treatment conditions. The concentration of benzodiazepine receptors (B_{max}; mean fmol/mg protein ± SEM) was significantly increased in the caudate nucleus (397 ± 19 vs 492 ± 39, $p<0.05$; saline vs cocaine treated) and the cerebellum (471 ± 21 vs 544 ± 17, $p<0.05$). In contrast, the concentration of benzodiazepine receptors was slightly reduced in the frontal cortex (876 ± 27 vs 800 ± 15, $p<0.05$) following cocaine treatment. However, cocaine did not affect benzodiazepine receptor binding in the diencephalon, hippocampus or brain stem. No significant changes in K_d values (affinity) were seen in any brain region examined.

The results of this investigation suggest that chronic cocaine administration can differentially affect benzodiazepine receptors in discrete brain regions, although the mechanism of action for this effect is unclear. A potential role for dopamine is currently being investigated.
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201.7 VISUALIZATION OF BENZODIAZEPINE AND T-BUTYLBICYCLOPHOSPHOROTHIONATE [(^{35}S)TBPS] BINDING SITES FOLLOWING ANXIOLYTIC AND ANTIDEPRESSANT TREATMENT. B.E. Suranyi-Cadotte, M.J. Meaney, S. R. Bodnoff, F. Lafaille*, M. Dalpé and R. Quirion. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Québec H4H 1R3.

Considerable evidence suggests that anxiolytic drug effects are mediated through an interaction with the benzodiazepine (BZ)/GABA receptor/chloride ionophore complex. In addition to the anxiolytic BZ's, several classes of antidepressants are also known to exert anxiolytic effects. Results from our laboratory indicate that chronic administration of tricyclic antidepressants, results in a decrease in BZ receptors. This suggests a possible role for the BZ/GABA receptor-ionophore complex in the actions of both antidepressants and anxiolytics. To extend these results, we investigated the effects of acute and chronic treatment with the benzodiazepine, diazepam, and the tricyclic antidepressant, desipramine (DMI) on BZ receptors and (^{35}S)t-Butylbicyclophosphorothionate (TBPS) binding to the GABA receptor/chloride ionophore in areas of rat brain using quantitative receptor autoradiography. Male Long Evans rats received once daily i.p. injections of either vehicle, or 2 mg/kg diazepam or 10 mg/kg DMI for 1 or 21 days. Four hours after the last injection, animals were decapitated, brains rapidly removed and frozen. Serial sections of brain from control and drug-treated animals were processed for in vitro ^{3}H-flunitrazepam or (^{35}S)TBPS autoradiography as described previously by Young and Kuhar (Nature 280:393-395, 1979) and by Wamsley et al (Life Sci. 33:2321-2329, 1983) respectively. Various brain regions from control and drug-treated animals were compared after acute and chronic treatment using computer-assisted quantitative densitometric analysis. Chronic, but not acute DMI treatment elicited a significant reduction in BZ receptor densities in lateral septum (28%), olfactory tubercle (16%), and CA_3 region of hippocampus (20%). In addition to the above regions chronic diazepam caused decreased BZ binding in several other brain areas, including cortex (20%), n. accumbens (20%) and hypothalamus (24%). DMI, like diazepam produced a significant decrease in (^{35}S) TBPS binding sites (24% and 28% respectively) after chronic treatment. This decrease was observed in the hippocampus, but not in other regions where the drug-induced decreases in BZ receptors occurred. Thus, hippocampal TBPS binding sites, may be an important site of interaction between antidepressants and anxiolytics. These data also indicate that anxiolytic and antidepressant agents are capable of differentially regulating BZ receptors and GABA-gated chloride ionophore binding sites.

201.8 MODULATION OF GLUCOCORTICOID BINDING CAPACITY IN SELECTED BRAIN REGIONS AND PITUITARY OF ADRENALECTOMIZED RATS BY VARIOUS STEROIDS. A. Sarrieau*, W. Rostene, T. Antakly, D.H. Aitken and M.J. Meaney. (SPON: J. Bradwejn). INSERM Unité 55, Paris, France and Departments of Anatomy and Psychiatry and Douglas Hospital Research Ctr., McGill Univ., Montreal, Canada.

Glucocorticoid receptors (GR's) are widely distributed in the rat brain. These receptors bind with high affinity to glucocorticoids, mineralocorticoids, and, to a lesser extent, to progesterone. GR concentrations in brain and pituitary are regulated by endogenous corticoids, such that long-term (>4 days) adrenalectomy (ADX) results in a large (>100%) increase in GR concentrations. In the experiments presented here, we have examined the ability of a wide range of naturally occurring and synthetic steroids to regulate GR concentrations in principle glucocorticoid target regions, including the pituitary hypothalamus, hippocampus, and cortex.

Adult, male Long-Evans rats were ADXed and treated with one of the following steroids for 4 days: RU 28362 (a GR agonist), corticosterone (the principle glucocorticoid in the rat), dexamethasone, progesterone, estradiol, or spironolactone (a mineralocorticoid receptor antagonist). All steroids were dissolved in absolute ethanol and added to the animals' drinking water (0.9% NaCl) to a final concentration of 100 µg/ml. The animals were then sacrificed by decapitation and the frontal cortex, hippocampus, hypothalamus, and pituitary were dissected and frozen on dry-ice. GR binding capacity was measured in soluable fractions using an exchange assay. Cytosol was incubated with 10 nM [^{3}H]dexamethasone for 20 h at 2°C alone or in the presence of a 500-fold excess of cold corticosterone in TEGMD buffer (30 mM Tris, 1 mM Na EDTA, 10 mM Na molybdate, 10% (v/v) glycerol, and 1 mM dithiothreitol; pH adjusted to 7.4). Bound from unbound steroid was separated on Sephadex LH-20 columns.

In all regions examined RU 28362 and dexamethasone decreased [^{3}H]dexamethasone binding (in comparison to ADX + NaCl controls) by approximately 85 and 70%, respectively. In the hippocampus and frontal cortex corticosterone and progesterone produced a 50% down-regulation of [^{3}H]dexamethasone binding, but were less effective (~30%) in the hypothalamus and pituitary. Neither spironolactone nor estradiol influenced [^{3}H]dexamethasone binding in any region examined. These data indicate a region-specific, differential pattern of GR regulation that is consistent with descriptions of two types of GR sites in the rat brain and pituitary. These findings are also discussed in terms of the affinity of the various steroids for the GR sites as well as the ligand-receptor interactions necessary for GR regulation.

201.9 THE OCCURRENCE OF [^{3}H]-IMIPRAMINE BINDING INHIBITOR(S) IN HUMAN BLOOD PLASMA. A. Strijewski*, J. Chudzik* and S.W. Tang. Psychopharmacol. Unit, Clarke Inst. of Psychiat., Toronto, Canada M5T 1R8

In our attempt to isolate the postulated endacoid for [^{3}H]-imipramine (IMI) binding site, platelet-free human plasma was first fractionated according to Cohn. Fractions II/III and VI were found to contain high inhibitory activities of IMI-binding which could be partially attributed to the presence of non-specific plasma protein inhibitions. We observed that various blood plasma proteins (e.g. α_1-acid glycoprotein, α_1-antitrypsin, IgG and lipoproteins) affect imipramine binding to platelet membranes via non-specific ionic or hydrophobic interactions. After separation of non-specific proteins by several chromatographic steps, an additional inhibitory activity, associated with an as yet unidentified protease, was revealed. This enzyme is heat stable, digests casein at pH 7.4 and is inhibited by α_1-antitrypsin. Several synthetic chromogenic peptides were tested as potential specific protease substrates but these experiments failed to provide any positive identification of the protease. However, the presence of thrombin, plasmin, urokinase, or trypsin-, chromotrypsin- and elastase-like protease activity can be excluded. When protease fractions of DEAE-Sepharose chromatography were subjected to further purification by ultrafiltration (PM-10 ultrafilter), followed by Sephadex G-25 and Bio-Gel P-2 gel filtration, a partial separation of IMI-binding inhibitor from protease was achieved. However, the protease fraction still retained a substantial inhibitor activity. TLC analysis of both fractions on cellulose plates showed a complex composition of the analyzed material. At least 8 ninhydrin positive compounds were detected.

Persistent occurrence of the protease and inhibitor in the same fractions at various purification steps raises the possibility of the existence of some kind of protease-inhibitor interaction. It cannot be excluded that the protease itself may modulate the receptor binding site thus altering its binding properties. Further investigation should lead to the purification and identification of the protease and [^{3}H]-IMI binding inhibitor.

201.10 CHRONIC PHENYTOIN TREATMENT DOWN-REGULATES SPECIFIC 3H-PHENYTOIN BINDING SITES IN RAT BRAIN. J. FRANCIS AND L. SPERO. Dept. of Pharmacology, University of Toronto, Toronto Ontario, Canada M5S 1A8.

3H-Phenytoin has been previously shown to have specific, satureable binding to rat, calf and human brain (1,2). The present study was conducted to look at the effects of chronic modulation of these binding sites by phenytoin (PHT). Male Long-Evans rats were treated initially with 25 mg/kg PHT, or its vehicle alone, by intraperitoneal (i.p.) injection once daily for 14 consecutive days. Rats were sacrificed on the 15th day by decapitation and their brains were removed and frozen in dry ice-ethanol until use. Synaptosomal (P2) fraction of whole brain homogenates were assayed for specific 3H-PHT binding, as previously described (1). A second series of rats were treated for 14 days by daily i.p. injections with one of vehicle only or 10 mg/kg, 35 mg/kg, or 50 mg/kg PHT in vehicle. Upon sacrifice, these latter rat brains were dissected into whole cortex and cerebellum (regions previously shown to be high in specific 3H-PHT binding sites) and assayed for specific 3H-PHT binding.

Whole brain, one-site analysis of 3H-PHT binding, using the program LIGAND (3) on an IBM-PC computer, revealed a decreased density of binding sites (Bmax) in the treatment versus vehicle control groups from the first series of rats. There was no change in the binding dissociation constants (Kd) from either group. In the second series of rats, 3H-PHT Bmax was lower in the cortices from the treatment groups versus the vehicle controls while there was minimal change in the cerebellar Bmax's amongst these groups. Kd's in both cortex and cerebellum were not changed in the treatment versus vehicle control groups. There did not appear to be a dose-dependent change in the Bmax's of the three PHT dosage groups.

These results would suggest a down-regulation of specific 3H-PHT binding sites following chronic PHT treatment. In light of the chronic nature of clinical phenytoin therapy, this down-regulation may occur in man and be important to the clinical anticonvulsant actions of PHT.

(Supported by MRC Grant MT-7119 and an OMHF Studentship (JF))

(1)Burnham, W.M. et al. (1981) Can. J. Physiol. Pharmacol. 59: 402-407
(2)Spero, L. (1985) Can. J. Physiol. Pharmacol. 63: 517-518.
(3)Munson, P.J. et al. (1980) Anal. Biochem. 107: 220-239.

201.11 CHRONIC LITHIUM TREATMENT REDUCES AGONIST-STIMULATED INOSITOL PHOSPHOLIPID HYDROLYSIS IN RAT BRAIN. T.L. Casebolt* and R.S. Jope, Department of Pharmacology and Neuropsychiatry Program, University of Alabama at Birmingham, Birmingham, AL 35294.

Lithium is widely used as a therapeutic agent for bipolar affective disorders, but its mechanism of action is unknown. Altered catecholaminergic and cholinergic function has been implicated in the etiology of affective disorders and some experimental evidence indicates that lithium may alter the activity of these neurotransmitter systems. Because it has been suggested that inositol phospholipid metabolism may mediate the therapeutic action of lithium, we investigated the effects of chronic dietary lithium on norepinephrine (NE), serotonin (5HT), and carbachol-stimulated inositol phospholipid hydrolysis in rat cortical, hippocampal and striatal slices.

Sprague-Dawley rats were fed either commercial diet or diet containing LiCl (1.696g/kg diet) with ad libitum water and saline for 30 days. After decapitation, brain regions were rapidly removed and cross-chopped cortical, hippocampal, and striatal slices (0.3mm) were rinsed and preincubated in HEPES-bicarbonate buffer containing 10 mM LiCl at pH 7.4 and 37°C for 45 minutes. After incubation with 0.53μM [^{3}H]inositol for one hour, inositol phospholipid hydrolysis was measured by the method of Berridge et al. (Biochem. J., 206: 587, 1982).

Chronic lithium treatment reduced NE-stimulated [^{3}H] MIP production an average of 36% in cortex. Smaller reductions were observed in carbachol and 5HT-stimulated [^{3}H] MIP production, with carbachol being least affected by the treatment. Similar changes were observed in striatum and hippocampus. Incorporation of [^{3}H]inositol into phospholipids was not altered significantly after chronic lithium treatment.

These results demonstrate that chronic lithium treatment alters the activity of receptors coupled to inositol phospholipid hydrolysis, and that adrenergic-mediated inositol phospholipid hydrolysis is affected to a greater extent than either cholinergic or serotonergic mediated responses.

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RECEPTOR REGULATION: CHOLINERGIC

202.1 SOLUBLE NON-NEURONAL CELL FACTORS REGULATE RECEPTOR EXPRESSION BY SYMPATHETIC NEURONS AND PC12 CELLS. K.E. Smith*, N.E. Kremer, K. Spiegel and J.A. Kessler (SPON: E. Masurovsky). Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Neurotransmitter receptor as well as neurotransmitter phenotypic expression is influenced by the neuronal microenvironment. The present study examines factors which regulate muscarinic and nicotinic receptor development and alpha-bungarotoxin binding in sympathetic neurons and in pheochromocytoma (PC12) cells. Previously we reported that treatment of sympathetic neurons with rat fibroblast conditioned medium (RFCM) simultaneously reduced muscarinic receptor number and stimulated cholinergic transmitter traits such as choline acetyltransferase (CAT). To further characterize actions of RFCM on receptor expression, alpha-bungarotoxin binding and nicotinic receptor (alpha subunit) mRNA levels were examined after treatment. Treatment of sympathetic neurons with RFCM resulted in no change or a slight increase in ^{125}I-alpha-bungarotoxin binding while decreasing muscarinic receptor number (determined by ^{3}H-QNB binding) by at least 50%. RFCM treatment of PC12 cells exposed to NGF elevated alpha-bungarotoxin binding by more than 75% while simultaneously decreasing muscarinic receptor numbers by 65%. The factor mediating these effects on muscarinic binding was heat-labile and had a MW greater than 30kD as determined by Amicon ultrafiltration. Thus RFCM contained a soluble factor(s) which simultaneously decreased muscarinic receptor binding while stimulating alpha-bungarotoxin binding. Using Northern blot analysis and a cDNA clone encoding a neural nicotinic receptor alpha subunit (Boulter et al., 1986) nicotinic receptor mRNA levels were measured after RFCM treatment of sympathetic neurons and PC12 cells. Treatment did not significantly alter levels of the two RNA species in sympathetic neurons which are recognized by this probe. Treatment of PC12 cells with NGF elevated levels of both transcripts by 2-3 fold compared to untreated PC12 cells, but additional treatment with RFCM produced no significant changes. These observations indicate that soluble factors produced by non-neuronal cells may alter the proportions of different receptors expressed by the neuron in a manner analogous to previously described effects of such factors on transmitter expression.

202.2 Differential Regulation of Muscarinic Receptor Subtypes in the Cerebral Cortex by Chronic Tetrahydroaminoacridine Administration. D.D. Flynn, A. Suarez,* A. Cordoves,* and D.C. Mash, Departments of Pharmacology and Neurology, University of Miami School of Medicine, Miami, FL 33101.

Tetrahydroaminoacridine (THA), a potent central acetylcholinesterase inhibitor, is being used in cholinergic replacement therapy for the treatment of senile dementia of the Alzheimer type. Chronic esterase inhibition in rodents leads to a down regulation of total muscarinic receptor numbers. Comparable changes in patients given esterase inhibitors may diminish the efficacy of these agents in degenerative dementias. We have examined the effect of chronic THA administration on muscarinic receptor subtypes in the rat cerebral cortex.

Male Long Evans rats (150-200 g) were injected intraperitoneally with THA dissolved in saline for a minimum of ten days (2.5 mg/kg starting dose, increased in increments to a peak dose of 10 mg/kg). Eighteen hours after the last injection, animals were sacrificed by decapitation and the brains were rapidly removed. Cortical tissue was dissected and homogenized in 50 mM $NaPO_4$ buffer (pH 7.4) containing Na_3EDTA. Membranes were collected by centrifugation and resuspended in the appropriate buffers for assays of total muscarinic receptors and M1 and M2 receptor subtypes. The prevalences of 'uncoupled' M1 and M2 receptors were determined by carbachol-[3H]-quinuclidinyl benzilate (QNB) competition. Carbachol binding to high-and low-affinity pirenzepine sites in the cerebral cortex was also measured in these experiments.

Chronic THA administration resulted in a 20-74% decrease in M1 receptors assayed by [^{3}H]-pirenzepine. This decrease in the number of [^{3}H]-pirenzepine sites was positively correlated with the duration of drug treatment. Carbachol-[^{3}H]-pirenzepine competition revealed no change in the ratio of high-and low-affinity agonist states of the M1 receptor. Total muscarinic receptors showed a 13-20% decrease, whereas M2 receptors, measured directly with [^{3}H]-OXO-M or estimated by carbachol-[^{3}H]-QNB competition, were unchanged.

These results demonstrate a selective regulation of the M1 receptor subtype by chronic THA administration. Down regulation of M1 receptors by chronic agonist occupancy, if accompanied by a reduction in the M1-mediated hydrolysis of phosphoinositides, may lead to diminished efficacy of long-term THA treatment in Alzheimer's disease.

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202.3 PHOSPHOINOSITIDE METABOLISM LINKED TO MUSCARINIC RECEPTORS IN PURIFIED MYELIN. F. GOLLY*, J.N. LAROCCA* and R.W. LEDEEN (SPON W.T. NORTON). Dept of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

The original view of myelin as a metabolically inert membrane has undergone radical revision in light of the numerous intrinsic enzymes that have been revealed over the past two decades. These enzymes are involved in lipid metabolism, ion transport, protein acylation, phosphorylation / dephosphorylation etc. Phosphoinositide metabolism in myelin has been reported (Deshmukh and al., J. Neurochem., 36:594, 1981) and recent studies have shown that incorporation of ^{32}P into phosphatidic acid, phosphatidylinositol-4-phosphate and 4,5-bisphosphate (PIP and PIP_2) could be increased in myelin by cholinergic stimulation of brain slices (Kahn and Morell, Trans. Am. Soc. Neurochem. 17:446, 1986). Previous studies of our laboratory (Larocca and al., J. Neurosci., in press) have shown the presence of high affinity muscarinic receptor(s) in purified myelin from rat brainstem. These receptors appear to be linked to both adenylate cyclase and phosphoinositide metabolism. 5'-guanylylimidodiphosphate (Gpp(NH)p), a non metabolisable analog of GTP, decreased the agonist affinity for N-methylscopolamine binding sites in myelin, indicating a linkage to a second messenger system via a G regulatory protein. Treatment of freshly isolated myelin (from rat brainstem prelabeled by intracerebral injection of [^{3}H]-inositol) with carbachol plus Gpp(NH)p resulted in an increase of inositol di- and trisphosphate (IP_2 and IP_3). This increase was more pronounced for IP_3. We found no change in inositol monophosphate, suggesting that only polyphosphoinositides are involved in this phenomenon. This was done in the presence of lithium, known to block phosphatase activity. We have found shorter times of incubation (5 to 15 min) to give better results than longer time (30 min). In the absence of calcium (with EGTA), carbachol plus Gpp(NH)p induced formation of IP_2 and IP_3, but the effect was more prononced with calcium; the response was dose-dependant (10 to 100 nM). We have found that GTP was also effective, but less so than Gpp(NH)p, in increasing formation of IP_2 and IP_3.

These results suggest that some myelin phosphoinositides are functionnaly linked to muscarinic receptor(s) in the myelin membrane and involved in transmission of extracellular signals.

202.4 DOWN-REGULATION OF MUSCARINIC CHOLINERGIC RECEPTORS FOLLOWING SHORT-TERM TREATMENT IN MOUSE NEUROBLASTOMA CELLS IN SUSPENSION. C.L. Cioffi and E.E. El-Fakahany. Department of Pharmacology, University of Maryland, School of Pharmacy, Baltimore, MD, 21201.

We have reported that short-term desensitization of muscarinic receptors in mouse neuroblastoma cells (clone N1E-115) maintained in monolayer results in approximately 50% decrease in the specific binding of the muscarinic antagonist [^{3}H]N-methylscopolamine ([^{3}H]-NMS) and that this receptor population which is highly susceptible to rapid regulation by agonists includes both the pirenzepine high-affinity and the agonist low-affinity receptor conformations (Cioffi and El-Fakahany, J. Pharm. Exp. Ther.,238: 916, 1986). It has previously been suggested that this agonist-sensitive subset of muscarinic receptors might be the receptor subpopulation linked to receptor-mediated increases in cyclic GMP in this same neuronal clone (McKinney et al., Mol. Pharmacol., 27: 223, 1985) The present study was undertaken to determine if down-regulation of [^{3}H]-NMS binding could be observed under the same conditions in which desensitization of the cyclic GMP response occurs.

For the desensitization experiments, mouse neuroblastoma cells were prelabeled with [^{3}H]guanosine, diluted with iso-osmotic HEPES buffer and distributed into conical tubes where the cells were desensitized for 2 to 16 minutes with 1 mM carbamylcholine (CBC) at 37° C. The cells were then centrifuged, washed with HEPES buffer, resuspended and distributed into wells. Cells were then incubated at 37° C for 5 min before stimulation with 1 mM CBC for 30 sec. The time course of desensitization of the cyclic GMP response was very rapid. For binding experiments, cells were also desensitized with 1 mM CBC at 37° C for up to 30 min, harvested and washed. This treatment resulted in a time-dependent decrease in the specific binding of 1 nM [^{3}H]NMS to muscarinic receptors in intact cells measured at 15° C. Scatchard plots of saturation curves using 0.02-1.0 nM concentrations of [^{3}H]NMS in control and desensitized cells resulted in a significant decrease in Bmax in the desensitized cells with no change in the k_d. These results indicate that challenging mouse neuroblastoma cells in suspension with muscarinic receptor agonists elicits both down-regulation of [^{3}H]NMS binding sites and desensitization of receptor-mediated cyclic GMP responses. (Supported by contract #DAAG-29-85-K-0123 from the U.S. Army Research Office).

202.5 MUSCARINIC RECEPTOR REGULATION IN SLICES OF RAT CEREBRAL CORTEX F. Van Huizen*[1], C. Shaw*[1], M. Wilkinson[2], and M.S. Cynader[1] (SPON: R.A. Leslie) [1]Dept. of Psychology and [2]Physiology and Biophysics, Dalhousie Univ., Halifax, N.S., B3H 4J1

Neurotransmitter receptor up- or down-regulation in response to alterations in bioelectric activity or receptor-ligand interactions have been well documented. Down-regulation may involve both receptor internalization and degradation. We have examined regulatory mechanisms of muscarinic acetylcholine receptors (mAChR) in slices of rat cerebral cortex.

Rats were killed by decapitation and the brains rapidly removed and placed in cold Dulbecco's phosphate buffered saline, pH 7.3, to which glucose (1 mg/ml), 0.004% H_2O_2 and Hepes (10 mM) was added (Dulb$^+$). Blocks of neocortex were dissected and sliced at 400 μm. Trypan blue exclusion tests showed that about 30% of the neurons in the 100 μm from each cut edge were dead (approx. 15% of total). Electron microscopy revealed healthy tissue in the middle of the slice, but after several hours in Dulb$^+$, swollen dendrites were seen without cytoplasmic organelles. Axon terminals, however, were still attached to these processes. Electrophysiological single unit recordings showed spontaneous action potentials in these slices.

For receptor binding experiments, slices were incubated in Dulb$^+$ (0.5 ml) containing either [^{3}H]N-methyl scopolamine ([^{3}H]-NMS), a hydrophilic mAChR ligand which does not penetrate the cell membrane, or the lipophilic [^{3}H]quinuclidinyl benzylate ([^{3}H]-QNB). Binding of both ligands was saturable, specific and could be displaced by cold atropine sulphate, NMS and QNB. In these experiments, bioelectric activity of the slices was raised for 2-4 hours at 30°C by either veratridine (10^{-5} M), which increases sodium-channel dependent depolarizations, L-glutamate (10^{-5} M), picrotoxin (10^{-5} M) or high K^+ (50 mM). Increases in neural activity decreased [3H]-NMS labelled mAChR number, with the largest effects seen for veratridine (-28.3±9.6; N=4). This decrease was not accompanied by any change in K_d. [^{3}H]-QNB labelled mAChR number was also lower (-9.8%) following veratridine. This implies an internalization and partial degradation of mACh receptors after this treatment. Stimulation of mAChR by ACh (10^{-5} M) or carbachol (10^{-3} M) also lowered mAChR number. Tetrodotoxin, a blocker of sodium-dependent neural activity, gave an insignificant increase in mAChR number.

The present results suggest that mAChR number is down-regulated following increases in neural activity or agonist activation of the receptor. These data may support the idea that 'rapid' alterations of synaptic responsiveness following altered activity are induced by alterations in receptor number, e.g. inside/outside ratio. (Supported by Killam postdoctoral fellowship to FvH and MRC grants to MW and MSC.)

202.6 ACETYLCHOLINE RECEPTOR mRNA LEVELS ARE INCREASED BY THE CALCIUM IONOPHORE A23187. J.P. Alsobrook II* and J.L. Pinkham** (SPON: C. Scheffey). *Dept. of Human Genetics and **Section of Molecular Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The mouse muscle cell line BC3H1 can be induced to exit the mitotic cycle and express acetylcholine receptors (AChR) on the cell surface. Induction is accomplished by allowing cells to grow to confluence or by incubation in low (0.5%) serum media. Levels of receptor subunit mRNAs have been shown to increase after such treatments.

We report that the calcium ionophore A23187 causes an increase in the levels of AChR mRNA. A23187 is known to increase the intracellular availability of calcium. After a 30 minute exposure to 1uM ionophore, BC3H1 cells have a steady-state level of AChR alpha subunit mRNA approximately equal to that seen in 3-day low serum induced cells. After 120 minutes the mRNA level is reduced to that seen in mitotic cells. These results point to calcium as a second messenger in the differentiation of muscle cells. Since the increased AChR mRNA level is not sustained by continuous exposure to ionophore, other signals may be required for maintenance of the differentiated phenotype.

202.7 PARAMETERS AFFECTING THE UP-REGULATION OF BRAIN NICOTINIC RECEPTORS BY DIETARY CHOLINE. B.J. Morley, L.L. Garner*, C. Larsson*, D.L. Fleck*, and C.L. Murrin. Research Division, Boys Town National Institute, Omaha, NE 68131, and the Department of Pharmacology, Univ. of Nebraska Medical School, Omaha, NE 68105.

The administration of dietary choline increases brain acetylcholine levels[1,2] and alters certain post-synaptic events[3,4]. The chronic administration of dietary choline also increases the concentration of nicotinic receptors, as measured by ^{125}I-α-bungarotoxin binding[5]. Several studies were carried out to further describe parameters affecting the increase in nicotinic receptors by dietary choline and to determine the mechanism by which choline produces the up-regulation. The studies indicate that the effect of choline on the concentration of nicotinic receptors (1) is attributable to choline-supplementation rather than choline-deficiency; (2) occurs rapidly (within 24 hrs); (3) is reversible (over a period of days) upon the elimination of choline; (4) is dose-dependent and (5) is age-dependent. Dietary choline affects the concentration of receptors without altering binding kinetics. Dietary choline and lecithin have been ineffective in improving functional improvement or objective cognitive test scores in Alzheimer's disease patients or elderly individuals[6]. One possible reason is that high-affinity uptake sites are decreased in number in these individuals. To test the hypothesis that the effect of dietary choline on the concentration of nicotinic receptors is related to the integrity of the high-affinity choline uptake (HACU) system, we administered intraventricular injections of AF64, a choline analogue that irreversibly blocks HACU, and measured the concentration of HACU sites and the concentration of nicotinic receptors in the hippocampus and cortex. The results of these studies will be presented.

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202.8 THE REGULATION OF BRAIN α-BUNGAROTOXIN BINDING SITES BY NICOTINIC CHOLINERGIC AGENTS. C. Larsson*, D.L. Fleck*, and B.J. Morley. Research Division (SPON: G.R. Farley), Boys Town National Institute, Omaha, NE 68131.

Nicotine up-regulates and diisofluorophosphate (DFP) down-regulates high-affinity acetylcholine binding sites in rat brain (Schwartz, R.D. and Kellar, K.J., *J. Neurochem., 45:*427, 1985). Up-regulation of mouse brain α-bungarotoxin (BuTX) receptors was observed following the constant infusion of high doses of nicotine but the administration of nicotine by injection produced no change in the concentration of BuTX binding in rat brain (Marks, M.J., Stitzel, J.A., Romm, E., Wehner, J.M., and Collins, A.C., *Mol. Pharmacol., 30:*427,1986).

We now report the effects of the chronic administration of several cholinergic agents, including nicotine, DFP and mecamylamine, on the concentration of rat brain BuTX receptors. Drugs were administered by injection or osmotic mini-pumps in several doses and for several time periods.

The lowest effective dose of nicotine (tartrate) was 3.0 mg/kg/day administered in 2 injections. The effect was observable within 3 days of administration. Upon withdrawal of nicotine, the concentration of receptors returned to baseline in approximately 24 hrs in most brain areas. There was some evidence for a re-bound effect (decreased receptor concentration) in certain areas upon the withdrawal of nicotine.

DFP reliably decreased the concentration of BuTX binding sites in the cortex but not in the hypothalamus nor hippocampus. The effect was reversible upon the withdrawal of DFP treatment.

Mecamylamine affected the concentration of BuTX receptors in a complex manner, apparently depending upon dose, length of of treatment, and type of administration (injection or mini-pump).

REGULATION OF AUTONOMIC FUNCTION III

203.1 ORGANIZATION OF VISCERAL SENSORY THALAMUS IN THE RAT. D.F. Cechetto and C.B. Saper, Department of Pharm. & Physiol. Sci. and Neurology, University of Chicago, Chicago, IL 60637.

The insular cortex of the rat contains a topographically organized visceral representation. We have studied the organization of its thalamic relay nuclei by injecting antero- and retrograde tracers at physiologically characterized sites in cortex and thalamus.

Injections of WGA-HRP at the sites of neurons in the dysgranular insular cortex that were selectively responsive to gustatory stimulation resulted in anterograde and retrograde labeling in the ventroposterior medial parvicellular (VPMpc) thalamic nucleus. Injections into cardiopulmonary responsive sites in the posterior granular insular cortex resulted in labeling in a parvicellular region, lateral to VPMpc, along the surface of the medial lemniscus, that we term the ventroposterior lateral parvicellular (VPLpc) nucleus.

Injections of WGA-HRP or fluorescent dyes into VPMpc and VPLpc have confirmed their complementary relationship to the different subfields of insular cortex. In addition, these experiments confirmed our earlier reports that the ventrobasal thalamus receives ascending afferents from the medial and ventrolateral parabrachial subnuclei. Injections of anterograde tracers (PHA-L and tritiated amino acids) into the parabrachial nucleus showed that the projection to the visceral thalamic relay nuclei originated primarily from the contralateral external medial subnucleus. Within this cell group, the rostrodorsal portion projected primarily to VPMpc and the caudoventral part to VPLpc. The remainder of the parabrachial-thalamic projection was mainly directed at the intralaminar nuclei that are adjacent to the visceral relay nuclei.

Recordings of single units in the VPLpc confirm that they are selectively responsive to cardiopulmonary (but not somatic sensory) stimulation. Our results indicate that there is a continuum of parvicellular neurons along the medial lemniscus that is viscerotopically organized for the relay of special and general visceral afferent information to the insular cortex. Earlier studies of this system may have overestimated the extent of the parabrachial input to the thalamic relay nuclei, and overemphasized the gustatory representation compared to the other visceral modalities.

203.2 PEPTIDERGIC PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT TO THE PARABRACHIAL NUCLEUS. H. Herbert, M.M. Moga, C.B. Saper, Dept. Pharm. & Physiol. Sci., University of Chicago, 947 E. 58th Street, Chicago, IL 60637

The parabrachial nucleus (PB) is the main relay for ascending visceral information from the nucleus of the solitary tract (NTS) to the forebrain. The NTS consists of a number of anatomically and functionally discrete subnuclei. Neurons in the rostral (r) NTS respond to gustatory stimulation, those in ventrolateral (vl) NTS are driven by pulmonary stimuli, and those in medial (m) and commissural (c) NTS respond to cardiovascular and gastrointestinal afferents.

Using anterograde and retrograde axonal tracers, we found previously that the functional specificity of the NTS is maintained in its projections to PB by the selective termination of the fibers in particular PB subnuclei. Briefly, rNTS was found to project to parts of the medial PB, particularly to the caudal portions of PB around the dorsomedial end of the superior cerebellar peduncle (waist area); vlNTS is reciprocally connected with the Kolliker-Fuse nucleus of PB, and also innervates part of the adjacent external lateral PB subnucleus; finally mNTS and cNTS project to a distinct terminal field involving parts of the external and central lateral PB and a second small field in dorsal lateral PB.

We now report the results of experiments combining retrograde (Fast Blue) and anterograde (PHA-L) tracing with immunohistochemistry to examine the neuropeptide specificity of some of these connections. In addition to SOM-, CCK-, APP-, ENK-, NT-, SUB P-, TH- and DBH- like immunoreactivity (li) that have previously been identified in the projection from mNTS and cNTS to PB (Manthy & Hunt '84, Milner et al. '84, Milner & Pickel '86a, b), we have found GAL-, AII- and NPY-li in this system. These neuropeptides may allow chemical coding in relaying specific types of visceral information to PB.

(H. Herbert is a recipient of a DAAD/NATO Research Training Fellowship)

203.3 PROJECTIONS OF THE CANINE PARABRACHIAL COMPLEX. C.H. Block, C.L. Chernicky and C.M. Ferrario. Department of Brain and Vascular Research, The Research Institute of The Cleveland Clinic Foundation, Cleveland, OH 44106.

The parabrachial nuclear complex (PB) is a pontine structure that is involved in the regulation of autonomic and chemosensory function. In the rat, afferent input to the PB has been demonstrated from the dorsomedial medulla, a site that functions in autonomic regulation and includes the nucleus of the tractus solitarii (nTS), the area postrema, and the dorsal motor nucleus of the vagus. In addition, cells in the PB project to the periventricular preoptic/hypothalamic area (PV), a forebrain region involved in hydromineral balance. The present study was designed to determine the anatomical relationship of the PB with the dorsomedial medulla and PV in the dog.

Microinjections of wheat germ agglutinin (WGA) were placed in the PV of 3 mongrel dogs. In these same animals, cholera toxin-horseradish peroxidase (HRP) was microinjected into the area postrema. The animals survived 48-72 hours, at which time they were perfused with a solution of picric acid, paraformaldehyde, and glutaraldehyde. Frozen sections were cut in the coronal plane at 30 μm and processed for histochemical localization of HRP and immunocytochemical localization of WGA. Projection fields were analyzed microscopically and mapped with an X-Y plotter interfaced to the microscope stage.

Injections of HRP into the area postrema resulted in local labeling of fibers and scattered cells within both the area postrema and the adjacent medial nTS. No retrograde or anterograde label was observed elsewhere in the brain. Injections of WGA into the PV resulted in a label of a distinct population of neurons in the dorsolateral aspect of the PB bilaterally. Scattered neurons were observed within the medial PB, the dorsal cap of the brachium conjunctivum, and in the adjacent locus coeruleus.

These results suggest that efferent projections from the area postrema do not extend beyond the medial aspect of the dorsomedial medulla and also do not include the PB. Although the present findings are consistent with the results from fiber degeneration studies in the dog by Chernicky et al., (1979) and Morest (1967) in cat, they are not consistent with the observation of abundant area postrema input to the PB in the rat (Shapiro and Miselis, 1985). In contrast, the distribution of PB cells that project to PV is similar to that reported in the rat by Block, et al.,(1984). From these anatomical observations it appears that the canine PB can influence sites in the forebrain that are critical to hydromineral balance.

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203.4 PROJECTIONS OF THE PERIVENTRICULAR HYPOTHALAMIC/ PREOPTIC REGION IN THE CANINE BRAIN. C.L. Chernicky, C.H. Block and C.M. Ferrario. Department of Brain & Vascular Research, Research Institute of The Cleveland Clinic Foundation, Cleveland, OH 44106

The periventricular region of the anterior portion of the third ventricle (PV) is critically involved in hydromineral balance. Lesions of specific periventricular nuclei result in hypernatremia, adipsia, and a reduction of the pressor response to intravenous infusion of angiotensin II (Lopes et al., 1986). Since little is known of the interrelated connections of this region in the dog, the present anatomical study sought to examine the afferent projection field of the PV region.

Wheat germ agglutinin alone (WGA) or coupled to horseradish peroxidase (HRP) was injected into the PV of 3 mongrel dogs. After 48 hours, the animals were perfused and the tissue was cut at 30 μm and processed for HRP or WGA and for immunocytochemical localization of the catecholamine-synthesizing enzymes, tyrosine hydroxylase (TH) and phenylethanolamine-N-methyl transferase (PNMT). The location of these cells was mapped using an X-Y plotter interfaced to the microscope stage.

Injections of WGA or HRP placed in the PV region resulted in a retrograde filling of neurons in the forebrain, mesencephalon, pons, and medulla. Specifically, retrogradely-labeled neurons were found ipsilaterally within the bed nucleus of the stria terminalis, lateral septum, medial amygdaloid nuclei, nucleus medianus, and medial preoptic area. In the mesencephalon, bilateral retrograde labeling was found in the peripeduncular region, however the ipsilateral label was more intense. In the pons, retrogradely-labeled cells were observed bilaterally in the dorsolateral aspect of the parabrachial nucleus. Scattered retrogradely-filled neurons were found within the locus coeruleus, medial parabrachial nucleus, and the dorsal cap of the brachium conjunctivum. Of these, only a few cells within the medial parabrachial nucleus also contained TH immunoreactivity. In the medulla, retrogradely-labeled neurons were found within the dorsal motor nucleus of the vagus and the medial nucleus tractus solitarii. Several of these neurons also contained TH. Scattered cells within the ventral aspect of the medulla were found to project to the PV and contain either TH or PNMT. In addition, a population of retrogradely-labeled cells was located in the medullary reticular formation, extending dorsoventrally between the hypoglossal nucleus and the nucleus ambiguus.

These results demonstrate a diverse afferent input to the PV of the dog from both forebrain and brainstem regions that are involved in autonomic and chemosensory regulation. Additionally, it appears that the brainstem afferents to the PV may contain catecholamines. These pathways may provide the anatomical substrates through which brainstem structures influence forebrain mechanisms of fluid balance.

(Supported by NIH grants HL - 6835 and HL - 37927).

203.5 PROJECTIONS OF THE RAT CENTRAL NUCLEUS OF THE AMYGDALA TO THE VENTROLATERAL MEDULLA. R.L. Thompson* and M.D. Cassell* (Sponsored by J.R. West). Dept. of Anatomy, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

The central nucleus of the amygdala (CNA) is known to project to autonomic areas of the brainstem including the parabrachial nucleus, the dorsal motor nucleus of the vagus (DVN) and the nucleus tractus solitarius (NTS). Though the anatomical data suggest a predominantly parasympathetic relationship for the CNA, physiological studies indicate a strong sympathetic component in its role in autonomic regulation. The CNA does not appear to project to preganglionic sympathetic neurons in the spinal cord, and sympathetic components of CNA function may be mediated via intermediate cell groups in the brainstem. The present study is part of a series of experiments designed to determine anatomical relationships between the CNA and sympathetic structures in the brainstem. One area of the brainstem whose connections with the CNA have not been studied in detail is the A1 catecholaminergic cell group in the medulla. Small injections of 0.05μl of either bisbenzimide or Fast Blue were made in the region of the A1 catecholaminergic cell group in male Sprague-Dawley rats. The animals were sacrificed after appropriate survival times and the brains and brainstems were removed and sectioned. The injection sites were found to be confined to the ventrolateral medulla and did not infiltrate the NTS/DVN or the nucleus ambiguus. In the CNA, retrogradely labeled cells were located throughout the rostrocaudal extent of the nucleus and were restricted to its medial part. Comparing the distributions of labeled neurons with counterstained material indicates that almost all of the neurons are confined to the medial subdivision of the CNA. The distribution pattern of CNA-ventral medulla neurons was compared to the distribution pattern of neurons labeled following bisbenzimide or fast blue injections into the NTS/DVN. CNA neurons projecting to the dorsal medulla were found not only in the medial subdivision but in considerable numbers in the lateral and ventral subdivisions. The results indicate that the distributions of CNA neurons projecting to the dorsal or ventral medulla are different but overlap in the medial subdivision. This may indicate a common innervation of both populations by afferents ending in the medial subdivision but suggests that a sizeable proportion of CNA neurons projecting to the dorsal medulla may be innervated by different afferents. This may represent an important anatomical substrate by which parasympathetic and sympathetic components of CNA function are affected differentially. (Supported in part by BRSG RR05372 from NIH.)

203.6 CORTICOSTERONE-INDUCED NUCLEAR LOCALIZATION OF GLUCOCORTICOID RECEPTORS IN CA1 NEURONS OF ADRENALECTOMIZED RATS: AN ULTRASTRUCTURAL ANALYSIS. C. Gonzales*, J.-Å. Gustafsson*, B. Nathan*, K. Fuxe and M. Kalia (SPON: W.H. Vogel). Dept. of Pharmacology, Thomas Jefferson Univ., Philadelphia, PA 19107(CG, BN, MK); Dept. of Medical Nutrition, Huddinge Univ. Hosp., Huddinge, Sweden (J-ÅG); Dept. of Histology, Karolinska Institute, Stockholm, Sweden (KF).

Light microscopic examination of a number of regions of the telencephalon and diencephalon of the adrenalectomized rat by means of a monoclonal antibody raised against glucocorticoid receptors (GR) has demonstrated that GR translocate from the cytoplasm to the nucleus in the presence of exogenously administered corticosterone (Fuxe et al, '85). A recent study by McEwen et al ('86) has demonstrated that receptors for adrenal steroids in the hippocampus are negatively regulated by corticosterone. The glucocorticoid receptors appear to be unique in their ability to translocate from the cytoplasm to the nucleus in the presence of their target hormone (corticosterone) since recent studies on the estrogen receptor have yielded negative data regarding the translocation hypothesis (King and Greene, '84; Welshons et al, '84). A crucial step in understanding the mechanism of action of steroid receptors in general is the "activation process," ie, the process whereby steroid receptors acquire the ability to bind to cell nuclei or DNA leading to their involvement in the regulation of gene expression. Although some differentiation of nuclear vs. cytoplasmic labeling is possible with high magnification light microscopy, definitive details of the localization of glucocorticoid receptors in the nucleus or the cytoplasm under different conditions can be revealed only with electron microscopy. We examined the ultrastructure of the CA1 region of the hippocampus of bilaterally adrenalectomized (ADX) rats (3 days post-ADX) with and without corticosterone treatment (10 mg/kg injected IP) administered 2 to 4 hours prior to perfusion. Non-corticosterone treatment was achieved by injecting an equal volume of the vehicle IP. The acrolein fixation protocol of Pickel ('85) (acrolein 3.75% in 2% paraformaldehyde in phosphate buffer) was used. Control studies in sham operated animals were included in each experimental series. Serial 50 μm thick vibratome sections of hippocampus were incubated in different (serial) dilutions of a mouse monoclonal antibody raised against molybdate-stabilized glucocorticoid receptors (Okret et al, '85). We analyzed tissue immunoreacted with 1:8000 dilution of the GR antibody and used the biotin/avidin system for demonstration of positive immunoreactivity. No colchicine pretreatment nor the addition of Triton X-100 to increase the immunoreactivity and penetration of the antibody, respectively, was used. Serial 70-80 μm ultrathin sections on formvar-coated slot grids were examined under a JEOL 100CX electron microscope with an accelerating voltage of 60 volts.

Intense GR positive immunoreactivity was detected in the nuclei of cells in the CA1 region of the hippocampus of adrenalectomized rats treated with corticosterone. Adrenalectomized rats without corticosterone treatment (vehicle treated) showed nuclei without any GR positive immunoreactivity. Sham operated animals appeared similar to corticosterone treated animals. These results demonstrate that corticosterone induces the localization of glucocorticoid receptors within the nucleus of CA1 neurons. In the pyramidal layer of the CA1 region GR positive immunoreactivity was limited to the pyramidal cells which had the following ultrastructural characteristics: large central nuclei with a completely smooth contour, prominent nucleoli and nuclear inclusions; the average nuclear-cytoplasmic ratio was 1.7:1; the cytoplasm contains a number of arrays of rough endoplasmic reticulum, lysosomes, a well-developed Golgi apparatus, free ribosomes, multivesicular bodies, scattered mitochondria and agranular vesicles. A number of small axon terminals containing clear, round vesicles (6 per ultrathin section) made synaptic contact with the somal surface. (Supported by USPHS Grants HL30991, HL31997, HL33632 to MK.)

203.7 ASCENDING AXONAL PROJECTIONS OF THE NUCLEUS TRACTUS SOLITARIUS IN THE PIGEON. M. L. Berk. Dept. of Anatomy, Marshall Univ. Sch. of Med., Huntington, WV 25704.

The nucleus tractus solitarius (NTS) has an important role in integrating peripheral autonomic inputs. In the pigeon, the visceral afferent fibers to the NTS are partially segregated to specific NTS subnuclei (Katz and Karten, '83). In this study, the NTS projections by which visceral sensory information can be relayed to the forebrain and pons was investigated by the anterograde transport of WGA-HRP. Injections (5-10nl) of 2 percent WGA-HRP were placed at several rostral-caudal levels of NTS. Anterogradely labeled fibers leave NTS by coursing ventrolaterally into the plexus of Horsley in the medullary lateral reticular formation. At pontine levels, a great number of fibers sweep dorsally into the parabrachial region (PB). Many of these fibers appear to terminate within the medial part of the PB. Labeled NTS fibers ascend into the midbrain tegmentum and enter the medial forebrain bundle in the posterolateral hypothalamic area (PLH). These fibers are interspersed amongst PLH neurons, which have descending projections to NTS. A dense, oval shaped field of axonal termination is located in the posteromedial hypothalamus just ventrolateral to the paraventricular organ. The NTS fibers in the medial forebrain bundle ascend into the ventral basal telencephalon and terminate amongst the cells of the bed nucleus of the stria terminalis - ventral striatum, which also have descending projections to NTS. Labeled NTS fibers also course into the periventricular zone of the anterior hypothalamus - preoptic area, where fibers were found amongst the cells of the avian homologue of the paraventricular nucleus. The majority of NTS fibers pursue an ipsilateral course, although contralateral projections were observed including projections to the contralateral NTS.

The NTS perikarya of origin of the projections to the PB and basal forebrain were determined by the retrograde axonal transport of WGA-HRP. All basal forebrain nuclei and PB are mostly innervated by NTS perikarya within subnuclei medialis superficialis (pars posterior) and the part of NTS caudal to the obex. Visceral sensory input to subnucleus medialis superficialis has not been described, while the caudal NTS could act as a relay of esophageal and gastric information. A few cells in the NTS subnucleus lateralis dorsalis (pars posterior) were labeled. This subnucleus could act as a relay for baroreceptive information to the basal forebrain and PB. In addition, the lateral PB receives fibers from a few cells of the lateral parasolitary subnucleus. This pathway could provide a relay for pulmonary information to the PB. Supported by NS 20512, BRSG 05870, and AHA, WVA affiliate.

203.8 THE EFFECT OF REDUCING THE SODIUM CONCENTRATION INTO THE III CEREBRAL VENTRICLE ON THE RENAL EXCRETION OF SODIUM AND WATER IN THE RAT. A. Guevara-Rojas*, P. Vergara-Aragón*, M.P. Rosas-Arellano*, and B. Barrera-Mera. (SPON: Dr. J. Roig-Varela). Dept. of Physiology. Fac. of Medicine. U.N.A.M. Apdo. Postal 70250, 04510 México, D.F.

Evidence provided by several experimental groups indicates the existence of hypothalamic receptors which respond to variations in the extracellular concentration of sodium. For some authors these are osmoceptors involved in the regulation of the water intake, — while for us, these sodium sensors are part of an antinatriuretic mechanism specifically in charge of the bodily sodium contents.

To solve the doubt we planned to study the effect on the sodium and water renal excretion of a transient reduction of the sodium - concentration into the III cerebral ventricle (III CV), preferably without any osmolar change.

Female Wistar rats of 250-300 g. of body weight (b.w.), with a chronically implanted canula into the III CV and another into the bladder were used. They had free access to water and food until the begining of the test. During the test, they remained quietly resting, conscious and unrestrained. Their urine was collected in 15 min. periods and after the 2 initial ones, a water load (3% - b.w.) was generally given to maintain a convenient urine flow. At the end of one or two more periods, 5µl of isotonic glucose solution or distilled water were injected in 12 min. into the III CV. The urine volume, very small in the control periods, usually began to increase before the end of the first experimental period, after the water load was given. This increase frequently coincided with a marked increase of the sodium and chloride excretion which, like the urine volume, was proportional to the rat's ingestion of food and water before the test. In 15-30 min. this rise reached its -- maximum and then decreased. The III CV injection, especially that of distilled water, always was immediately followed by a fall of the sodium and chloride excretion rates to values lower than those of the control periods, but without change in the water diuresis. We interpret this intense antinatriuresis as the response of the hypothalamic sodium sensing mechanism to a sudden decrease of the extracellular sodium concentration, in order to preserve the bodil sodium contents, as we had postulated.

203.9 EFFECTS OF INTRACEREBROVENTRICULAR COLCHICINE ON ARTERIAL PRESSURE AND BRAIN CYTOCHROME OXIDASE ACTIVITIY IN NORMO- AND HYPERTENSIVE RATS. T.L. Krukoff and D. Vincent*. Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Canada. T6G 2H7.

Intracerebroventricular (i.c.v.) colchicine injection facilitates immunohistochemical localization of neuropeptides in neurons. Little is known, however, about the physiological effects of this procedure. We have studied the effect of i.c.v. colchicine on arterial pressure (AP) in normotensive Sprague-Dawley (SD) and Wistar Kyoto (WKY) rats and in spontaneously hypertensive rats (SHR); its effects on cellular metabolism in brain were also assessed by cytochrome oxidase (COX) histochemistry. APs of SD, WKY, and SHR were measured for three consecutive days using standard tail cuff techniques. Rats were anesthetized (sodium pentobarbital, 40 mg/kg i.p.) and 70 ug colchicine in 20 µl saline were injected into the right ventricle of the brain. APs were measured 1 and 2 days after injection. Rats were then reanesthetized and perfused through the heart with 50 ml saline and 200 ml Zamboni's fixative. Brains were removed, frozen, sectioned in a cryostat (50 µm), and prepared for COX histochemistry (Krukoff et al., Brain Res. 280: 160, 1983). Pre-injection AP values (mm Hg, mean ± S.E.) were: SD, 140 ± 2 (n=9); WKY, 156 ± 2 (n=14); SHR, 195 ± 3 (n=11). As there were no differences in measurements made 1 or 2 days after injection, these values were combined. Post-injection values were: SD, 124 ± 5; WKY, 133 ± 4; SHR, 145 ± 8. These values were all significantly lower than pre-injection values and reductions were greatest in the SHR. As colchicine blocks axonal transport and may affect neural transmission, these results support the hypothesis that the CNS is at least partly responsible for maintaining elevated AP in the SHR. Colchicine-induced cellular changes assessed by COX histochemistry were similar for all strains: neurons in the paraventricular and supraoptic nuclei of the hypothalamus, nucleus circularis, and the dorsal motor nucleus of the vagus were shrunken and more intensely stained for COX than in controls. Colchicine induces mitochondria to migrate to the perinuclear cytoplasm in placental cells 'in vitro' (Addai & Ockleford, J. Anat. 147: 219, 1986); similar factors may explain the changes in neuronal COX activity as COX is a mitochondrial respiratory enzyme. Why only select groups of neurons undergo these changes is not known. These results suggest that i.c.v. injection of colchicine has widespread physiological effects in the CNS and that caution should be exercised in interpreting results obtained after its use.

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203.10 **VASOPRESSIN INHIBITS SYMPATHETIC GANGLIONIC TRANSMISSION BUT POTENTIATES SYMPATHETIC NEUROEFFECTOR RESPONSES IN HINDLIMB VASCULATURE OF RABBITS.** K.P. Patel and P.G. Schmid. V.A. Med. Ctr., C.V. Ctr., & Dept. Int. Med., Univ. of Iowa, Iowa City. IA 52240.

Vasopressin (AVP) has been shown to modify sympathetic nervous system responses. However, the site (sympathetic ganglia, sympathetic terminals, or sympathetic adrenergic receptors) of action of AVP is not clear. In addition the AVP receptor or receptors (V_1 or V_2) involved in this response is/are not identified. To determine whether AVP affects vasoconstrictor responses to electrical stimulation of sympathetic nerves or intra-arterial norepinephrine (NE), changes in perfusion pressure were measured during lumbar sympathetic nerve stimulation (LSNS, 1-8 Hz), or administration of NE (50 - 200 ng), in an isolated constant-flow perfused hind limb of chloralose anesthetized rabbit (n=7) before and after intra-arterial infusion of AVP (0.65 mU/Kg/min). At this dose AVP produced an increase in perfusion pressure of 5.3 ± 0.9 mm Hg and no detectable changes in systemic arterial pressure or heart rate. AVP significantly potentiated responses to LSNS (relative potency (RP) = 1.59) and to NE (RP = 5.17). The potentiation of LSNS and NE to AVP infusion was abolished by AVP antagonist ($d(CH_2)_5[Tyr(Me)^2]$AVP, 400 ng) (n = 6).

Since there was a significant difference between the RP of LSNS (stimulation of both preganglionic and postganglionic nerves) and NE (direct effect on the vascular smooth muscle), we reasoned that this difference might represent disparate actions of AVP on the ganglia and sympathetic neuro-effector sites. To evaluate responses to stimulating only the postganglionic sympathetic nerves, we repeated the above study in animals with ganglionic blockade (hexamethonium - 25 mg/kg, supramaximal dose for ganglionic blockade). After ganglionic blockade the responses to LSNS were reduced to 22% of control. In the presence of ganglionic blockade, AVP potentated responses to LSNS (RP = 4.09) (n = 6). Potentiation was not different for postganglionic LSNS and NE.

We draw the following conclusions from these data: 1) AVP potentiates vasoconstrictor responses to LSNS and NE, 2) AVP mediated potentiation of vasoconstrictor response to these stimuli is mediated via V_1 receptors, 3) AVP seems to have disparate actions ie., inhibition at the sympathetic ganglia and potentiation at the neuro-effector site.

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203.11 PARTICIPATION OF ALPHA$_2$ ADRENOCEPTORS AND VASOPRESSIN IN CARDIOVASCULAR RESPONSES INDUCED BY CENTRAL ANGIOTENSIN II IN RATS. L.C. Lopes*, W.A. Saad, L.A.A. Camargo*, A. Renzi*, L.A. De Luca Jr.*, J.V. Menani*, H. Milani* and William A. Saad*. Dep. of Physiology and Pathology. Faculty of Dentistry, UNESP, Araraquara, SP, 14.800 and Dep. of Surgery, Faculty of Medicine, USP, São Paulo, SP.

Intracerebroventricular (ICV) injection of angiotensin II (AII) produces an increase in mean arterial pressure (MAP) and heart rate (HR). In a recent communication we demonstrated that adrenodemedullation reduces the pressor effect, whereas previous treatment with guanethidine prevents the tachycardia due to ICV AII injection. In this study we investigated the influence of previous treatment with prazosin* or arginin vasopressin antagonist (AVPa) on the MAP and HR increase caused by AII injected ICV. Holtzman rats with delay cannulae implanted into the lateral ventricle had the jugular vein and femoral artery cannulated. Twenty minutes after intravenous injection of the antagonist, AII was injected ICV and MAP and HR were recorded. Intravenous injection of prazosin (0.5 mg/kg) reduced the MAP of rats (n=10) from 110 ± 2 mmHg (control) to 92 ± 2 mmHg, whereas HR was increased from 395 ± 17 bpm to 488 ± 12 bpm. The increase in MAP by ICV injection of AII (12 ng) after intravenous injection of prazosin (8 ± 2 mmHg) was smaller than that observed in control before prazosin (14 ± 2 mmHg). The tachycardic response to ICV injection of AII was not modified by prazosin (HR increased by 20 ± 10 bpm in control versus 17 ± 10 bpm after prazosin). Intravenous injection of AVPa (10 µg/kg) also reduced the pressor response produced by ICV injection of AII (12 ng) from 14 ± 1 mmHg in control to 5 ± 1 mmHg after AVPa treatment (n=7). The tachycardia observed with ICV injection of AII before AVPa (11 ± 7 bpm) was not altered after AVPa treatment (10 ± 3 bpm).The basal values of MAP (107 ± 4 mmHg) and HR (346 ± 15 bpm) was not altered by AVPa treatment. The results demonstrated that vasopressin and α_2-adrenergic receptors participate in the pressor response induced by centrally injected AII, but neither participate in the tachycardic response.

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*Gentle Supplied by Pfizer.

203.12 HISTAMINE AND EPINEPHRINE MICROINFUSIONS INTO THE PARAVENTRICULAR NUCLEUS CAUSE HYPERGLYCEMIA IN RATS. M.W. Gunion, S. Miller*, B. Butler*, and B. Shryne*. GRECC, Sepulveda V.A. Med. Ctr., Sepulveda, CA 91343.

Histamine has recently been localized in neurons of the paraventricular nucleus of the hypothalamus, which is thought to be involved in glucoregulation. Epinephrine terminals have also been located in this region. To determine if paraventricular histaminergic neurons and adrenergic terminals may be involved in central glucoregulation, histamine and epinephrine were microinfused into the paraventricular nucleus, and their effects on circulating glucose determined.

After 24 hr food deprivation, male Sprague-Dawley rats (225-350 g; Hilltop) were anesthestized with enflurane-methoxyflurane and given bilateral microinfusions of histamine (0, 10, 100 nmol/rat) or epinephrine (0, 10, 30, 100 nmol/rat) into the paraventricular nucleus or caudate-putamen (0.5 ul/1.0 min/side). Blood samples (120 ul) were taken from the tail tip immediately prior to anesthetization and 15, 30, 60, 90, and 120 min postmicroinfusion.

Microinfusion of histamine into the paraventricular nucleus caused mild hyperglycemia which was not statistically significant until 60 min postmicroinfusion (10 nmol=+23, 100 nmol=+25 mg/dl, both p .05). Hyperglycemia was still present at 120 min postmicroinfusion after the 100 nmol dose (+17 mg/dl, p .05). Epinephrine caused significant hyperglycemia at 15 min postmicroinfusion when given into the paraventricular nucleus (10 nmol=+43, 30 nmol=+43, 100 nmol=+58 mg/dl, all p .01). Statistically significant hyperglycemia was still present at 90 min only at the highest dose (+20 mg/dl, p .01), and was not present at any dose at 120 min (all p .05). Neither histamine nor epinephrine microinfusion into the caudate-putamen had any significant effect on blood glucose levels (both p .05).

The results suggest that histaminergic neurons and adrenergic terminals in the region of the hypothalamic paraventricular nucleus may be involved in glucoregulation.

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203.13 HYPERGLYCEMIA EVOKED BY CENTRAL INJECTION OF EPINEPHRINE IS LINKED TO A NICOTINIC MECHANISM IN RAT BRAIN. L. Console and R.F. Orzechowski*. Phila. College of Pharmacy and Science, Phila., PA 19104.

We previously reported that central injections (intracerebroventricular, ICV) of epinephrine (EPI) evoke dose-related hyperglycemia in both conscious and anesthetized rats (Fed. Proc. 46 (3):699, 1987). In the present study, we used ganglionic blocking drugs and adrenergic antagonists to explore possible mechanistic aspects of this phenomenon. EPI was centrally injected into conscious, freely moving rats that had been pretreated either centrally or peripherally (intraperitoneal injection, IP) with one of the ganglionic or adrenergic blockers.

Central drug injections were made via stainless steel (30 gauge) cannulae stereotaxically implanted in the right lateral cerebral ventricle. Drugs were given at the following doses in a volume of 3ul: EPI (50 nmol), hexamethonium (83 nmol), propranolol (34 nmol) and tolazoline (51 nmol). Peripheral (IP) pretreatment with ganglionic blockers (hexamethonium, 8 mg/kg or mecamylamine, 5 mg/kg) involved repetitive dosing, 15 and 5 minutes prior to and 60 and 90 minutes after EPI injection, to maintain continuous ganglionic blockade.

Peripherally administered mecamylamine, but NOT hexamethonium, abolished EPI-induced increases in plasma glucose. Centrally administered hexamethonium also completely prevented the hyperglycemic response to ICV EPI. Hexamethonium, unlike mecamylamine, is a quaternary amine which does not readily cross the blood-brain barrier. Therefore, blockade of the EPI-induced hyperglycemic reaction by IP administration of mecamylamine may be a centrally mediated effect.

Preliminary data from experiments with adrenergic antagonists suggest that ICV injections of either the beta-blocker propranolol or the alpha-blocker tolazoline only partly suppress EPI-induced hyperglycemia. Neither caused abolishment of the response as was observed when rats were pretreated with ganglionic blockers. The results of this study suggest a link between central adrenergic and central nicotinic mechanisms in the mediation of EPI-induced hyperglycemia in conscious rats.

203.14 IMPAIRED GLUCOSE TOLERANCE IN RATS WITH SUBSTANTIA NIGRA LESIONS. B.J. Davis, S.E. Hawes*and T.H. McNeill. Departments of Neurology and Neurobiology and Anatomy, Univ. Rochester School of Medicine and Dentistry, Rochester, New York, 14642.

Previous studies showed that bilateral lesions of the substantia nigra (SN) in rats led to reduced pancreatic islet volume due to impaired growth of the insulin-producing B cells (Davis and Smith, 1984, 1985). In the present study, we asked whether loss of dopamine (DA) cells in the SN was associated with altered insulin responses to glucose or to impaired glucose tolerance. Young adult male Sprague-Dawley rats were food restricted preoperatively and were given limited access to a high fat diet postoperatively to minimize lesion-associated decrements of body weight as described previously. SN lesions were produced by stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the SN bilaterally (12ug/hemisphere).Sham operated rats underwent identical dietary and surgical manipulations except that no drug was injected. Insulin responses to intravenous glucose (40mg in 0.5ml saline) and intragastric glucose tolerance tests (150mg/100gm body weight) were administered to separate groups of rats 3-10 weeks following surgery. Basal levels of glucose, insulin and glucagon were determined at the time of sacrifice. Brains were processed for formaldehyde-induced fluorescence (FIF) or for tyrosine hydroxylase immunocytochemistry (TOH-ICC) to determine the extent of loss of DA cells in the SN.

Compared with sham operated rats, rats that received intranigral 6-OHDA showed bilateral reductions of DA cells as revealed by a loss of TOH immunoreactive or fluorescent perikarya and processes in the zona compacta of the SN. Reductions of DA cells were most pronounced in the caudal and medial aspects of the SN. Consistently the extent of cell loss appeared greater in brains analyzed with FIF when compared with brains analyzed with TOH-ICC.

When compared with sham operated rats, SN lesioned rats showed impaired insulin responses to glucose as reflected by the absence of acute insulin responses and significantly elevated glucose levels at 5 and 10 minutes following IV glucose. SN lesioned rats also showed significantly higher glucose levels than the sham operated rats at 30 and 60 minutes following intragastric glucose loads. While basal levels of glucose were comparable between sham operated and SN lesioned rats, the lesioned rats showed significant reductions of basal serum insulin levels and significantly elevated basal serum glucagon levels.

These results provide a functional correlate for our previously reported morphologic investigation of islet changes following SN lesions. The mechanisms leading to alterations of the pancreatic islets and to impaired glucose tolerance following SN lesions are not known, but altered autonomic input to the islets may be a contributing factor. Supported by AG 05937.

203.15 EFFECTS OF GLYCINE AND MILACEMIDE IN DDT INDUCED HYPERTHERMIA. D.D. Truong*, J.J. Claude, G. Pezzoli and S. Fahn (SPON: R. Burke). Department of Neurology, Columbia University, New York, New York.

The DDT toxic syndrome consists of tremor, myoclonus, running seizures and hyperthermia. Decreased brain glycine levels have been reported in the DDT syndrome (Kar and Matin, Eur. J. of Pharmacol. 25, 36, 1974). We tested the effects of glycine i.c.v. (26 umol in 10 ul) and milacemide, a glycine prodrug, in the DDT induced hyperthermia in adult Sprague-Dawley rats who simultaneously received DDT 600 mg/kg (or corn oil control) by orogastric intubation. Rectal temperature was measured every half-hour for 4 hours in controlled room temperature using a Noralco digital thermometer. Results reported are average of mean body temperature (C°) (MBT) $\pm$ S.E.M. between 0.5 and 3 hours. In the DDT treated rats, MBT increased to 39.0 $\pm$ 0.11 vs. control 37.77 $\pm$ 0.22 ($p<.05$). When DDT treated rats received i.c.v. glycine, MBT decreased to 35.27 $\pm$ 0.16 vs. 38.36 $\pm$ 0.16 ($p<.05$) in DDT controls, which received iso-osmolar and pH corrected saline. DDT rats treated with milacemide 200 mg/kg or 400 mg/kg i.p. showed a decrease of MBT to 37.38 $\pm$ 0.11 and 36.31 $\pm$ 0.08, respectively, vs. DDT rats of 39 $\pm$ 0.16 ($p<.05$). In non-DDT rats i.c.v. glycine decreased MBT to 34.72 $\pm$ 0.27 vs. control 37.72 $\pm$ 0.16 ($p<.05$) Milacemide 200 mg/kg and 400 mg/kg showed a dose dependent decrease in MBT in naive rats. We conclude that glycine and milacemide have hypothermic effects and its action on the DDT-induced hyperthermia may be non-specific.

203.16 FEVER IN GUINEA PIGS WITH KNIFE CUTS IN THE BRAINSTEM. C.M. Blatteis. Dept. of Physiol. Biophys., Univ. of Tenn. Coll. of Med., Memphis, TN 38163.

Although the preoptic-anterior hypothalamus (POA) is thought to be the main site of the febrigenic action of interleukin-1 (IL1), several studies have shown that fever can develop after destruction of this region and, indeed, extra-POA sites responsive to IL1 have been localized in the lateral hypothalamic area (LHA), pons (PON) and medulla oblongata (MO) of guinea pigs. These experiments were undertaken to examine the possible interactions among these secondary sites by disconnecting them by microsurgery, then injecting IL1 either i.v. or directly into these sites. When wide bilateral coronal microcuts were made between the POA and LHA, no fever developed after IL1 was microinjected into the LHA either above or below the transections; however, IL1 injected i.v. induced significant fever. Similarly, microinjections of IL1 into the PON after coronal separation of the midbrain reticular formation from the brainstem, both above and below the cuts, also did not evoke pyrogenic responses; yet fevers were produced by i.v. injections of IL1. By contrast, sharp fevers of similar magnitudes were elicited when IL1 was microinjected into the MO of these operated animals or when administered i.v. It is concluded that, although in intact guinea pigs locally applied IL1 evokes fever not only from the POA, but also from the LHA, PON and MO, only the medullary site appears capable of activating fever independently of connections with the POA. It may be, therefore, a true subsidiary, autonomous controller of fever, activated particularly when the POA is impaired. (Supported by NIH grant NS-14929.)

203.17 THERMOSENSITIVITY OF PREOPTIC NEURONS *IN VITRO* IS INDEPENDENT OF TEMPERATURE. D.S.F. Ling*, N.L. Geller*#, H.M. Geller. (SPON: G.S.F. Ling). Depts. of Pharmacology and Biomedical Engineering, UMDNJ-Robert Wood Johnson Medical School and Rutgers University, Piscataway, N.J. 08854; #Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

The preoptic anterior hypothalamus (POAH) is a region of the brain considered important in thermoregulatory function. Many neurons in this region are thermosensitive, responding to alterations in environmental temperature (T) with a change in action potential generation. In this study, we examined the effects of thermal stimulation on neuronal firing rate (FR). We compared the effects of stimulation over a wide range of temperatures (25 - 45°C) to the effects of stimulation over more physiological temperatures (37-39°C). This was done to test the hypothesis that POAH neurons are thermosensitive over the physiological range of temperatures.

POAH tissue explant cultures were prepared from neonatal Sprague-Dawley rats and incubated for 2 to 4 weeks prior to extracellular recording. Cultures were placed in a 0.5 ml recording chamber and perfused with a heated, balanced salt solution at 1 ml/min. As bath temperature was varied, the temperature and interspike interval (msec) were recorded on-line by a Data General Eclipse S/140 minicomputer.

Using the statistical technique of simple linear regression, the relationship between neuronal firing rate and temperature was examined by both linear and semi-log models. Two measures of thermosensitivity were thus used: the thermal response coefficient (TRC, slope of the regression line of FR on T) and Q_{10} (inverse log of 10 times the slope of the regression line of the log of FR on T). Three groups of neurons were found. Those with TRC > 0.6 Hz/°C or Q_{10} > 2 were considered warm-sensitive (WS). Those with TRC < -0.6 Hz/°C or Q_{10} < 0.5 were classified as cold-sensitive (CS). All others were considered temperature insensitive (TI).

Records from 43 neurons in 14 cultures were analyzed. When the analysis was performed over the wide temperature range, 10 neurons were found to be WS by both TRC and Q_{10} criteria. 2 neurons were classified as CS. The remaining 31 neurons fit the criteria of TI. When the analysis was confined to a narrow (and more physiological) range of temperatures, 7 neurons exhibited WS characteristics as determined by TRC and 6 were found to be WS by Q_{10}. Only 1 neuron was classified as CS by TRC and Q_{10}. 36 neurons were found to be TI by TRC criterion and 35 by Q_{10}.

In conclusion, this comparison of correlated data appears to show that the thermosensitive characteristics of POAH neurons does not change significantly between narrow and wide ranges of temperatures. This suggests that the temperature sensitivity of preoptic area neurons is independent of temperature and that they may act as thermosensors over the physiological range.

203.18 REVERSIBLE INHIBITION OF THE NASOPHARYNGEAL REFLEX IN THE MUSKRAT. W.M. Panneton. Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Stimulation of nasopharyngeal areas in mammals causes an abrupt apnea and bradycardia, but little is known of the brainstem circuits mediating these responses. The fact that the nasal cavity and upper pharynx is innervated by branches of the trigeminal nerve suggests that neurons located within the trigeminal sensory complex are obligate links for relaying the stimulus to respiratory and cardiac neurons. The purpose of the present study was to locate the trigeminal region which mediates the nasopharyngeal reflex.

The common muskrat, Ondatra zibethicus, displays dramatic cardiorespiratory adjustments to nasal stimulation and was used for these experiments. Muskrats were anesthetized with either urethrane or alpha chloralose, or a mixture of them, and their tracheas transected below the larynx. The distal tracheal stump was intubated while a cannula was passed rostrally through the proximal trachea to the posterior nasal cavity. The animal was secured in a stereotaxic unit and the caudal medulla exposed. Both heart rate and respirations were monitored via subcutaneous electrodes and recorded on a physiograph. Ammonia vapors were used as the stimulus; the vapors were pulled with gentle suction through the nasal cavity from a cotton ball soaked in a solution of ammonium hydroxide and held in front of the nose. A 2% solution of lidocaine hydrochloride was injected bilaterally into the rostral spinal and medullary dorsal horns through a micropipette. Both the apnea and bradycardia normally induced by ammonia stimulation were either partially or totally blocked for 20-30 minutes by lidocaine injections into middle and rostral parts of the medullary dorsal horn but were uneffected by injections into the rostral cervical spinal cord and spinomedullary junction. Injection sites were verified histologically.

In those trials where the responses were blocked successfully, intravenous injections of 10-20µg of phenylephrine were administered to induce the baroreceptor reflex to insure that the inhibition was at the level of the trigeminal neurons rather than nearby cardiac or respiratory neurons or the fibers of the vagus nerve running through the spinal trigeminal nucleus.

These results indicate neurons in the medullary dorsal horns as mediators of autonomic reflex behaviors induced by nasal stimulation.

203.19 SOMAN-INDUCED CHANGES IN MEDULLARY RESPIRATORY-RELATED UNIT DISCHARGES AND DIAPHRAGMATIC EMG IN AWAKE, BEHAVING GUINEA PIGS. F.-C.T. Chang, R.E. Foster, T.J. Kerns, E.T. Beers, D.L. Rickett, and M.G. Filbert. U.S. Army Medical Research Institute of Chemical Defense. Aberdeen Proving Ground, Maryland 21010-5425

Knowledge of the relative contributions of CNS, peripheral and other actions to soman-induced respiratory depression is essential for development of medical countermeasures to its toxicity. Definitive data are not available since findings from earlier research either lacked sufficient information or were confounded by the presence of anesthetics. To elucidate these mechanisms, we developed a model system that permits electrophysiological studies of soman-induced respiratory failure in awake, behaving animals.

Guinea pigs were chronically instrumented under barbiturate anesthesia so as to allow concurrent monitoring of medullary respiratory-related unit (RRU) activity, diaphragmatic electromyogram (DEMG), and heart rate. At least seven days post-surgery, and following control period recording, bolus doses of soman were administered (15 ug/Kg, s.c.) at 10-min. intervals until all signs of respiration had ceased.

A hyperpneic profile consistently appeared as the first sign of soman-induced respiratory perturbation. Electrophysiologically, this profile was characterized by augmenting RRU burst frequency, decreasing interspike interval, and brief but increasingly robust DEMG oscillations. As the dose was increased, the hyperpneic profile began to show: a) progressively degenerative RRU-DEMG phase synchrony (phase shift and phase reversal); b) increasingly variable RRU discharge duration and periodicity; c) high within-burst RRU spike frequency; d) increase in background medullary neuronal activities; and e) diaphragmatic muscle fasciculations. Shortly before respiratory arrest, RRU and DEMG cycle frequencies showed a dramatic decrease. Seconds before respiratory arrest, RRU's and background medullary neurons invariably began to engage in erratic discharges. Ineffective respiratory attempts were always observed after respiratory failure. They occurred, however, between long intervals (tens of seconds), and were considered functionally and physiologically inconsequential. It is noteworthy that with doses of soman sufficiently high to produce respiratory arrest, diaphragm muscles were still responsive to occasional centrally mediated respiratory drive.

Our findings show that many, but not all, electrophysiological events associated with soman-induced respiratory failure in awake animals are qualitatively similar to those seen in anesthetized preparations. The effects of anesthesia on various aspects of toxic responses, relative central versus peripheral actions, and other pathophysiological changes will be discussed.

203.20 VENOMOTION IN MAN DISPLAYED BY A NON-INVASIVE METHOD. J. García Ramos. Lab. of Neurophysiology, Escuela de Medicina. U. A. Q. Querétaro, Qro. MEXICO.

Veins possess smooth muscle with sympathetic innervation. Mechano-receptors in their walls play a role in reflex venomotion. Carotid sinus reflexes are accompanied by venoconstriction. Venoconstriction by cold or injury are well recognized. More subtle reflex activity can be displayed by a non-invasive method employing one or more photoelectric transducers. The method was used for the superficial veins of the dorsum of the hand or on the forearm in twenty volunteers. Venomotion was followed at rest during normal respiration and after several experimental maneuvers. Venous caliber showed spontaneous fast rythmic oscillations and slower and prolonged reductions after an amotional situation even a very light one. Venous distension by occlusion of its central end, or by a sudden change in its position from the horizontal were followed by an active and prolonged venoconstriction. Normal respiration, but particularly a deep inspiration, the pasive raising of the arms above zero venous pressure, a small muscular effort, or the Valsalva maneuver elicited also venoconstrictions that appear locally and may produce distension of more peripheral portions which in turn provokes a second constriction of these parts. Venous pulsations may occur during an active and prolonged constriction. Nicotine administered by tobacco smoking produced a clear vanoconstriction which may not have the same temporal course of the arteriolar vasoconstriction followed by the simultaneous d.c. recording of the capillary pulse at a finger. These observations show that peripheral veins in humans present tonic and phasic contractions in responses of locally elicited reflexes and also from central influences. These responses occur in many physiological conditions. The mechanisms involved appear to be very sensitive. Venoconstriction and arteriolar constriction not necessarily occur simultaneously and with the same degree.

REGULATION OF AUTONOMIC FUNCTION IV

204.1 CORRELATION BETWEEN BLADDER ACTIVITY AND NEURONAL FIRING IN THE PONTINE MICTURITION CENTER OF THE CAT. H. Noto,* J.R. Roppolo, and W. C. de Groat (Spon: J. Krier). Dept. of Pharmacology, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

A variety of studies suggest that a group of neurons located in the dorsolateral pontine tegmentum are essential for reflex micturition. This area has been called the "pontine micturition center" (PMC). The present study examined the response characteristics of individual neurons in the PMC to a variety of stimuli, including: rhythmic reflex bladder contractions, pelvic nerve afferent (PN) stimulation, and somatic afferent stimulation (cutaneous, joint).

Precollicular decerebrate cats were used in these studies. Bladder pressure was recorded via a urethral catheter. Single neuron activity was recorded with either NaCl micropipettes or with carbon fiber microelectrodes.

A total of 243 single neurons were recorded in 8 animals at stereotaxic coordinates: AP -1.5 to -3.0, ML 2 to 3, H +1 to -4. Of these 168 neurons were isolated for sufficient time to be studied for at least three bladder contractions; these neurons are reported here. Four basic patterns of activity were seen: (1) Direct neurons (DIR) (20% of our sample), which increased their firing frequency (5-30 Hz) just prior to or at the onset of a bladder contraction, maintained their firing throughout most of the contraction, and then decreased their firing rate as the bladder began to relax. (2) Inverse neurons (INV) (50%), which were active (5-30 Hz) during bladder relaxation and were inhibited during bladder contraction. (3) On-off neurons (5%), which increased their firing frequency at the onset of a bladder contraction and at the onset of bladder relaxation. (4) Independent neurons (25%), which had no relationship to bladder activity. Within each group of neurons various subtypes were seen. For example, some DIR neurons had background spontaneous activity while others had none. Likewise the activity of some INV neurons were completely inhibited during a bladder contraction while others had only a reduction in firing frequency. Neurons encountered dorsal to the PMC did not exhibit firing correlated with bladder activity.

Electrical stimulation (2-10 V, 0.05 msec, 20 msec trains at 100 Hz) of pelvic nerve afferents (PN) excited some DIR neurons at a latency of 30 to 60 msec, while PN stimulation inhibited INV neurons at a latency of 20-150 msec. Somatic afferent stimulation activated some of the DIR (15%) and INV (30%) neurons.

These studies indicate that several populations of neurons in the PMC are involved in the regulation of the urinary bladder. Since some PMC neurons receive PN afferent input and fire prior to bladder contractions it is possible that these cells are involved in the initiation of micturition.

204.2 DOPAMINERGIC AND CHOLINERGIC MODULATION OF BLADDER REFLEXES AT THE LEVEL OF THE PONTINE MICTURITION CENTER IN THE CAT. J.R. Roppolo, H. Noto*, B. Mallory and W.C. de Groat. Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Pharmacological studies using intravenous administration of drugs suggest that dopaminergic and cholinergic mechanisms may be important in modulating bladder reflexes, at sites within the CNS. Histochemical and receptor binding studies are consistent with the view that an area of the rostral pons known as the "pontine micturition center" (PMC) may be one site for these pharmacological effects. Thus the present study was undertaken to examine the effects of dopaminergic and cholinergic agonists and antagonists injected directly into the PMC.

In precollicular decerebrate cats voiding cystometrograms (CMG) and isovolumetric rhythmic bladder contractions were recorded via a catheter in the dome of the bladder. Electromyograms (EMG) were recorded from the external uretheral sphincter (EUS). The PMC was identified in each animal by: (1) stereotaxic coordinates (AP = -1.5 to -3, ML = 2 to 3, H = +1 to -3), (2) electrical stimulation (2-10 V, 0.5 msec. 300 msec train, at 50 Hz) which produced short latency ($<$2 sec) voiding, and (3) chemical stimulation using excitatory amino acid L-glutamic acid (100 mM, 0.2 to 0.4 ul). The site which gave the best voiding response to L-glutamic acid was then used for further experiments.

Dopamine (DA) 100 mM (0.1-0.8 ul) injected unilaterally into PMC reduced the capacity of the bladder 10-60% as measured by CMG and increased the frequency of rhythmic contractions recorded at constant volume. Apomorphine (APO) 50mM (0.1-0.6 ul), a DA agonist, elicited a similar reduction in capacity. The effects of both DA and APO could be partially reversed by haloperidol (HAL) 13 mM (0.5-1 ul) given into the PMC or given I.V. (0.5-1 mg/kg). Pretreatment with HAL either I.V. or into PMC prevented the effects of DA or APO. HAL also elicited a small (10-20%) increase in bladder capacity. DA, HAL, or APO had little effect on EUS activity.

Carbachol (CARB) 10 and 100 mM (0.05 to .4 ul), a muscarinic cholinergic agonist, increased the capacity of the bladder (10-150%), inhibited isovolumetric bladder contractions and reduced EUS activity. Atropine (ATRP) 100 mM (0.4 to 1 ul) a muscarinic antagonist, partially reversed the effects of CARB. Pretreatment with ATRP into the PMC prevented all the effects of CARB, while ATRP alone caused only a small (15%) decrease in capacity.

These data suggest that dopaminergic and cholinergic mechanisms in the pontine micturition center may be important in modulating reflex bladder activity.

204.3 PONTINE MICTURITION CENTER IN RAT REVEALED BY RETROGRADE TRANSPORT OF RHODAMINE-LABELLED BEADS INJECTED INTO THE SACRAL INTERMEDIOLATERAL COLUMN. Irving Nadelhaft and Cecelia Devenyi*. VA Med Cent. and Univ. of Pittsburgh Med. Sch., Depts. of Neurological Surgery and Pharmacology, Pittsburgh, PA.

About 30 nl of rhodamine-labelled latex microspheres were injected into the intermediolateral column (IML) in the L6-S1 spinal cord. One week later, the animal was sacrificed and perfused with 4% paraformaldehyde. The brain was frozen-sectioned into 30 micron sections which were treated in one of three ways: mounted for viewing with the fluorescent microscope, stained with neutral red, or reacted with an antiserum to dopamine beta hydroxylase (DBH) to reveal noradrenergic neurons.

At the injection site (L6 segment) rhodamine label was concentrated mainly in a small area about 300 microns in diameter covering the IML and extended almost 1 mm in the rostrocaudal direction.

In the brainstem DBH-labelled cells of the locus coeruleus (LC) were observed along the lateral floor of the fourth ventricle for a rostrocaudal distance of about 2 mm. A small nucleus of rhodamine-labelled neurons was located slightly medial and ventral to the neurons of the LC. In transverse sections the center of this nucleus was situated about 1 mm lateral to the midline and at a depth of about 250 microns below the surface of the fourth ventricle. The nucleus was round and had a diameter of about 150 microns. Consistent with previous reports (Satoh et al.(1978) Neurosci. Lett. 8:9-15) we identify this nucleus as the pontine micturition center (PMC). Following a unilateral injection into the sacral IML, PMC neurons were labelled bilaterally in approximately equal numbers. There were about 350 neurons per side. PMC neurons extended over a distance of about 1 mm and were located in the middle of the rostrocaudal extent of the LC. The PMC neurons were small and round in transverse sections. A measurement of a sample of 50 PMC neurons resulted in an average diameter of 13.9 +/- 3.2 microns.

204.4 URETHRAL AND PELVIC FLOOR FUNCTION IN THE CHRONIC SPINAL DOG. James S. Walter, John S. Wheeler, Jr*., Charles J. Robinson, Robert D. Wurster. Rehabilitation R&D Center, Hines V.A. Hospital, Hines IL 60141, and Dept. of Urology and Physiology, Loyola Medical Center, Maywood, IL. 60153.

Eight spinal dogs (complete T8-9 transection) were urologically managed once a day for 8 weeks with sacral stimulation using either surface electrodes over sacral foramina or epidural electrodes implanted adjacent to sacral nerves. This study was in accord with NIH and AAALAC guidelines for the humane care and use of laboratory animals and each dog remained in excellent health throughout the experiment. Post-stimulus voiding was obtained using 10 to 35 pps applied for 1 to 3 sec. Currents ranged from 1 to 5 ma for implanted electrodes and 20 to 40 ma for surface electrodes. At 4 weeks post-injury (i.e., after resolution of transient hyporeflexia) voiding of 10 to 60 ml occurred following each stimulation period.

Since one factor that influenced voiding with sacral stimulation was urethral resistance, we evaluated urethral responses by EMG, cystoflouroscopy and cystometry. Contrast medium (150 ml) was placed in the bladder and bladder pressure was recorded with a small urethral catheter. Bladder pressure rose to 40-60 mmHg during stimulation. Voiding started immediately, or within 2 sec after the end of stimulation, but before voiding commenced there was a large amount of pelvic foor EMG activity, and the contrast medium filled the prostatic urethra but not the sphincteric urethra. Voiding commenced with the opening of the sphincteric urethra distal adjacent to the prostate, and voiding continued for 5 to 15 sec as bladder pressure slowly declined. The stream of urine was pulsatile with 2 to 4 squirts per sec. Correlated with these pulses was a spiking pelvic floor EMG and an opening and closing of the sphincter that set up a peristaltic movement down the urethra, in the area from 1 to 5 cm distal to the prostate just prior to the level of the pelvic floor; (flouroscopic and post-mortum measurements). The pulsatile rate significantly increased ($P<0.05$) during voiding from 2.5 ± 0.4 during the first two sec to 3.8 ± 0.2 during the last two seconds of voiding. A strong urethral closure and high voltage EMG was seen at the end of voiding. Two urethral flexures were noted, one just distal to the prostate and a second at the entrance to the pelvic floor.

These observations indicate important aspects of lower urinary tract physiology: 1) In addition to the continence function of the sphincter urethra, the peristaltic urethral activity suggests a second role promoting urine flow, 2) The sphincter urethra activity above the pelvic floor indicates that intrinsic urethral muscle (rhabdosphincter) and not the pelvic floor is necessary for sphinteric activity, 3) Flexures probably allow for urethral length adjustments, 4) Pulsatile urine flow was seen in the spinal intact dog, and in the early and late spinal dog, indicating a spinal organization of this urethral reflex, 5) The independent activity of the rhabdosphincter and pelvic floor can expain why the spinal dog may not be a good model for the vesical-sphincter dyssynergia that is seen in spinal cord injured (SCI) humans. However, pulsatile urine flow that is seen in SCI humans may involve in part some of the mechanisms described here. Supported by funds from Hines Veterans Administration Hospital, Rehabilitation R&D Center, and V.A. Rehab. R&D Grant 8394-A.

204.5 SEROTONERGIC- AND NORADRENERGIC-LIKE IMMUNOREACTIVE TERMINALS IN THE THORACIC INTERMEDIATE GREY MATTER OF THE RABBIT PROJECT TO RETROGRADELY IDENTIFIED SYMPATHETIC PREGANGLIONIC NEURONS. P.L. Vera, D.R. Liskowsky, P.M. McCabe & N. Schneiderman. University of Miami, Psychology Dept., P.O.B. 248185, Coral Gables, Fl. 33124.

Serotonin (5-HT) and noradrenaline (NA) have been localized in the intermediate grey matter of the spinal cord of several species and have been implicated in the regulation of sympathetic preganglionic neuron (SPNs) activity. Sympathoadrenal preganglionic neurons receive 5-HT terminals in the rat (Holets & Elde, 1982, Neurosci., 7:1155) and tyrosine hydroxylase-like terminals in the rabbit (Blessing et al,1987,JANS, 18:121). In the present study we examined the distribution of these two transmitters in the thoracic spinal cord of the rabbit and their relationship with retrogradely identified SPNs projecting to a different autonomic target.

All rabbits were perfused with 4% paraformaldehyde in phosphate buffer (0.1 M, pH=7.4) at 4^{o} C. Serial horizontal sections were taken of segments T1- T12 of 2 rabbits and alternate segments were exposed to antisera against 5-HT (1:5000; 48 hr) or DBH (1:800; 5 d.) at 4^{o} C. The tissue was processed according to the avidin-biotin procedure using DAB as a chromagen. In an additional 4 rabbits, both cervical sympathetic trunks were exposed to horseradish peroxidase 72 hrs prior to perfusion. The retrogradely labeled SPNs were visualized using DAB with the nickel intensification procedure in serial horizontal sections (20 um). T2-T5 segments were then processed for 5-HT (n=2) or DBH (n=2) immunoreactivity, since these segments contain most SPNs projecting to the superior cervical ganglion .

5-HT- and DBH-like immunoreactivity was observed at all levels of the thoracic cord studied. Longitudinal fibers were present in the lateral funiculus, very close to the surface. In addition, fibers were also seen in the lateral funiculus perpendicular to the horizontal axis and oriented towards the intermediate grey matter. A very heavy accumulation of fibers and terminals was observed in the intermediolateral nucleus (IML) in the upper thoracic levels (T2-T5), in agreement with findings in the cat (Krukoff et al, 1985,JCN,240:103). This innervation appeared continuous at these levels and contrasted with lower thoracic levels where the termination pattern in IML was patchy. 5-HT- and DBH-like immunoreactive terminals were observed in close apposition to both retrogradely and non-retrogradely labeled SPNs in the IML. The intermediolateral fasciculus did not contain immunoreactive fibers. Immunoreactive fibers were also observed in the intercalated nucleus at all levels, and they appeared to contact retrogradely labeled SPNs present in that area .Transverse bundles of fibers extending from IML towards the intercalated nucleus pars paraependymalis (ICpe) were observed starting at the level of T2, and were most prominent at the level of T5-T9. A longitudinal bundle of fibers was observed in the ICpe region, lateral to the central canal at all levels of the cord. In addition, fibers were observed crossing into the contralateral side via the dorsal grey commissure, and were also present in the ventral aspect of lamina X.The present findings confirm a serotonergic and catecholaminergic innervation of SPNs. In addition, the greater innervation of IML by these two transmitters at the upper thoracic levels suggests a rostro-caudal differentiation, in which 5-HT and NA might play a greater functional role in regulating the activity of rostrally located SPNs (projecting to the superior cervical and stellate ganglion) than more caudally located SPNs projecting to the adrenal gland. Supported by NIH HL0742608 & HL36588.

204.6 DORSOLATERAL PATHWAYS IN CERVICAL SPINAL CORD MAY TONICALLY INHIBIT ADRENAL SECRETION OF CATECHOLAMINES D.A.Bereiter, A. Benetti*, and D.S. Gann. Depts Surg. & Neuroscience, Brown Univ., Providence, R.I. 02902.

Tonic sympathetic nervous system (SNS) activity depends on supraspinal descending inputs that in turn, differentially influence various sympathetic efferent nerves. Although electrophysiological methods have been used extensively to estimate the relative contributions of different spinal cord pathways on SNS activity, few studies have directly assessed the acute influence of spinal pathways on adrenal secretion of catecholamines. The purpose of this study was to determine the early effects of dorsolateral (DL) cervical spinal cuts at C1 made ipsilateral or contralateral to the adrenal sample on the secretion of catecholamines in the anesthetized cat.

Adult cats were anesthetized with alpha-chloralose (75mg/kg, iv), paralyzed with gallamine triethiodide (5mg/kg, iv), and respired artificially. Blood pressure, heart rate and expiratory CO_2 were monitored. Catheters were placed in the cephalic vein (drugs), femoral vein (peripheral blood samples), and thoracic aorta (blood pressure). Selective sampling of adrenal venous blood was obtained via a catheter placed in the left lumboadrenal vein (Bereiter et al, 1986) and timed sampling permitted estimates of adrenal blood flow to be made. Adrenal venous plasma catecholamines were determined by HPLC with EC after extraction. Peripheral plasma ACTH was determined by RIA. Cats were placed in a stereotaxic frame and high cervical (C1) DL quadrant cuts were made ipsilateral or contralateral to the adrenal vein sample with a scalpel blade. Blood samples were obtained prior to transection and at 1, 5, and 60 min postcut.

Ipsilateral DL cuts caused a prompt increase in secretion of epinephrine by 1 min that remained elevated at 60 min ($+161\pm55$ ng/min, n=4). In contrast, contralateral DL cuts had no significant effect on secretion of epinephrine by 60 min ($+2.7\pm1.1$ ng/min, n=4). Secretion of norepinephrine increased after ipsilateral cuts by 60 min ($+33.8\pm20$ ng/min, $P<.05$), but not after contralateral cuts ($+5.7\pm3.4$ ng/min). The E/NE secretory ratio was increased after ipsilateral cuts (precut= 2.4 vs postcut= 7.8, $P<.001$), but was not affected by contralateral cuts (precut=1.5 vs postcut=1.2). Adrenal blood flow was not influenced consistently by ipsi- or contralateral cuts. Cuts of either side caused similar transient increases in blood pressure and heart rate that returned to precut levels after 5 min. Plasma ACTH was not affected by DL cuts at 60 min. To assess the effect of spinal cuts on brain stem-evoked adrenal secretion, microinjections of substance P (30 ng) were made into lamina I-II of trigeminal nucleus caudalis and preliminary results indicate increased secretion of epinephrine in response to substance P before and after ipsilateral DL cuts.

These results suggest that descending pathways in the ipsilateral DL cervical cord tonically inhibit the adrenal secretion of catecholamines. Phasic adrenal secretory responses evoked by n. caudalis are not blocked by such cuts. The apparent release from inhibition by ipsilateral cuts preferentially increases the secretion of epinephrine over that of norepinephrine and does not affect adrenal blood flow. These data further indicate the non-uniform influence of supraspinal pathways on autonomic function. Supported by NIH grants AM-26831 and GM-27946.

204.7 INTERACTION OF ALPHA$_1$- AND ALPHA$_2$-ADRENOCEPTOR ANTAGONISTS ON A CNS AUTONOMIC REFLEX. J.A. Hey, T. Ito* and M.C. Koss. Departments of Pharmacology and of Ophthalmology and Dean A. McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Reflex pupillary dilation (parasympatho-inhibition) was elicited by electrical stimulation of the afferent sciatic nerve (ScN) or by stimulation of the posterior hypothalamus in pentobarbital anesthetized cats. Inhibition of oculomotor tone due to ScN stimulation is mediated by a monoamine (perhaps NE) whereas that following hypothalamic activation utilizes a non-monoaminergic inhibitory neurotransmitter (Koss et al., J. Auton. Nerv. Syst. 10:55-68, 1984). In the present experiments, intravenous administration of the alpha$_2$-adrenoceptor blocker idazoxan (30-300 µg/kg) blocked ScN-induced mydriasis in a dose-related fashion. In contrast, the selective alpha$_1$-adrenoceptor antagonist prazosin (3-300 µg/kg, i.v.) produced a dose-dependent enhancement of the reflex pupillary dilation. Idazoxan produced about 70% inhibition, whereas prazosin caused about 50% potentation of the reflex mydriasis. Neither antagonist significantly altered the mydriasis elicited by stimulation of the posterior hypothalamus. A CNS site of action of these drugs was confirmed by direct recordings made from the short ciliary nerves.

To further delineate possible modes of interaction between these alpha-adrenoceptor antagonists on this system, prazosin and idazoxan were administered concurrently with the effect on ScN mydriasis observed. Idazoxan (30-300 µg/kg, i.v.) given after prazosin (1 mg/kg, i.v.) still reversed the pupillary dilation in a dose-dependent manner. Conversely, prazosin (3-1000 µg/kg, i.v.) given after pretreatment with idazoxan (300 µg/kg) continued to potentiate the reflex parasympatho-inhibition.

These results support earlier studies which indicate that activation of an ascending inhibitory mechanism involves alpha$_2$-adrenergic receptors in that ScN-induced mydriasis is blocked by alpha$_2$-adrenoceptor antagonists. The potentiation of this reflex parasympatho-inhibition by alpha$_1$-adrenoceptor antagonists, even in idazoxan pretreated cats, suggests that alpha$_1$-adrenoceptors either 1) directly stimulate oculomotor tone or 2) provide a brake on the alpha$_2$-adrenoceptor inhibitory input. We hypothesize that the potentiating effect of alpha$_1$-adrenoceptor antagonists is mediated primarily by a facilitation of ascending inhibitory tone. (Supported by Research to Prevent Blindness and Grant # RII-8610676 from the National Science Foundation)

204.8 BOMBESIN CENTRAL NERVOUS SYSTEM SITE OF ACTION TO INCREASE PLASMA CONCENTRATIONS OF EPINEPHRINE. K. Carver* and M.R. Brown (SPON: P. Plotsky). Autonomic Physiology Laboratory, UCSD Medical Center, San Diego, CA 92103.

Bombesin administered into the lateral cerebroventricle (icv) has previously been demonstrated to increase plasma concentrations of epinephrine greater than norepinephrine. The current study has been performed to identify the central nervous system site(s) of action of bombesin that mediate changes of adrenal epinephrine secretion. Experiments have been performed in awake, male Sprague-Dawley rats equipped with chronic icv and right atrial catheters. Microinjections of bombesin (10-100 ng/200 nl) into brain parenchymal sites were made through 28 ga cannulae. Cannulae placements were verified histologically. The effects of injections of bombesin into selected brain regions on plasma epinephrine levels are shown in the following table:

	3V	AHy	Ce	DPB	PnC	Amb	NTS
Treatment	Plasma Concentrations of Epinephrine pg/ml						
Control	60±14	52±16	44±8	50±17	90±14	64±2	52±14
BOM, 1ng	--	--	--	--	--	--	163±63
BOM, 10ng	--	--	--	--	--	--	619±225
BOM, 50ng	675±26	144±22	44±10	156±12	1036±620	1936±448	3710±686

3V=Third Cerebroventricle; AHy=Anterior Hypothalamus; Ce=Central Amygdaloid Nucleus; DPB=Dorsal Parabrachial Nucleus, PnC=Pontine Reticular Nucleus, caudal part; Amb=Nucleus Ambiguus, NTS=Nucleus of the Solitary Tract (AP -2.3).

To further define the site of action of bombesin within the area of the NTS, the medulla at AP -2.3 and -1.3 (interaural) was divided into quadrants. Bombesin (10 ng) was then injected into one of these areas or into the 4th ventricle. The medial quadrant at AP -2.3 was the most responsive site. Parenchymal injections made at AP -1.3 or the 4th ventricle at -2.3 or -1.3 resulted in minimal elevations of plasma epinephrine levels.

Previous studies have demonstrated somatostatin-28 to decrease bombesin-induced elevation of plasma concentrations of epinephrine. Co-injection of SS-28 (300 ng) into the NTS attenuated bombesin-induced elevation of plasma epinephrine levels. These studies are consistent with the conclusion that a potent site of action for bombesin exists within or near the rostral NTS. Using the present methodologies, more caudal sites could not be assessed.

204.9 MULTIPLE NEUROPEPTIDES IN VAGAL AND GLOSSOPHARYNGEAL AFFERENT NEURONS IN THE RAT. K.M. Hill* and C.J. Helke (SPON: E.Frey). Dept. of Pharmacol., Uniformed Services Univ. of the Health Sciences, Bethesda, MD. 20814.

Visceral afferent neurons of the vagus and glossopharyngeal nerves transmit sensory information from major organ systems of the thorax and abdomen to the nucleus of the solitary tract. The cell bodies reside in the nodose (NG) and petrosal (PG) ganglia of the vagus and glossopharyngeal nerves, respectively.

The presence and localization of neuropeptides in NG and PG neurons were examined with immunocytochemistry in tissue from 4% paraformaldehyde perfused rats. Calcitonin-gene related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin (SOM), and neurotensin (NT)-immunoreactivities (ir) were studied. With the exception of neurotensin, ir cells in NG and PG were seen with each antiserum. Peptide-ir neurons were scattered throughout the NG; however, the greatest numbers were generally found in the rostral part of the NG. In the NG, the relative number of peptide-ir neurons was SP>CGRP>VIP>>CCK>SOM. CGRP and SP showed very similar distributions with scattered cells in the NG and numerous groups in the PG. VIP-ir neurons were also scattered throughout the NG, but in relatively larger numbers than seen in PG. Whereas numerous SP and CGRP-ir cells were detected in the PG, fewer CCK, VIP and SOM neurons were seen. VIP-ir cells were also found in the glossopharyngeal nerve 1-2mm caudal to the PG. Following intranodose injections of colchicine, accumulations of SP, CGRP, VIP, and CCK-ir were seen in caudal nodose and vagal fibers. SOM-ir fibers were not detected after colchicine and very few SOM-ir cells were seen in NG or PG. To date, double-labeled SP and VIP-ir in the NG have been seen following retrograde transport of Fluoro-Gold from the cut end of the cervical vagus. IR for each peptide was blocked by preabsorption of the antisera with the homologous antigen. In addition, as reported in spinal and trigeminal ganglia (Neurosci. Lett. 68:305, 1986), CCK-ir was preabsorbed with CGRP raising the possibility that NG and PG CCK-ir represents endogenous CGRP.

These data demonstrate the presence of several different peptide-ir neurons in vagal and glossopharyngeal afferents and suggest a multiplicity of visceral sensory neurotransmitters. (Supported by NIH Grant #NS20991.)

204.10 MONOSYNAPTIC CONTACTS WITHIN THE NUCLEUS TRACTUS SOLITARIUS BETWEEN VAGAL AFFERENT AND EFFERENT NEURONS INNERVATING THE STOMACH IN RAT. L. Rinaman* and R.R. Miselis (SPON: P. Hand). Depts. Neurosci. and Animal Biology, Univ. Pennsylvania, Philadelphia, PA 19104.

It has recently been demonstrated that neurons within the dorsal motor nucleus of the vagus (DMV) which innervate the stomach in rat have dendrites which penetrate discrete subregions of the nucleus tractus solitarius (NTS), where they are coextensive with the central terminals of gastric primary sensory neurons (Shapiro and Miselis, 1985, JCN 238: 473). This coextensive distribution provides indirect evidence that monosynaptic contacts between gastric vagal afferent and efferent neurons may occur and thereby provide a substrate for reflex regulation of vagal motor outflow. In the present investigation we have accrued data which provide the first direct ultrastructural evidence that these proposed gastric monosynaptic vagovagal contacts do in fact exist. The DMV and NTS were analyzed by light and transmission electron microscopy three days following injection of 70 ul CT-HRP into the musculature of the stomach of adult male Sprague-Dawley rats. TMB was used as the chromagen to demonstrate CT-HRP labeled neuronal profiles within the DMV/NTS complex at both light and EM levels. Light microscopy confirmed the presence of retrogradely labeled DMV neurons bilaterally whose dendrites extended into dorsomedial subfields of the NTS where they overlapped with labeled gastric afferents; specifically, the subnucleus commissuralis, the subnucleus gelatinosus, and the medial solitary subnucleus. EM examination of these regions revealed discretely labeled dendritic and terminal profiles. Transganglionically labeled gastric afferents routinely formed asymmetric synaptic contacts with the labeled dendrites of backfilled DMV gastric neurons, as well as with unlabeled dendrites. The majority of these synaptic contacts were on distal dendrites and their appendages. Labeled dendrites exhibited characteristic fine structure and also received dense synaptic inputs from unlabeled afferents. Labeled afferents were characterized by a homogeneously dense axoplasm that was filled with large numbers of lucent, spherical vesicles (40 nm) and scattered dense core vesicles (60 nm). Our data demonstrate unequivocally that central monosynaptic connections exist between vagal afferents and efferents whose peripheral axons innervate the stomach in rat. These monosynaptic circuits can provide the bases for a feedback relay of visceral interoceptive information directly to the appropriate visceral effectors. Supported by NIH # GM-27739 to R.R.M., an Office of Naval Research Fellowship to L.R., and E.I. duPont de Nemours and Co., Medical Products Dept.

204.11 ELECTROPHYSIOLOGICAL STUDY OF GASTRIC VAGAL AND SPLANCHNIC INTERACTIONS IN THE BRAINSTEM OF THE CAT. C.S. Yuan*, W.D. Barber and T.F. Burks. (SPON: S. Hsiao). Departments of Anatomy and Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Gastric vagal projections to the dorsomedial region of the caudal brainstem were examined electrophysiologically in cats anesthetized with halothane and nitrous oxide supplemented with oxygen. Single shock stimulations (0.3 msec, 500 uA, 0.5 Hz) were applied to the gastric vagal branches which serve the proximal stomach, and to the left greater splanchnic nerve. To date, single unit activity was recorded extracellularly from 80 neurons in both sides of the caudal brainstem during gastric vagal nerve stimulation. Based upon stereotaxic coordinates and electrophysiological correlates, the results showed that the units responding to gastric nerve stimulation were located in the region of nucleus solitarius, in the same area where responses to gastric distention were previously reported in our laboratory (W.D. Barber and T.F. Burks, 1983). Stimulation of gastric vagal branches and the splanchnic nerve simultaneously resulted in an inhibition in 34 neurons of a total of 210 brainstem unitary discharges activated by gastric vagal stimulation. These results indicate that 16% of the brainstem neurons activated by electrical stimulation of the gastric vagal branches also received splanchnic input which was inhibitory. Further analysis showed that when the splanchnic nerve stimulation was increased to a frequency of 20 Hz, it produced a weak inhibitory effect upon some brainstem neurons. These data provide evidence that gastric vagal and splanchnic interactions occur upon the same cell in the brainstem. Brainstem neurons which shared vagal and splanchnic inputs were always inhibited when electrical stimulation of the splanchnic nerve and gastric vagal branches were paired. These data provide evidence in the cat of brainstem neurons in the region of nucleus solitarius which share gastric vagal and splanchnic input. The differences in quality or precise nature of sensory information conveyed via the splanchnic and gastric vagal fibers is not known at this time. This study adds important information concerning the organization of gastric vagal and splanchnic visceral neuronal interactions at the level of the caudal brainstem. (Supported by USPHS Grant AM 31804).

204.12 **BRAINSTEM SENSORY AND MOTOR PROJECTIONS FROM THE ESOPHAGUS IN THE RAT: VISCERAL TOPOGRAPHY.** **S.M. Altschuler*, X. Bao*, and R.R. Miselis, Departments of Pediatrics and Animal Biology and Institute of Neurological Sciences. University of Pennsylvania, Philadelphia, PA 19104.**

In contrast to the stomach, the medullary organization of afferent and efferent connections from the esophagus has not been extensively studied. Although retrograde labeling of efferent cell bodies has been reported, little information concerning the dendritic architectonics of retrogradely labeled neurons and location of afferent terminal fields is available. We employed the neural tracer cholera toxin-horseradish peroxidase (CT-HRP) to simultaneously determine the afferent and efferent connections of the esophagus within the medulla of the rat. CT-HRP (0.25% - Lowry assay) was injected in either the cervical or subdiaphragmatic esophagus of anesthetized rats. Tracer diffusion from the injection site was limited by isolating the esophagus using sterile saline-soaked gauzes and by diluting the injection site surfaces. Fresh gauzes were left in place, isolating the injected structures during the survival period. Control injections were made in the stomach, larynx, and trachea. After a 72-hour survival the animals were sacrificed for histological analysis using the TMB protocol. Afferent terminal labeling was limited to the medial portion of the nucleus of the tractus solitarius (NTS) at a level just anterior to the area postrema, in an area medial-ventromedial to the tractus solitarius, particularly in the subnucleus centralis (Ross et al., JCN 242:511, 1985). Other areas of the NTS previously shown to receive terminals of gastric vagal afferent fibers (Shapiro and Miselis, JCN 238:473, 1985) contained no labeling. Following both cervical and subdiaphragmatic esophageal injections, retrogradely labeled perikarya were observed in the rostral portion of the nucleus ambiguus (NA). Dendrites of labeled NA neurons were limited to the NA. Subdiaphragmatic injections also resulted in labeling of predominantly the left dorsal motor nucleus (DMN) neurons with dendrites confined to the boundaries of the DMN. Labeled DMN neurons were observed simultaneously with afferent terminal field labeling confined to the subnucleus centralis as opposed to the stomach injections, in which case there was occasional specific caudal NA and DMN labeling and labeling of afferent terminal fields in the subnucleus gelatinosus. Our findings suggest that gastrointestinal afferents project to the medial NTS with a degree of viscerotopic organization. The cervical esophagus receives motor innervation entirely from the rostral NA. The subdiaphragmatic esophagus receives motor innervation from both the rostral NA and left DMN. The innervation by the DMN may provide for coordination of activity of the lower esophageal sphincter and stomach during deglutition. Supported by N.I.H. grants GM27739 and DK01747.

204.13 ALTERATION OF GASTRIC MOTILITY BY PICOMOLE QUANTITIES OF GLUTAMIC ACID AND TRH IN THE DORSAL VAGAL COMPLEX (DVC) IN RATS. L.J. Jakobsen*, H.E. Raybould*, Y. Tache and D. Novin. Center for Ulcer Research and Education, VA Wadsworth Medical Center, Dept of Pyschology and Medicine, Brain Research Institute, University of California, Los Angeles, CA 90024.

Electrical and chemical stimulation of the nucleus of the solitary tract (NTS), where vagal afferents terminate, has demonstrated the importance of this region in control of gastric function. We further evaluated excitatory and inhibitory influences on gastric motility from this region by microinjection of glutamic acid (glu) and TRH. Tonic and phasic changes in intragastric pressure (IGP) were measured in chloralose-urethane anesthetized rats using a catheter placed in the body of the stomach and secured at the pylorus. Glu and TRH (0.1-10 mM) were injected in 1-10 nl volumes by micropressure ejection from glass multibarrelled micropipettes. Gastric responses were monitored while pipettes were advanced from surface to a depth of 1 mm of the dorsomedial medulla in 100 μm increments. Track positions were marked by 2.5% pontamine blue and verified histologically. Injections of glu (1-100 pmol) caused rapid onset, dose dependent decreases in tonic IGP (0.5 - 4 cmH_2O) at 32 of 35 sites within NTS and attenuated phasic contractions. Injection of TRH at these sites (0.5 - 100 pmol) increased tonic IGP by up to 8 cmH_2O and increased amplitude of phasic contractions. Bilateral cervical vagotomy abolished responses to glu and TRH. Glu and TRH were most potent when injected into the NTS but were effective throughout the DVC.

Conclusions: Injection of Glu into DVC produces predominantly inhibitory effects on gastric motility. In contrast, injection of TRH at the same loci increases gastric motility. Subpopulations of neurons in the DVC may therefore be activated by specific neurotransmitters having opposite effects on gastric function.

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204.14 GASTRIC ACID SECRETORY EFFECTS OF INJECTION OF NEUROPEPTIDE Y INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS OF RATS. G.A. Humphreys*, J. Davison* and W.L. Veale. (SPON: K.E. Cooper). Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Injection of neuropeptide Y (NPY) into the paraventricular nucleus of the hypothalamus (PVN) of rats enhances feeding behavior (Stanley, B.G. and Leibowitz, S.F., Proc. Natl. Acad. Sci. 82: 3940, 1985). The PVN has bidirectional neural connections with medullary autonomic areas known to influence gastric acid secretion. Experiments were done to determine if NPY given to the PVN of anaesthetized rats alters the autonomic regulation of a feeding related organ such as the stomach. The response of interdigestive levels of total gastric acid output, systemic arterial blood pressure and gastric mucosal blood flow were measured to injections of NPY (200 pmole) in rats anaesthetized with ketamine. Total acid output was also measured in rats that had received subdiaphragmatic vagotomy. Acid output was measured using a modification of the perfusion method of Ghosh and Schild (Brit. J. Pharmacol. 13: 54, 1958); arterial blood pressure using a femoral artery cannula connected to a pressure transducer and strip chart recorder; and mucosal blood flow by clearance of hydrogen gas. Total acid output dropped by 7.1 ± 1.4 μeq/10 minute interval, 20 minutes following injection of NPY into the PVN ($p < 0.01$ compared to saline injected controls). Arterial blood pressure was elevated by 9.8 ± 4.8 mm Hg only during the period of NPY injection, after which it promptly fell to baseline levels. This elevation was not statistically different from saline injected controls. Preliminary results indicate that gastric mucosal blood flow was not altered by injection of NPY into the PVN ($p=0.78$ paired with saline injected levels). Trunkal vagotomy abolished acid secretory response to PVN injections of NPY. These results indicate that injection of NPY into the PVN of anaesthetized rats decreases the interdigestive levels of total gastric acid output in a vagally dependent manner and that the effect is not dependent on alteration of mucosal blood flow.

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204.15 RECIPROCAL CONNECTIONS BETWEEN THE CENTRAL NUCLEUS OF THE AMYGDALA AND THE MEDULLARY VAGAL COMPLEX: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT.

R.C.Rogers, Department of Physiology and the Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

Reciprocal connections between the CNA and the dorsal vagal complex (DVC - i.e. the solitary and dorsal motor nuclei) have been implied from anatomical studies. These direct amygdalo-autonomic connections may play a role in modulating gastric acid secretion and motility as well as other visceral functions. This study sought to determine the functional parameters and general organization of this CNA-DVC projection.

Our electrophysiological studies on the urethane anesthetized rat verify this conclusion in that microstimulation of the DVC region causes both antidromic and orthodromic activation of neurons in the CNA [antidromic latency = 56 +/- 10 msec (SD), orthodromic latency = 62 +/- 7 msec (SD), conduction velocity ~ 0.2 meters/sec] . The distribution of CNA neurons activated by DVC stimulation suggests that the dorsomedial CNA provides input to the dorsal medulla while the ventrolateral portion of the CNA receives input from the solitary nucleus within the DVC.

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204.16 DORSAL MEDULLARY SOMATOSTATIN CAUSES AN INCREASE IN GASTRIC ACID SECRETION. G.E. Hermann and R.C. Rogers , Department of Physiology and the Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

Immunohistochemical and neurophysiological evidence supports the existence of a somatostatinergic projection from the central nucleus of the amygdala and the dorsal medulla. Since intracerebro-ventricular injections of somatostatin have been shown to increase gastric acid secretion, it seems likely that this CNA-dorsal medullary path may be involved in the parasympathetic control of gastric function. The observation that CNA stimulation usually produces an increase in gastric acid secretion adds further support to this view.

If somatostatin acts on the dorsal motor nucleus of the vagus (DMN) as an excitatory neurotransmitter to regulate gastic function, then the injection of very small amounts of this putative peptide neurotransmitter into the DMN should increase gastric acid secretion.

Urethane anesthetized rats were prepared for perfusion with 0.9% saline at a rate of 2 ml/min. Perfusate was collected and titrated to neutrality with 0.05M NaOH to determine the rate of acid production by the stomach. After mounting the rats in a stereotaxic frame, the dorsal medulla was exposed and a combination stimulation electrode/micropipette placed in the DMN under physiological guidance. Though artificial cerebrospinal fluid injections (2 nl) had no effects, somatostatin (10 picomole in 2nl) increased acid output considerably. Intraperitoneal atropine (1mg/kg) completely suppressed the effects of intra - DMN somatostatin.

These results support the view that somatostatin acts on vagal efferent neurons to control gastric function.

204.17 *IN VITRO* TRACING OF MICROCIRCUITS BY RETROGRADE AND ANTEROGRADE TRANSPORT OF FLUORO-GOLD: PROJECTIONS OF SUBMUCOSAL NEURONS TO THE MYENTERIC PLEXUS OF THE GUINEA PIG SMALL INTESTINE. M. D. Gershon, G.M. Mawe, and A. L. Kirchgessner*. Department of Anatomy and Cell Biology, Columbia Univ., Coll. of P & S, New York, NY 10032.

The enteric nervous system (ENS) is a unique division of the PNS because it can mediate reflex activity without input from the brain or spinal cord. The ENS thus contains intrinsic primary afferent neurons and complete microcircuits that link mucosal sensory receptors with motor neurons in the myenteric plexus. Neither the sensory receptors, nor the primary afferent neurons of these microcircuits have been identified. Pseudounipolar and bipolar neurons (Schofield, 1968), as well as neurons that appear physiologically to receive no synaptic input (Suprenant, 1984), have been described in the submucosal plexus. These cells may be intrinsic primary afferent neurons; however, although the submucosal plexus is known to innervate the intestinal mucosa, where enteric sensory receptors are known to be located, no projections of submucosal neurons to the myenteric plexus have previously been found. In order to determine whether such projections exist, the submucosal plexus was examined following the microinjection of a retrograde tracer into a single ganglion of the myenteric plexus. The mucosa was removed from the guinea pig ileum, the remainder of the wall of the bowel was stretched flat with the serosal surface facing up, and the tissue was superfused in a chamber on the stage of an inverted microscope with nifedipine-containing (1.0μM) Krebs solution at 37°C. Individual myenteric ganglia were visualized using differential interference contrast optics and were injected with the fluorescent tracer Fluoro-Gold (FG; 4%). Injections were made with pressure pulses (100 msec in duration at 8 psi for 1.5 min at a frequency of 1.0 Hz) from the beveled tip (12 μm) of a glass micropipette; 2.5-3.0 hrs were allotted for retrograde transport *in vitro*. Preparations were then fixed with 4 % formaldehyde (from paraformaldehyde), dissected into layers containing the submucosal or myenteric plexuses, and examined as whole mounts by fluorescence microscopy. In the myenteric plexus, the injection sites could readily be visualized and were restricted to a portion of single ganglion. Some spread of FG beyond the confines of the injected ganglion occurred and led to staining of the surrounding connective tissue and smooth muscle. As previously observed following the microinjection of SITS, a small number of the neurons of the injected ganglion were fluorescent and additional neurons in distant ganglia (predominantly oral, but not immediately adjacent to the injection site) were also retrogradely labeled. In the submucosal plexus, scattered neurons deep to, but not directly underneath, the injected myenteric ganglion showed an intense granular cytoplasmic fluorescence indicative of retrograde labeling by FG. About 5-6 submucosal neurons were labeled/myenteric ganglion injected and only rarely was there more than one labeled neuron in a submucosal ganglion. Varicose terminal axons were also fluorescent in the submucosal plexus (not necessarily in ganglia that also contained labeled cell bodies), suggesting that anterograde, as well as retrograde transport, occurred. Controls were done to ensure that labeling of submucosal neurons was due to retrograde transport of FG from myenteric ganglia and not diffusion of the tracer into the submucosal layer. When FG was injected into the adjoining muscle, instead of a ganglion, occasional myenteric neurons in a ganglion near the injection site became labeled; however, there was no labeling of neurons in the submucosal plexus. Similarly, if connections between the myenteric and submucosal plexuses were severed by dissection before injecting FG, no neurons were labeled in the submucosal plexus. It is concluded that submucosal neurons project to the myenteric plexus. These observations are consistent with the hypothesis that intrinsic enteric primary afferent neurons reside in the submucosal plexus. Supported by NIH grants NS 12969, NS 07062, and the PMAF.

204.18 MARKERS SHARED BETWEEN SUBSETS OF DORSAL ROOT GANGLION (DRG) AND ENTERIC NEURONS A. L. Kirchgessner*, J. Dodd, and M. D. Gershon. (SPON: L. Coté). Dept. of Anatomy and Cell Biology, Columbia Univ., Coll. of P & S, New York, NY 10032

Alone among peripheral organs, the gut is able to mediate reflex activity when cut off from the CNS; therefore, the bowel must contain intrinsic primary afferent neurons. These enteric primary afferent cells have not yet been identified. The current study was undertaken to determine whether antigenic determinants or other markers common to primary sensory neurons could be found in enteric neurons. Functional subpopulations of cutaneous sensory neurons of the rat DRG can be identified by their expression of structurally defined lactoseries and globoseries carbohydrate differentiation antigens. Lactoseries antigens have also been found on other primary sensory neurons and sensory transduction cells; therefore, in dissected laminar preparations and cryostat sections of the rat small intestine, the presence of epitopes recognized by mouse monoclonal antibodies (MAbs) against lactoseries (α-SSEA-1, AC4, 1B2/1B7, LD2, LA4, TC6) and globoseries (B2) antigens was studied immunocytochemically. Dual-label immunofluorescence was used on single sections to demonstrate the labeling pattern of rabbit antibodies against VIP, substance P [SP], calcitonin gene related peptide [CGRP], enkephalin [ENK], and the MAbs described above, in order to study the coincident expression of neuropeptides and carbohydrate differentiation antigens. Preparations were used from animals treated 24 hrs previously with colchicine (5mg/kg). Carbonic anhydrase activity, also present in a subset of DRG neurons, was visualized histochemically in similar preparations of gut. Subsets of enteric neurons were labeled by α-SSEA-1, AC4,1B2/1B7, and carbonic anhydrase. α-SSEA-1/AC4$^+$ neurons were found (~1% of the total) in the myenteric plexus. These cells were morphologically classified as Dogiel type 1 (many short clubby dendrites) neurons. α-SSEA-1/AC4 co-localized with ENK, but the correspondence was not 1:1. 1B2/1B7$^+$ neurons were found in both the submucosal (~50% of neurons) and myenteric plexuses (~5% of neurons). All submucosal VIP$^+$, but no other submucosal neurons were marked by 1B2/1B7. In the myenteric plexus 1B2/1B7 was found in ~50% of VIP$^+$ and NPY$^+$ neurons and in additional neurons, the transmitter of which has not yet been identified. Carbonic anhydrase activity was intense in ~50% of the neurons of the submucosal plexus and moderate in a small number of myenteric neurons. A further evaluation of the correspondence between enteric neurons and DRG neurons labeled with antibodies to lactoseries antigens, was to administer capsaicin (50 mg/kg) to newborn rats. Two months later, 1B2/1B7, VIP, and SP immunoreactivities were depleted in DRG and the dorsal horn of the spinal cord. In the bowel of the same animals 1B2/1B7 immunofluorescence was lost from all submucosal and most myenteric neurons, but VIP immunoreactivity was depleted only from submucosal nerve fibers and not cell bodies. Enteric α-SSEA-1 was not affected by capsaicin. It is concluded that subsets of enteric neurons share properties with subsets of DRG neurons (α-SSEA-1$^+$, 1B2/1B7-VIP$^+$ [sensitive to capsaicin], carbonic anhydrase activity). The hypothesis that some of these enteric neurons function as primary afferents remains to be confirmed. Supported by NIH grants NS12969, NS 07062 and the PMAF.

204.19 GALL BLADDER GANGLIA: PROJECTIONS, INPUTS, PUTATIVE TRANSMITTERS, AND ENTERIC PROPERTIES. G.M. Mawe & M.D. Gershon, Dept. of Anatomy and Cell Biology, Columbia University, P&S, New York, NY 10032

The structure and chemistry of the ENS differ from those of the remainder of the PNS. Like the CNS, the ENS is supported by enteric glia and lacks internal collagen and basal laminae. Although the gall bladder originates as an outgrowth of the fetal gut, its innervation differs from that of the bowel in that the gall bladder has one, rather than two ganglionated plexuses. Nevertheless, it is not yet clear whether the intrinsic ganglia of the gall bladder are like those of the ENS or whether, instead, they resemble extraenteric autonomic ganglia. Moreover, the possibility that communication between the gut and the gall bladder may be mediated by nerves, as well as by the endocrine release of hormones, such as cholecystokinin, has not previously been examined. The current study of the guinea pig gall bladder was therefore undertaken to determine whether the ganglia of the gall bladder are part of the ENS. Studies were done inMarkers that were used included the immunocytochemical demonstration of laminin and 5-hydroxytryptamine (5-HT). Laminin is present in the basal lamina that surrounds enteric ganglia, but not in their interior. In contrast, laminin is abundant inside all other peripheral ganglia. 5-HT is found in the cell bodies of neurons of the myenteric, but not in the submucosal plexus of the gut or in extraenteric parasympathetic ganglia. In order to locate gall bladder ganglia, neurons were simultaneously visualized by the immunodetection of microtubule associated protein II (MAP II). Immunoreactivity (IR) was demonstrated using rabbit polyclonal or mouse monoclonal primary antibodies followed by biotinylated species-specific secondary antisera in combination with avidin-Texas Red or species-specific secondary antisera directly coupled to fluorescein isothiocyanate. To determine whether neurons in the duodenum project to the gall bladder, Fluoro Gold (FG) was injected into the wall of the gall bladder and the duodenum was examined after a 48 hour survival period.for the presence of neurons labeled by retrograde transport. MAP II-IR was found in the somata and dendrites of all gall bladder neurons, but not in their axons. The size of the ganglia and the shape of their neurons resembled submucosal, rather than myenteric ganglia. On the other hand, 5-HT-IR was present in the varicosities and somata of some gall bladder neurons, although these neurons represented a relatively small proportion of the neurons of the gall bladder. All gall bladder ganglia, were devoid of internal laminin-IR, whereas intense laminin immunofluorescence was seen in the basal laminae surrounding ganglia, smooth muscle cells, and blood vessels. Other putative transmitters found in gall bladder ganglia by immunofluorescence included, NPY, VIP, substance P, and CGRP. The detection of fibers with tyrosine hydroxylase-IR showed that gall bladder ganglia receive a noradrenergic sympathetic innervation. When FG was injected into the wall of the gall bladder, FG-fluorescence appeared in the perikarya of a subset of myenteric neurons in duodenal ganglia near the sphincter of Oddi. Some varicose FG-fluorescent terminal axons were also seen in these ganglia; thus, the duodenal myenteric plexus appears to be labeled both by retrograde and anterograde transport of FG from the gall bladder. Neurons in the celiac ganglion were also labeled by retrograde transport of FG, and as in the myenteric plexus, FG-fluorescent varicose fibers were observed in the celiac ganglion.

These observations indicate that the intrinsic ganglia of the gall bladder should be considered part of the ENS because they are enteric in structure, contain transmitters characteristic of the ENS, and they receive an input from and project to the duodenal myenteric plexus. Moreover, like the myenteric plexus, the gall bladder ganglia also appear to be innervated by the celiac ganglion and to send fibers centripetally to it. Further studies are needed to determine the role played by gall bladder ganglia in the physiology of the organ as well as to ascertain the functional significance of gut-gall bladder neural communication . Supported by grants NS 12969, NS 07062 and the PMAF.

204.20 SYNAPTIC BEHAVIOR OF MYENTERIC NEURONS IN THE GUINEA-PIG STOMACH IN VITRO. M. Schemann* and J.D. Wood. Department of Physiology, Ohio State University, Columbus , Ohio 43210

Much is known about synaptic behavior of myenteric neurons in the small intestine and less is known in the large bowel. However, there are no reports on synaptic behavior in the myenteric plexus of the stomach. Neurally controlled behavior of effector systems in the stomach differs from behavior in the intestine suggesting that the neurophysiology of the gastric nervous system may also be different. The aim of the present study was to investigate synaptic correlates of neurally controlled gastric behavior. Conventional methods were used for intracellular recordings in 156 neurons from 51 animals. The results revealed that electrical and synaptic behavior of gastric neurons differed significantly from the small intestine. Gastric neurons could be divided into three classes: Type I cells (n=64) were highly excitable, discharged multiple spikes on depolarizing current pulses and had relative high input resistance. Type II cells (n=63) were less excitable, discharged one spike only at the onset of depolarizing current pulses and had relative low input resistance. Type III cells (n=30) were inexcitable to intrasomal injection of depolarizing current pulses and showed the lowest input resistance. All cells showed fast excitatory postsynaptic potentials (fEPSP's) that were evoked by focal electrical stimulation of interganglionic fiber tracts. The mean amplitude of the fEPSP's was 7.9mV in Type III cells, and 15mV and 16mV in Type I and Type II cells, respectively. The mean duration of the fEPSP's was 18.2msec in Type III cells, and 25msec and 22.1msec in Type I and Type II cells, respectively. In Type I cells fEPSP's often reached threshold for spike discharge. Spontaneous fEPSP's occurred in 36% of Type I cells, 27% of Type II cells and 10% of Type III cells. All fEPSP's were suppressed by Hexamethonium (200μM), Tetrodotoxin (0.1μM) and in the presence of elevated Mg^{++} and reduced Ca^{++}. Estimated reversal potential for the fEPSP's was at a membrane potential of -20mV to +10mV. Microapplication of acetylcholine mimicked the fEPSP. During microapplication of acetylcholine the amplitude of fEPSP's was suppressed. Suppression of the fEPSP's was not produced by application of substance P or 5-HT. The fEPSP's followed high stimulus frequencies (>20Hz), without showing the "run-down" phenomenon, and sometimes summated to spike-threshold. Antidromically evoked potentials were recorded in only 7% of the cells. The results suggest that gastric neurons have characteristic electrical and synaptic behavior different from small intestinal neurons. This may be a reflection of the specialized motor and secretory functions of the stomach.
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204.21 **MORPHOLOGY OF THE POSTERIOR VAGAL PLEXUS AND ITS NODULAR PARAGANGLION.** J.C. Prechtl and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue University, West Lafayette, IN 47907

The concept that the posterior celiac and gastric branches of the rat vagus are formed by a simple bifurcation of the posterior vagal trunk is based on dissection studies and is inaccurate. Previously, we reported (Prechtl & Powley, 1985, J. Comp. Neurol., 235: 182) on the number and size of the posterior vagal branch components. In the present whole-mount study, we describe the organization of the branching, the intricate plexus it forms, and its association with an identifiable paraganglion.

Nerve whole-mounts were prepared from 13 adult male Sprague Dawley rats. The whole-mounts were stained with osmium tetroxide vapor, cleared in xylene, and mounted in permount. Four additional specimens were embedded in Epon and serially sectioned at 0.5-9 μm, in order to re-construct the nodular paraganglion and calculate its volume.

The whole-mounts revealed that the celiac branch was comprised of 4.8±0.4 nerve bundles which ramified separately from the posterior trunk within a rostro-caudal interval of 0.3 to 1.1mm. The gastric branch was formed by the division of the residual vagal trunk into 2.8±0.2 bundles at a level usually about 0.3mm distal to the first celiac bundle, but sometimes (23% of cases), at or before the celiac branching. Within 1mm of their branch point, some bundles (celiac or gastric) contributed to a plexus, by forming anastomoses. Included in this plexus of every specimen, were extrinsic (non-vagal) bundles which coursed between, and anastomosed with, distal segments of the two vagal branches. Despite some variations in this posterior vagal plexus, it always contained a large nodular paraganglion (vol.=113±21 X 10^4 μm^3, whole-mount dimensions -- 86 by 171 μm), which was regularly found attached to the side of the posterior vagal trunk opposite the celiac branch point. The paraganglion contained hundreds of glomus cells and 2-7 neuronal somata. Whole-mounts and serial reconstructions showed that it was traversed by 3-6 distinct nerve bundles, some of which could be traced to the posterior vagus. Numerous smaller paraganglia were also present in the branch plexus, but their sizes and locations were less predictable.

Extrinsic fibers have also been found in anterior vagal branches (Prechtl and Powley, 1987, Anat. Embryol., 176), and may represent sympathetic or enteric nerve bundles. The function of abdominal vagal paraganglia remains uncertain. The nodular paraganglion reported in this study is as large as any we have seen in the subdiaphragmatic vagus (see same ref.); because it would be identifiable in vitro, it could be adopted as a model for physiological studies. Supported by NIH Grant DK27627.

205.1 DISTRIBUTION AND POSSIBLE ROLE OF A GALANIN-LIKE PEPTIDE IN MUDPUPPY CARDIAC TISSUE. D.S. Neel* and L.M. Konopka* and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Galanin, a 29 amino acid peptide, initially isolated from pig intestine, has recently been found in many regions within the CNS and PNS. Further, galanin immunoreactivity coexists with many other peptides and transmitter substances. Although previously found in numerous ANS targets, we found no reports of galanin immunoreactivity in cardiac tissue. However, the results of the present study demonstrate the presence of a galanin-like peptide in cells and fibers in the mudpuppy (Necturus maculosus) cardiac septum, which contains the parasympathetic cardiac ganglion. Whole mount septal-sinus venosus preparations and atrial preparations were stained immunohistochemically to determine the distribution of galanin-like peptide. Galanin immunoreactive fibers were found coursing diffusely across the septum and sinus venosus, and forming complex networks over cardiac muscle strands. Individual muscle strands in atrial preparations also appeared to be densely innervated. Galanin immunoreactive fibers were present in the nerve trunks connecting clusters of ganglion cells and could be seen making close appositions to individual ganglion cells. The majority of the parasymapthetic postganglionic neurons in the cardiac ganglion exhibited galanin immunoreactivity. Many of the small intraganglionic neurons (SIF-like cells) also exhibited galanin immunoreactivity. The density and distribution of galanin immunoreactive fibers was unchanged in preparations taken from animals pretreated with 6-hydroxydopamine, indicating that the fibers were not sympathetic postganglionic axons. The staining pattern also suggested that the galanin-like peptide was not contained in processes of primary afferent fibers. However, the pattern of galanin immunoreactivity, both in ganglion cells and innervating cardiac muscle, was very similar to the staining pattern obtained for AChE. Galanin in the range 1×10^{-7} to 1×10^{-6}M decreased the spontaneous rhythm of isolated sinus venosus preparations. Cardioinhibition was obtained in many preparations exposed to 1×10^{-6}M galanin. Galanin also produced a concentration dependent (1×10^{-7} to 1×10^{-6}M) decrease of the twitch tension development in electrically stimulated atrial preparations. Local application of galanin produced hyperpolarization of cardiac muscle fibers in both isolated septal-sinus venosus preparations and atrial preparations. The response to local application of galanin to individual parasympathetic ganglion cells varied between neurons; some neurons being depolarized whereas others were hyperpolarized. We conclude that a galanin-like peptide is contained in the parasympathetic neurons and further that it may work in conjunction with ACh to regulate cardiac function. Supported by PHS RO1-23978 and NSF BNS-8605611.

205.2 INNERVATION OF SEPTAL CARDIAC MUSCLE IN N. Maculosus. R.M. Kriebel, G.M. Hendricks*, L.M. Konopka, D.S. Neel*, and R.L. Parsons. Department of Anatomy and Neurobiology, The University of Vermont College of Medicine, Burlington, VT 05405.

The major constituents of the septal area stretching between the two limbs of the sinus venosus are neuronal: postganglionic parasympathetic neurons, preganglionic vagal fibers, sympathetic fibers, sensory fibers, and intrinsic neurons. Among these neuronal elements, there are also numerous bundles of cardiac muscle. These cardiac muscle fibers receive branches from aminergic nerve fibers coursing through the septum and may also receive an innervation by peptidergic fibers. This electron microscopic study was done to examine the type of cardiac muscle which is contained in the septum and to study the innervation of the muscle which apparently is not active in pulsations of the heart chambers. Many of the cardiac muscle cells in the septum ultrastructurally were characterized by well organized cytoplasmic arrays of myofibrils and complex intercellular junctions. Interspersed with these fibers were bundles of cardiac muscle which were less spindle shaped and more importantly had poorly arranged and dispersed myofibrillar elements. The cytoplasm of these cardiac muscle cells was also less electron dense when compared to the other muscle type. These features are similar to those described for the specialized conduction type cardiac muscle found in hearts of other animals. Immunofluorescence staining for actin filaments also showed septal cardiac myocytes with well organized myofibrils and some myocytes with cytoplasmic clumping of actin filaments. Several bundles of unmyelinated nerve fibers were seen coursing in parallel with the cardiac muscle strands. These unmyelinated fibers branched and single nerve fibers were found coursing between the individual muscle cells. Varicose profiles containing clear and granular synaptic vesicles were enclosed by profiles of several myocytes. Terminals containing only clear vesicles were seen in close apposition to cardiac myocytes having well developed contractile protein organization. Generally, amphibian myocardium has been thought to contain a single type of myocyte specialized for force production. These studies suggest that a conduction-type myocyte may be present within the septal area of the heart in the mudpuppy. Supported by PHS RO1-23978 and NSF BNS-8605611.

205.3 PHARMACOLOGICAL EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE ON THE ISOLATED PERFUSED GUINEA-PIG HEART. D.B. Hoover. Dept. Pharmacol., Quillen-Dishner College of Medicine, East Tenn. State Univ., Johnson City, TN 37614.

Vasoactive intestinal peptide (VIP)-like immunoreactive nerves are found in right atrium, nodal tissue and coronary vessels of guinea-pigs (Weihe et al, Cell Tissue Res 236:527-540, 1984). This suggests that VIP plays a role in the neuroregulation of the heart and coronary circulation. Other investigators have shown that porcine VIP is a coronary vasodilator in the dog. Positive inotropic and chronotropic effects have also been demonstrated with porcine VIP in several species. In the present investigation, the pharmacological effects of guinea-pig VIP were examined using isolated perfused guinea-pig hearts.

Hearts from male Hartley guinea-pigs were perfused with a modified Krebs-Ringer bicarbonate solution (8 ml/min; 37° C; pH 7.4) containing 0.1% BSA. Experiments were started after a 30 min stabilization period. Perfusion pressure (mmHg), isometric ventricular contractions (g) and ventricular rate were monitored. Guinea-pig VIP (Peninsula) was given by bolus injection in 100 µl of saline containing 0.1% BSA. VIP (0.01-1.0 nmole) produced a dose-dependent increase in ventricular rate (40 ± 6 beats/min at 1.0 nmole dose, n=4). Perfusion pressure was reduced, but changes were small because of the low basal perfusion pressure (32 ± 1.3 mmHg, n=11). The vasodilator response to VIP was substantially larger when basal coronary tone was elevated with 1 µM [Arg^8]-vasopressin. VIP caused a delayed reduction in amplitude of ventricular contractions and, in many cases, increased baseline tension. Norepinephrine (0.1 and 0.5 nmole) had a marked positive inotropic effect in the same preparations. A brief positive ionotropic response has occasionally seen with 10 nmoles of VIP, but these contractions were not as impressive as those elicited by norepinephrine. Atenolol (3 µM) blocked positive inotropic and chronotropic responses to norepinephrine but did not affect responses to 1.0 nmole VIP. Tachyphylaxis occurred to guinea-pig VIP when doses were repeated at 15 min intervals. The positive chronotropic and vasodilator effects of guinea-pig VIP are consistent with actions previously demonstrated for porcine VIP. The failure of guinea-pig VIP to consistently evoke a positive inotropic response in guinea-pig heart was surprising and needs further study. The latter finding could represent a species difference or could be a consequence of the structural difference between porcine and guinea-pig VIP. (This work was supported by a Grant-in-Aid from the American Heart Association, Tennessee Affiliate.)

205.4 DEVELOPMENT OF PHYSIOLOGICAL RESPONSIVENESS TO ADENOSINE ANALOGS IN EMBRYONIC CHICK HEART. T.A. Blair*and T.F. Murray (Spon: R.A. Dodson) College of Pharmacy, Oregon State University, Corvallis, OR 97331

The developing chick heart has been used extensively to study the biochemical events involved in the activation and regulation of various neuroreceptors. In the present study we have used the embryonic chick heart to study the development of caridac adenosine receptors. Adenosine has been reported to have negative inotropic, chronotropic and dromotropic effects and has also been shown to be capable of inhibiting the myocardial effects of catecholamines. These effects of adenosine are competitively antagonized by methylxanthines indicating the involvement of specific receptors on the cellular membrane. We have determined the ontogeny of a physiological response to adenosine analogs by measuring atrial beating in embryos at various stages of development. Intact beating hearts were removed from the embryos and placed in buffered aerated media maintained at 37°C. Atria were separated from the ventricles and pinned to a Sylgaard-coated petri dish. Beating rates were determined visually with the aid of a dissecting scope. Atria isolated from 4-, 5-, and 6-day embryos were minimally responsive to 2-chloroadenosine (ClADO) with a 10 µM concentration inhibiting spontaneous beating rate by only 4-5%. Physiological sensitivity to ClADO-induced negative chronotropic response gradually increased from embryonic day 5 to day 12 at which time the beating rate was completely suppressed by ClADO (3-10 µM). Analysis of ClADO concentration response curves revealed that the development of physiological sensitivity to ClADO reflects a change in the maximal response and not the affinity for ClADO. A virtually identical ontogenetic pattern was demonstrated for R-phenylisopropyladenosine (R-PIA). The relative potencies of the adenosine analogs R-PIA (EC50 0.176 ± 0.065 µM), NECA (EC50 0.516 ± 0.009 µM), ClADO (EC50 0.789 ± 0.079 µM) and S-PIA (EC50 2.94 ± 1.74 µM) indicate the involvement of an A-1 type of adenosine receptor. 8-(p-sulfophenyl)theophylline (8-PST), a methylxanthine that does not cross the cell membrane, was capable of competitively inhibiting the actions of ClADO. Schild analysis of antagonist data demonstrated that 8-PST had a K_i of 0.287 ± 0.145 µM with a slope not significantly different than unity. These results suggest that A-1 adenosine receptors in the chick atrial myocardium are responsible for the physiological response to adenosine analogs and the developmental profile for this response is later than previously reported for the negative chronotropic effects of muscarinic agonists. (Supported by a grant from American Heart Association/ Oregon Affiliate)

205.5 DIFFERENTIAL EFFECTS OF RAPID EYE MOVEMENT AND SLOW WAVE SLEEP ON CORONARY HEMODYNAMIC FUNCTION. Debra A. Kirby* and Richard L. Verrier. Cardiovascular Laboratories, Harvard School of Public Health, Boston, MA 02115.

The sleep/wake cycle results in distinctive patterns of autonomic nervous system activity. Whereas the influence of these changes on systemic hemodynamic function has been characterized, little is known about the influence of sleep on the coronary circulation. Investigation of this issue is particularly germane since it could provide insights into the mechanisms involved in coronary vasospastic disease. In the present study we examined the effects of rapid eye movement (REM) and slow wave sleep (SWS) on coronary hemodynamic function in 5 chronically instrumented dogs. Mean arterial blood pressure (MAP) was measured via a catheter in the aorta, and coronary blood flow (CBF) was determined using Doppler probes placed around the left anterior descending or circumflex coronary artery. Mean coronary vascular resistance was calculated as the quotient of MAP and mean CBF. Instantaneous heart rate (HR) was obtained with a cardiotachometer. Identification of sleep stages was accomplished by implanting electrodes via the frontal sinus to record electro-oculogram, electromyogram, and electroencephalogram. After a surgical recovery period of 4 weeks the animals were studied in the afternoon hours during natural sleep. The cycles were divided into 1 minute epochs of either quiet wakefulness, SWS, or REM sleep. The following results were obtained (means ± SEM, * p<0.02 compared to awake and REM, and † p<0.02 compared to awake, SWS and REM).

	Awake	SWS	REM Baseline	REM Surge
MAP (mmHg)	107±4	106±4	105±7	111±7
HR (beats/min)	85±11	78±10*	83±10	102±9†
CBF (ml/min)	33±5	30±4*	34±4	52±8†
CVR (mmHg/ml/min)	3.6±0.7	3.9±0.7*	3.4±0.7	2.5±0.6†

These results indicate that during SWS there are moderate but significant reductions in HR and CBF and increases in CVR. In REM, the CBF baseline is moderately elevated compared to SWS and there are striking, episodic surges in flow. CVR is reduced correspondingly. HR and MAP are also elevated during the flow surges, indicating an increase in cardiac metabolic activity. Whether or not there is a component of primary coronary vasodilation remains to be determined.

205.6 **DEPRESSED VENTRICULAR INOTROPISM DURING PROLONGED MAXIMAL STELLATE GANGLIONIC STIMULATION IS NOT PRIMARILY DUE TO DECREASED ß-ADRENERGIC FUNCTION**

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Electrical stimulation of a thoracic sympathetic ganglion results in increased inotropism which rapidly peaks and then becomes diminished within 1-2 minutes, even though stimulation continues (inhibition of augmentation). This inhibition could be due to a decreased availability and/or responsiveness of myocardial ß-adrenergic receptors. To investigate this phenomenon, the acutely decentralized stellate ganglia of 8 dogs were stimulated (15V, 10 Hz, 4 ms) following administration of atropine sulphate (1 mg/Kg). Intramyocardial pressure (IMP) in the ventral midwall of the left ventrical was monitored. Noradrenaline (NOR) (0.025-2 µg/Kg) or isoproterenol (ISO) (0.025-1.2 µg/Kg) was administered into the left atrium (1) during the basal state (2) at the peak of inotropic response elicited by the stimulation (3) after 20 minutes of stimulation while stimulation continued and (4) after stimulation ceased. Ganglionic stimulation increased IMP from 98±7 to 257± 29 mm Hg initially but after 20 minutes of continuous stimulation it was 107±9 mm Hg. After the cessation of stimulation it fell to 69±7 mm Hg (p<0.005 vs all other pre-drug values). IMP was increased significantly after administration of NOR, except during peak stimulation when this drug had no significant effect on inotropism. Responses to these drug doses were less in the post-stimulation period (p<0.005). On the other hand, the NOR dose-response curve for IMP sensitivity (expressed as % of peak response) was similar for pre- and post-stimulation periods, but was shifted to the left at 20 minutes of stimulation. IMP responses to ISO were qualitatively similar to those for NOR. These results suggest that decreased ß-adrenergic receptor availability is not primarily responsible for the inhibition of augmentation noted with continuous maximal electrical stimulation, but that the sensitivity of these receptors and/or post-receptor events is increased at that time. Decreased ß-adrenergic function may be implicated in the post-stimulation depression in inotropism.

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205.7 BILATERAL STELLATECTOMY IN THE RAT PRODUCES A MORE SELECTIVE AND SUSTAINED CARDIAC SYMPATHECTOMY THAN 6-HYDROXYDOPAMINE. D.D. Lund, P.G. Schmid, C.L. Putz*, A.R. Subieta*, & B.J. Pardini. Vet Admin Med Ctr & CV Ctr, Dept Int Med, U of Iowa, Iowa City, IA 52242.

Cardiac sympathectomy in small animals is commonly achieved with systemic administration of 6-hydroxydopamine (6-OHDA). This technique is limited because it produces: 1) sympathectomy of all peripheral tissues, 2) incomplete sympathectomy, 3) temporary norepinephrine depletion, and 4) hyperplasia of the adrenal medulla. Our previous study (Fed. Proc. 45(3): 294, 1986) which demonstrated that over 90% of all cardiac sympathetic fibers originate bilaterally in the stellate ganglia implies that removal of the stellate ganglia should produce long term cardiac sympathectomy. Additionally, the sympathetic innervation to more caudal organs supplied by other ganglia should remain intact. The purpose of the present investigation was to compare the effectiveness of bilateral stellatectomy (BI-STEL) and 6-OHDA on regional cardiac norepinephrine concentration. Male, Sprague-Dawley rats were anesthetized with Equithesin and either injected with 6-OHDA (80 mg/Kg, i.v.) or atropinized and intubated in preparation for BI-STEL or sham surgery (CONTROL). After the experimental procedure, rats were allowed to recover and were sacrificed after 7 or 28 days. Norepinephrine concentration was measured by HPLC with electrochemical detection in 4 cardiac and 4 non-cardiac regions. The following data represent norepinephrine concentration (ug/g tissue, wet wt; mean ± SEM in CONTROL and % of control in experimental groups) in right (RAAP) and left (LAAP) atrial appendage and right (RV) and left (LV) ventricle, 7 or 28 days after surgery (n = 6 or 7):

AREA	CONTROL	BI-STEL 7	BI-STEL 28	6-OHDA 7	6-OHDA 28
RAAP	1.80 ± 0.15	5.5%	4.4%	9.9%	18.3%
LAAP	1.68 ± 0.16	2.3%	6.3%	8.4%	20.2%
RV	0.67 ± 0.05	11.1%	9.6%	25.0%	45.3%
LV	0.70 ± 0.05	4.8%	3.4%	22.0%	39.4%

Bilateral stellatectomy produced a greater reduction in cardiac norepinephrine than 6-OHDA in all cardiac regions and at all times. Furthermore, while cardiac norepinephrine after 6-OHDA demonstrated significant recovery by a two-fold increase in norepinephrine content from 7 to 28 days, hearts of BI-STEL rats remained depleted at 28 days. In 4 non-cardiac peripheral organs tested 7 days after BI-STEL, norepinephrine concentration in sub-diaphragmatic (spleen, left adrenal gland) but not supra-diaphragmatic (left intrascapular fat, left forelimb muscle) organs was preserved at control levels. The present evidence suggests that bilateral stellatectomy may provide a more selective and longer lasting cardiac sympathectomy than chemical sympathectomy with 6-OHDA. (Supported by Veterans Administration and NIH HL 35484, HL 38137, and HL 14388.)

205.8 CONTRIBUTION OF MUSCARINIC TRANSMISSION IN THE STELLATE GANGLION TO THE MAINTENANCE OF CARDIOACCELERATOR TONE. M. M. Caverson, M. Bachoo* and C. Polosa*. Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Muscarinic receptors are present on the sympathetic ganglion cell membrane, but their physiological role in ganglionic transmission is unclear. In the decentralized stellate ganglion (SG), preganglionic (SPN) stimulation after nicotinic block produces an atropine-sensitive cardioacceleration but in the absence of nicotinic block the evoked cardioacceleration is not modified by atropine (J.Physiol.191:271,1967). Presumably, when nicotinic ganglionic transmission is unimpaired and activation of the SPN input to the SG is highly synchronous, muscarinic transmission is redundant. Muscarinic transmission may have a role, however, under physiological conditions, i.e. when only a fraction (20-30%) of SPN axons are active and their activity is largely asynchronous (Can.J. Physiol. Pharmacol.46:887,1968). The present study was done to estimate the contribution of muscarinic transmission to the tonic activity of the sympathetic cardiac nerves from the effect of muscarinic antagonists on heart rate (HR). Cats were anesthetized with alpha-chloralose, vagotomized, sino-aortic denervated, paralyzed and artificially ventilated. Doses of the muscarinic antagonist scopolamine (SCO) as low as 10 µg/kg were found to block the cardioacceleration evoked by SPN stimulation in cats in which the SG was decentralized and its SPN input electrically stimulated during constant infusion of hexamethonium. These doses also blocked the bradycardia evoked by vagal stimulation in the same animals. When SCO in doses of 5-100 µg/kg was injected i.v. in cats with an intact SG input, a dose-dependent, sustained decrease in HR was observed in 6 cats. The maximum sustained decrease in HR was 25 ± 5 bpm (223 ± 24 to 198 ± 21 bpm). In these cases, i.v. administration of hexamethonium produced a further decrease in HR of 63 ± 13 bpm (198 ± 21 to 135 ± 22 bpm). SCO had no effect if the SG was decentralized, the inferior cardiac nerves (ICN) sectioned or the animals pretreated with propranolol. These doses of SCO had no effect on the cardioacceleration evoked by stimulation of the right ICN and on the tonic activity of the SPN input to the SG recorded from the T_3 white ramus. Close intra-arterial injections of SCO towards the right SG, using doses (<1 µg/kg) below that which produced an effect by i.v. administration, elicited a sustained decrease in HR (30 bpm). Further i.v. doses of SCO in these animals did not augment the bradycardia. These observations suggest that the SCO-evoked bradycardia is due to a decrease in the tonic activity of SG cells. Thus, during natural activity a component of the input to these cells is mediated by muscarinic receptors.
(Supported by the Medical Research Council of Canada and the Quebec Heart Foundation. MMC is a Fellow of the Canadian Heart Foundation)

205.9 NORADRENERGIC CARDIAC PROJECTIONS FROM THE STELLATE GANGLIA IN THE RAT. B.J. Pardini, P.G. Schmid, C.A. Whiteis*, and D.D. Lund. Veterans Administration Medical Ctr. and Cardiovascular Ctr., Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IA 52242.

We have previously demonstrated, using retrograde tracers, that the sympathetic postganglionic innervation of the rat cardiac ventricles originates bilaterally in the stellate ganglia (approximately 92% of all labeled cells), with lesser contributions from the superior cervical and fourth and fifth thoracic ganglia (Fed. Proc. 45(3):294, 1986). The innervation is primarily ipsilateral (approximately 60%) in nature, with significant (40%) contralateral contributions. Presumably, removal of left, right, or both stellate ganglia should produce reductions in cardiac norepinephrine concentrations consistent with the retrograde tracer results. The purpose of the present investigation was to determine the effect of unilateral and bilateral stellatectomy on regional cardiac norepinephrine concentration in the rat. Male, Sprague-Dawley rats were anesthetized with Equithesin, atropinized, and intubated in preparation for left (L-STEL), right (R-STEL), or bilateral (BI-STEL) stellatectomy, or sham surgery (CONTROL). The stellate (middle and inferior cervical) ganglia were approached from the dorsal aspect of the first and/or second intercostal space. After surgery rats were allowed to recover and were sacrificed 7 days later. Norepinephrine concentration was measured after separation by HPLC with electrochemical detection in four cardiac regions: right (RAAP) and left (LAAP) atrial appendage and right (RV) and left (LV) ventricle. The following data represent norepinephrine concentration (ug/g tissue, wet wt; mean ± SEM; n = 6 or 7):

AREA	CONTROL	R-STEL	L-STEL	BI-STEL
RAAP	1.68 ± 0.08	0.57 ± 0.07	1.36 ± 0.16	0.09 ± 0.04
LAAP	1.52 ± 0.10	1.01 ± 0.09	0.49 ± 0.11	0.04 ± 0.04
RV	1.19 ± 0.05	0.77 ± 0.04	0.55 ± 0.07	0.13 ± 0.04
LV	0.58 ± 0.02	0.38 ± 0.04	0.20 ± 0.04	0.03 ± 0.01

One week after bilateral stellatectomy regional cardiac norepinephrine was reduced by 89% to 97%, indicating the removal of almost all cardiac noradrenergic cells of origin and possibly similar fibers of passage from other ganglia. Unilateral stellatectomy indicates that the RAAP, LAAP, and LV receive bilateral, predominantly ipsilateral, innervation from the stellate ganglia, which is consistent with prior neuroanatomical results. In contrast with earlier results, the left stellate ganglion appears to contribute a majority of the norepinephrine to the RV. The present neurochemical evidence largely corroborates our previous neuroanatomical study and suggests that bilateral stellatectomy may be an effective method to produce cardiac sympathectomy. (Supported by Veterans Administration and NIH HL 35484, HL 38137, and HL 14388.)

205.10 THE INTERACTION OF INTRADENTAL NERVE ACTIVITY AND PULPAL BLOOD FLOW AS MEASURED BY XENON-133 AND LASER DOPPLER FLOWMETRY. G. BILOTTO, M-T. LIU*, K. MARKOWITZ* and S. KIM*. Lab of Oral Physiology, Columbia University, New York, NY 10032.

Previous reports (Soc. Neurosci. abstr. 12:226, 1986; IDR 65:210, 1986) have shown the effects of 3M NaCl and 0.756M KCl on intradental nerve activity (INA). However since pulpal nerve terminals contain vasoactive peptides the above substances via the activation of these terminals can alter the pulpal blood flow (PBF). The altered blood flow can in turn affect the A-δ nerve fibers since they are kown to be very sensitive to change in oxygen supply. The above substances were applied to canine teeth of 3 dogs or 1 cat anthesthesized with sodium pentobarbital. The canine teeth were prepared with two dentinal cavities (incisal and gingival cavities). The solutions were applied to the incisal cavity while the INA was monitored with Ag/AgCl electrodes from the same cavity. 2 μCi of Xenon-133 suspended in saline was given as a bolus injection in the maxillary artery. A specially designed extracorporal γ-counting probe detected the γ-emissions from the canine tooth. A Tracor Multichannel Analyzer provided the Xenon-133 washout curve. A two compartment non-linear model provided the time contants for the fast and slow components. The laser Doppler flowmetry consisted of a He-Ne laser directed at the incisal cavity with associated electronic circuitry measuring the Doppler shift and hence indicating the PBF changes (Med Pacific LD5000 Laser). The Xe-133 washout in dogs indicated that with 3M NaCl applied for 5 min in the dentinal cavity, the PBF decreased by an average of 5.26% whereas a 22 min application cause a 53% decrease from the control level of an intact tooth. The laser Doppler revealed a decrease in the PBF by an average of 14% 30 sec after a 2 min application of 3M NaCl both in dog and cat preparations. 0.756M KCl caused a decrease in PBF by 8.45% after 26 sec of a 2 min application of the KCl. Xenon-133 indicated a decrease of 28% in PBF when KCl remained in the cavity for 6 or 8 min. Electrical stimulation of the intact canine tooth with different stimulus parameters to preferentially activate A-δ fibers vs C-fibers indicated that at low stimulus current (80 μA, 1 ms, 100 Hz) applied for 6 min reduced PBF by 6%-8% whereas high current levels (350 μA, 10 ms, 10 Hz) applied for 3 min reduced PBF by 6-20%.

Therefore from the above preliminary results INA evoked either by chemical agents or electrical stimulation reduced the PBF. It is interesting to note that KCl which has a biphasic effect-first eliciting INA for a short time and then causing a longer depolarizing phase which reduces INA still caused a lowering of the PBF.

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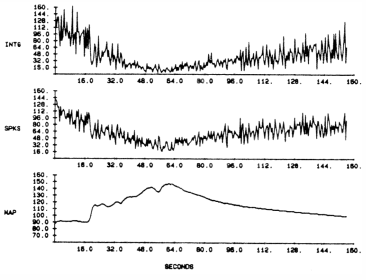
205.11 MICROCOMPUTER BASED DIGITAL ANALYSIS OF NERVE TRAFFIC. DR Brown*, DC Randall, and CF Knapp*. Center Biomed. Engin. & Dept. Physiol. & Biophys., Univ. Kentucky, Lexington, KY 40506.

Nerve traffic is often integrated or counted by analog methods. Digitizing amplified nerve traffic and using digital methods to analyze the resulting data would circumvent several problems with analog techniques. We developed a program which digitizes nerve traffic and performs "on-line" peak detection and integration. The nerve signal was digitized by a Data Translation 2821-F A-to-D converter at 10,000 samples/sec.; a Direct Memory Access controller on an 80286 microprocessor stored the digitized data alternately into two 5,000 sample memory buffers. The program determines the current buffer being filled and then scans the alternate (i.e., previously filled) buffer, digitally integrating and counting the spikes above a preset level. The integrated activity and spike frequency, calculated every 5,000 samples or .5 seconds, were stored on magnetic disks along with digitized mean arterial pressure. The reference level was determined by first digitizing and displaying the nerve signal on a video monitor and then setting the reference level above background noise. The figure below exemplifies the output of this system for percent changes in whole bundle renal sympathetic nerve activity during an increase in arterial pressure (phenylephrine injection) in a rat. The upper panel shows the change in integrated activity (INTG), the middle panel the percent change in spike frequency (SPKS), and the bottom panel the increase in mean arterial pressure (MAP, mm Hg).



This system enables "on-line" digital analysis of nerve traffic using conventional algorithms; it permits the eventual application of more complex analytical techniques to the digitized data. (Supported by NIH grant HL19343)

205.12 ANALYSIS OF THE COMPONENTS OF H_2 CLEARANCE CURVES IN PERIPHERAL NERVE. Timothy J. Day*, Phillip A. Low and Terrence D. Lagerlund* (SPON: E. H. Lambert). Dept. of Neurology, Mayo Foundation, Rochester, MN 55905

Hydrogen polarographic measurement of nerve blood flow (NBF) was performed in healthy Sprague Dawley rats using H_2-sensitive Pt microelectrodes inserted in the sciatic nerve. Biexponential clearances were often observed and further studies were performed to determine the physiological mechanisms responsible for each component.

NBF measurement were made (1) before and after inferior vena caval (IVC) occlusion using a noose of polyethylene tubing; (2) before and after intravenous infusion of 5-hydroxy tryptamine (5HT) at rates of 5 and 10 μg base/kg/min. Both these maneuvers caused abolition or reduction in the weight of the fast clearance rate (80-120 ml/min/100 g) suggesting that arteriovenous (AV) shunts are responsible for this component.

H_2 clearance curves were also recorded after H_2-saturated rats were killed with KCl. Slow clearance rates (median 2.4 ml/min/100g) were observed indicating that diffusion to air (in this exposed, oil-covered preparation) is very slow and, therefore, unlikely to be confused with nutritive flow. Mathematical modelling of the diffusion component yielded very slow clearances of approximately 2 ml/min/100g).

Intermediate clearance rates (10-30 ml/min/100g) were consistently found in all healthy animals and compare well with values for tissue flow obtained from microsphere and iodo-antipyrine techniques. No other postulated clearance mechanisms produced rates in this range and, therefore, these rates are thought to represent nutritive (capillary) flow.

Mathematical modelling indicates that biexponential clearances are expected where near-arteriolar recordings are made and that the two exponents reflect capillary and arteriolar (or AV shunt) flow, respectively. H_2 consumption by polarographic oxidation, a theoretical cause of H_2 clearance, would be expected to produce extremely slow clearances.

Therefore, H_2 polarography can be used to quantitate nutritive NBF, and also the degree and rate of AV shunting in tissue by separate consideration of the exponents of the H_2 clearance functions. (Supported in part by grants from NINCDS (NS-14304; NS-22352) MDA, Mayo and Mogg funds.

205.13 EFFECTS OF 6-HYDROXYDOPAMINE ON CATECHOLAMINE RELEASE AND PRESSOR RESPONSES TO ADRENAL STIMULATION IN PITHED RATS - ROLE OF ANGIOTENSIN. K. Richter*, S.A. Meyers*, S.J. Fluharty*, S.E. Corey*, E.M. Stricker and R.R. Vollmer*. (SPON: E. Krimmer). Dept. of Behavioral Neuroscience, U. of Pgh., Pittsburgh, PA 15260 and Sch. of Vet. Med., U. of PA, Philadelphia, PA 19104.

Partial sympathectomy (6-hydroxydopamine, 6-HDA, 100 mg/kg, s.c., 1 wk. previously) is known to increase adrenal tyrosine hydroxylase activity via a centrally mediated increase in splanchnic nerve activity. The present experiments in the pithed rat were conducted to determine if the increase in adrenal synthetic capacity is associated with alterations in catecholamine release or the responsiveness of the cardiovascular system to released catecholamines.

The thoracolumbar portion of the spinal cord was electrically stimulated (4 and 16 Hz) to activate noradrenergic neurons and the adrenal medulla. Compared to control rats, the damage to noradrenergic neurons in 6-HDA treated animals was reflected by a decrease in the increment in plasma norepinephrine (NE, measured by HPLC with electrochemical detection) and an attenuation of the blood pressure rise produced during stimulation. Plasma epinephrine (EPI) levels and the blood pressure-dose response curves to exogenous NE or EPI were similar in control and 6-HDA treated animals.

In a separate series of experiments the adrenal gland was directly stimulated by placing the gland on bipolar platinum electrodes. Blood pressure-frequency response curves generated (2-32 Hz) were not different in 6-HDA and control animals. Moreover increments in plasma EPI during stimulation at 4 and 16 Hz were similar. Interestingly, the plasma NE responses to direct adrenal stimulation were augmented in 6-HDA-treated animals.

In agreement with our previous studies, blockade of the renin-angiotensin system (captopril, 5 mg/kg, i.v.) significantly reduced the blood pressure-response curves to electrical stimulation of the adrenal gland and to exogenous EPI. In rats treated with 6-HDA the extent of the reduction was greater; however, this enhanced sensitivity to captopril does not appear to be related to a decrease in adrenal catecholamine release since the plasma catecholamine responses to stimulation were similar in control and 6-HDA treated rats.

The results of the present study suggest that despite marked increase in catecholamine synthetic rate following 6-HDA treatment the amount of catecholamine released from the adrenal gland in response to preganglionic stimulation is not altered. In addition, endogenously formed angiotensin II facilitates responses to adrenal catecholamines in 6-HDA treated animals via a mechanism that is independent of catecholamine release. (Supported by grants from NIH HL26212, NS19608 and NSF BNS85-18035.)

205.14 EFFECTS OF ACUTE SINO-AORTIC DENERVATION ON CEREBRAL GLUCOSE UTILIZATION DURING ADMINISTRATION OF ANGIOTENSIN II. M. Kadekaro, M.L. Terrell*, H. Lekan*, H. Gary, Jr.* and H.M. Eisenberg. Div. of Neurosurg., Univ. of Tex. Medical Branch, Galveston, TX 77550

Angiotensin II (AII) infused intravenously elicits a pressor and drinking response and an increase in the rates of glucose utilization in the subfornical organ (SFO) and pituitary neural lobe of water-sated Sprague-Dawley rats (Gross, P.M. et al, Peptides 6 supp 1:145-152, 1985). The firing rates of units in the SFO vary according to the arterial blood pressure suggesting an input of baroreceptors to the SFO (Nicolaidis, S. et al, Brain Res. Bull., 10:357-363, 1983). The objective of the present experiments was to study, with the quantitative deoxyglucose method, the influence of baroreceptors on metabolic response of the SFO to AII. Our previous studies in chronically sino-aortic deafferented animals indicated that the influence of baroreceptors on glucose metabolism of the SFO may be too small to be detected with the deoxyglucose method. A compensatory metabolic adjustment may, however, occur in chronic preparations obscuring such an influence. We, therefore, investigated, in acute preparations, the effect of sino-aortic denervation on glucose metabolism of the SFO and neural lobe in response to AII.

Adult male Sprague-Dawley rats were anesthetized with halothane and the sino-aortic baroreceptors denervated according to Krieger's technique (Krieger, E., Circ. Res. 15:511-521, 1964). The deoxyglucose experiments were performed 2-4 h after the denervation. Four groups of animals were studied, 2 sham-operated and 2 with baroreceptor denervation; each group was infused with saline or AII (2.5 ug/min) for 40 min. The results showed that AII increases glucose utilization in the SFO to the same extent in the sham and denervated rats. The response of the neural lobe in deafferented animals was larger than in sham-operated animals, even though this difference was not statistically significant. This finding confirms our previous investigation in chronic preparations that the influence of baroreceptors on the functional activity of the SFO is negligible.

In the course of this study, we also investigated the effect of sino-aortic denervation on local cerebral glucose utilization. The results showed that glucose utilization was increased in the median eminence but was decreased in 11 out of 20 brain structures examined. These structures included the dorsal motor n. of the vagus, n. ambiguus, ventrolateral medulla, locus coeruleus, parabrachial n., medial forebrain bundle, paraventricular n. and periventricular n., medial and lateral preoptic area and diagonal band of Broca. These structures may be involved in the baroreflex control of blood pressure.

205.15 CENTRAL AND PERIPHERAL ACTIONS OF ALPHA-2 ADRENERGIC AGONISTS ON RENAL FUNCTION IN LONG-EVANS (LE) AND BRATTLEBORO (DI) RATS, D.R. Wallace and R. Dawson, Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610.

Systemic administration of clonidine, a prototypical alpha-2 (partial) agonist produces diuresis and natriuresis in the conscious rat. The exact mechanism of the alpha-2 adrenergic mediated diuresis and natriuresis is unknown, although a centrally mediated inhibition of vasopressin (AVP), an alteration in sympathetic neural function and a direct renal action have all been postulated. This study employed LE and rats deficient in (AVP) (homozygous DI rats) to examine the renal actions of alpha-2 adrenergic agonists that have both central and peripheral actions (guanabenz and guanfacine) and an alpha-2 agonist with very poor blood brain barrier penetration (ST-91).

Male LE (n = 10) and DI (n = 10) rats were housed in metabolic cages and acclimated to a daily 4 hour collection period. Saline injections were performed and stable baseline measures were obtained prior to the randomized presentation of various doses (0.1 or 1.0 mg/kg) of alpha-2 adrenergic agonists. Drugs were prepared fresh on the day of injection in saline vehicle and administered subcutaneously. Water intake and urine volume were measured and urinary sodium excretion was determined by an ion sensitive electrode technique.

Alpha-2 adrenergic agonists with both central and peripheral actions (guanabenz, guanfacine) significantly ($p < 0.01$) increased sodium excretion in a dose related manner (1.5-3 fold above baseline) in LE rats but had no significant effect on sodium excretion in DI rats. In contrast, the peripherally acting alpha-2 adrenergic agonist, ST-91, significantly ($p < 0.001$) increased sodium excretion in both LE and DI rats, although at 1.0 mg/kg the increase in the LE rats (5.4 fold) was significantly ($p < 0.05$) greater than in DI rats (2.9 fold). The alpha-2 adrenergic agonists all increased urine output in both LE and DI rats, however the magnitude of the increase was greater in the LE rat. Water intake was stimulated in both LE and DI rats by 0.1 mg/kg of either guanfacine of guanabenz. ST-91 significantly ($p < 0.001$) decreased water intake in both LE and DI at the 1.0 mg/kg dose and guanabenz (1 mg/kg) reduced water intake in DI rats.

The results of this study suggest that in the absence of vasopressin, centrally acting alpha-2 adrenergic agonists have limited natriuretic action, although peripheral activation of alpha-2 adrenergic receptors is sufficient to elicit natriuresis irrespective of the presence of AVP. The diuretic response to alpha-2 adrenergic agonists is independent of AVP or direct CNS involvement, suggesting a direct renal action. This project was supported by a grant from the Division of Sponsored Research of the University of Florida.

205.16 Single Units From Fibers in Rabbit Carotid Sinus Nerve With Both Baro- and Chemoreceptor Characteristics. Long Qu and Sherry L. Stuesse, Neurobiology Department, N.E. Ohio Univ. College of Medicine, Rootstown, OH 44272

Fibers in cardiac depressor nerves have traditionally been classified as either baro- or chemoreceptive based on their firing patterns to pressure increases and/or chemical activation. We recorded single unit activity from the carotid sinus nerve in 16 urethane anesthetized, spontaneously breathing, rabbits with a bilateral cervical sympathectomy. The carotid sinus area was vascularly isolated and perfused with oxygenated Locke's solution. Of 48 single units recorded, 18 fibers gave a typical baroreceptive response with a mean firing threshold of 47.5 $\pm$ 3.1mmHg. 10 of the fibers were chemoreceptive since they increased their discharge frequency in response to 5 hydroxytryptamine (5HT) but failed to respond to intrasinus pressure (ISP) changes from 0 to 100mmHg ($P>0.5$). Twenty single fibers, with a discharge pattern similar to the chemoreceptive fibers, were active (22.0 $\pm$ 2.7 impulses/sec) when ISP was 0mmHg. 5HT increased their activity significantly ($P<0.01$) but unlike traditional chemoreceptive fibers, their unit discharge frequency increased from 22.0 $\pm$ 2.7 to 30.2 $\pm$ 2.8 impulses/sec ($P<0.001$) with the pulse of peristaltic ISP. Thus fibers with both baro- and chemoreceptive characteristics may exist in rabbit carotid sinus nerve. (Supported by grants from the Ohio Board of Regents).

205.17 COMPARATIVE EFFECTS OF ACTIVATION OF THE TRIGEMINAL GANGLION AND THE SUPERIOR SAGITTAL SINUS ON CEREBRAL BLOOD FLOW AND SPINAL EVOKED POTENTIALS IN THE CAT. P.J. Goadsby, G.A. Lambert*, A. Zagami* and J.W. Duckworth* Department of Neurology, The Prince Henry Hospital, and School of Medicine, University of New South Wales, Sydney, Australia.

The pain of migraine and other vascular headache involves regions innervated by the trigeminal and cervical sensory systems, and is usually accompanied by changes in cerebral blood flow (Lance, J.W. et al., Headache, 23:258,1983). The cranial vasculature and some parts of the dura are pain sensitive, innervated by the trigeminal nerve and may be involved in migraine. Stimulation of the trigeminal ganglion leads to increases in regional cerebral blood flow (RCBF) limited to the frontal and parietal cortex (Goadsby & Duckworth, Am. J. Physiol., 1987, in press). In these experiments we stimulated one of the pain sensitive structures innervated by the trigeminal nerve the sagittal sinus to measure changes in RCBF and cervical cord electrical activity. RCBF was measured in the d-chloralose anesthetised cat using the freely diffusible tracer [^{14}C]-iodoantipyrine and brain dissection. Multiple unit and field potentials were recorded from the cervical cord using standard electrophysiological techniques. Data were collected on-line with a microcomputer for processing of results. The sagittal sinus was exposed, isolated from the surrounding brain and stimulated at a rate of 10/s with 500us duration, 100v square wave pulses. RCBF was increased in restricted areas in stimulated animals, the effect was bilateral and involved the frontal and parietal cortices and thalamus. The increases were larger than those seen with trigeminal ganglion stimulation. Stimulation of the sagittal sinus produced either a single component slow wave response seen maximally caudal to the C2 root entry dorsolaterally or a complex fast wave response seen maximally in a ventromedial location 4mm further caudal from the slow wave response. Stimulation of the trigeminal ganglion produced similar responses topographically but ones that were quantitatively smaller. We conclude that craniovascular pain input can have powerful effects on central nervous system processing and that these effects strongly interact with other trigeminal sensory components.

205.18 INCREASES IN CEREBRAL CORTICAL PERFUSATE ADENOSINE AND INOSINE CONCENTRATIONS DURING HYPOXIA AND ISCHEMIA. M.H. O'Regan*, G.A. Walter*, R.E. Stair*, J.W. Phillis (SPON: R.R. Gala). Dept. of Physiol., Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Changes in the partial pressure of oxygen in the brain evoke appropriate responses in the cerebral vasculature. Adenosine, a potent pial vessel dilator, has been proposed as a mediator of this metabolic regulation of cerebral blood flow. This study used the cerebral cortical cup technique to monitor changes in adenosine and its major metabolite, inosine, levels in the rat cerebral cortex during periods of hypoxia, anoxia, or hemorrhagic hypotension. Basal levels of adenosine and inosine in cortical perfusates of artificial CSF stabilized within 10 minutes at concentrations ranging from 30-50 nM and 75-130 nM respectively. These levels are significantly lower than published estimates using implanted dialysis fibers. Normal CSF, collected from the cisterna magna, gave values for adenosine and inosine comparable to our perfusate concentrations. In response to reductions in the oxygen content of inspired air (14%, 12%, 8%, 5% O_2) adenosine and inosine levels in the perfusates increased in a dose dependent manner (180%, 510%, 754%, and 1224% above control levels of adenosine respectively). Cerebral ischemia/anoxia, induced by 100% N_2 inhalation, resulted in a rapid increase in adenosine and inosine concentrations. However, no significant elevations in either adenosine or inosine levels were obtained upon hemorrhagic hypotension of a magnitude (46.1 ± 1.7 mmHg) greater than the hypotension that frequently accompanied hypoxia. Thus, in response to decreases in the pO_2, adenosine is released into the interstitial space of the cerebral cortex in concentrations capable of dilating vessels supplying the area. This supports the adenosine hypothesis of metabolic regulation of cerebral blood flow during hypoxia and ischemia, but the autoregulatory response to moderate hypotension, unaccompanied by hypoxia, does not appear to be mediated by adenosine.

205.19 HEMODYNAMIC RESPONSES TO INTRAVENOUS COCAINE ADMINISTRATION IN RATS. M.M. Knuepfer, D.M. Wehner* and A. Poklis*. Dept. of Pharmacology St. Louis University School of Medicine, St. Louis, MO 63104.

Cocaine is a potent CNS stimulant and local anesthetic that is widely abused for its euphorogenic effects. Cocaine is known to cause an increase in arterial pressure (AP) and in heart rate (HR). The hemodynamic effects of acute and chronic cocaine administration are unknown. These are particularly important since cocaine abuse is becoming more widespread and reports of related cardiovascular dysfunction are increasing. The present studies are designed to describe the hemodynamic effects of cocaine in unanesthetized (U) and Dial-urethane anesthetized (DUA) rats. Pentobarbital-anesthetized rats (N=6) were instrumented with arterial (carotid) and venous (jugular) cannula that were exteriorized at the nape of the neck. Miniaturized pulsed Doppler flow probes were placed around the superior mesenteric artery and on the abdominal aorta. These were connected to a socket mounted on the skull for measurement of hindquarter (HQ) and mesenteric (MES) vascular flow changes. Rats were treated with antibiotics and allowed to recover for two days. After acclimatization, rats were given cocaine (0.25-10 mg/kg, i.v.) and their responses were recorded on a chart recorder and on magnetic diskettes using a data acquisition system (PC Chart Recorder). Results were variable particularly at low doses but, in general, HQ vasodilation and MES vasoconstriction were observed at all dose levels. AP and HR were hardly affected at low doses (0.25-1 mg/kg) whereas increases were observed at higher doses. Behaviorally, increasing doses produced an increase in motor activity and apparent arousal. Administration of cocaine hourly produced progressively smaller changes in hemodynamic responses. A separate group of rats (N=10) were examined for responses under DUA to avoid vascular responses due to increased skeletal muscular activity. DUA rats were prepared as described above and allowed to stabilize for 30-60 minutes. Treatment with cocaine (5-10 mg/kg, i.v.) produced a prolonged fall in AP and HR accompanied by moderate HQ vasodilation. For example, 5 mg/kg cocaine caused the following effects in U (N=4) and DUA (N=6) rats 2 minutes after injection:

	AP	HR	HQ Resistance	MES Resistance
U	22%	45 b/m	-23%	136%
DUA	-5%	-25 b/m	-18%	11%

These results indicate that cocaine causes specific changes in regional vascular blood flows that contribute to its effect on AP. Tolerance to these effects develops rapidly. DUA prevents MES vasoconstrictor and behavioral responses but does not affect HQ vasodilation. The specific mechanisms for these changes and the effects of chronic cocaine administration will be determined.

(Supported by NIH grants HL37224 and HL38299 and by a grant from the American Heart Association, Missouri Affiliate.)

205PO CLONIDINE REDUCES THE AVP RESPONSE AND THE DOSE REQUIREMENT DURING NITROPRUSSIDE-INDUCED HYPOTENSION. L. QUINTIN, O. CALVILLO, M. GHIGNONE*. Anesthesiology, University of Pittsburgh, Pittsburgh, PA, 15261 and Neuropharmacology, School of Medicine Alexis Carrel, Lyon, France.

This study was designed to investigate the effect of moderate and severe hypotension (blood pressure BP=75 and 55 mmHg respectively) induced by sodium nitroprusside (SNP) on arginine vasopressin (AVP) and catecholamines (CA) response and on its modulation by clonidine.6 mongrel dogs were anesthetized with thiopentone and maintained with halothane in O_2 with controlled mandatory ventilation. Hemodynamic variables, plasma CA and AVP were measured as follows : baseline condition (BL1) ; during SNP infusion after 30 min of BP=75 mmHg (SNP1A) and BP=50 mmHg (SNP1B) ; 20 min after SNP1B (BL2) ; 20 min (CLO) and 90 min (BL3) after clonidine infusion 7µg.kg^{-1}iv ; during SNP (SNP2A and SNP2B) and 20 min post SNP2B (BL4). Similar reduction of BP and filling pressure were obtained with SNP, before and after clonidine. However clonidine induced a sustained reduction of 20% of HR and CO ($p<.01$) and of 50% of AVP levels at BL3. Furthermore it induced a significant reduction of AVP levels during SNP2A and 2B ($p<.01$) but not of plasma CA. The reduction of SNP requirement for a given hypotensive level were proportional to the suppression of AVP secretion ($p<.01$). Clonidine in this dose did not suppressed, peripherally, the activation of the sympathoadrenal and neuronal responses to hypotension in contrast to what was observed, centrally, in the caudal ventrolateral medulla (Quintin L., Soc Neuro Sci Abstract, 12 (2):1157, 1986). The significant reduction in AVP secretion after clonidine and the parallel reduction of SNP dose strongly indicates that AVP play a major role in the short term regulation of BP during hypotension. Clonidine increases the sensitivity to SNP by lowering AVP secretion. This may be secondary to an inhibitory effect on hypothalamic cells (Reid I.A., JPET 229:1, 1983). Clonidine may also interferes with the AVP dependent baroreceptor facilitation (Undesser K., Circ Res 58:882,, 1986).

206.1 UPREGULATION OF RENAL α_2 ADRENOCEPTORS IN NaCl LOADED SHR IS NOT RELATED TO RENAL NERVE ACTIVITY. W. Sripairojthikoon, S. Oparil, and J.M. Wyss. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

The density of renal α_2 adrenoceptors is increased in the spontaneously hypertensive rat of the Okamato strain (SHR) and the NaCl loaded Dahl NaCl-sensitive rat compared to their normotensive controls. It had been postulated that this increase is related to the sodium retention and the resulting hypertension displayed in both models. Further, renal nerve activity is relatively high in both models. In the present study, we tested the hypothesis that the increase in the number of renal α_2 adrenoceptors in the NaCl loaded SHR is the result of an interaction between dietary NaCl and renal nerve activity. Seven week old, NaCl-sensitive male SHR (Taconic Farms, IBU3 colony) were placed on diets containing 8% or 1% NaCl. One week later half the rats in each group received either a bilateral renal denervation or a sham operation; the operations were repeated at 11 weeks. Both NaCl loaded groups displayed significantly higher systolic blood pressure than their 1% NaCl fed controls, but renal denervation effected the same relative decrease in blood pressure in both the 8% and 1% NaCl fed rats.

		High Affinity (fmole/mg protein)	Low Affinity (fmole/mg protein)	Blood Pressure (mm Hg)
1% NaCl	Sham	20 ± 1	80 ± 4	201 ± 6
	Denervated	40 ± 2*	122 ± 7*	162 ± 5*
8% NaCl	Sham	32 ± 2 +	109 ± 6 +	236 ± 8 +
	Denervated	55 ± 6*+	151 ± 12*+	206 ± 10*+

*$p<.05$ compared to sham group; +$p<.05$ compared to 1% NaCl group

Analyses of renal α_2 adrenoceptors, using the agonist [^{3}H] para-aminoclonidine, revealed that both denervation and NaCl loading effected increases in α_2 adrenoceptor binding (both high and low affinity), but there was no interaction between the effects of renal denervation and NaCl loading. There were no significant between group differences in the agonist binding affinity (Kd) for either site. These data strongly suggest that in the NaCl-sensitive SHR, dietary NaCl loading and renal nerve activity have independent effects on renal α_2 adrenoceptor regulation.

206.2 DIFFERENCES IN BRAIN AMINES AND NEUROPEPTIDES IN INBRED HYPERTENSIVE AND HYPERACTIVE RAT STRAINS. V.R. Holets[1], T. Hökfelt[2]* and E.D. Hendley[3]. [1]Dept. of Neurological Surgery, Univ. of Miami, Miami, FL, 33136, [2]Dept. of Histology, Karolinska Institute, S-10401 Stockholm, Sweden and [3]Dept. of Physiology and Biophysics, Univ. of Vermont, Burlington, VT 05405

Two new rat strains have been developed to separate the hyperactivity trait from the hypertensive trait, as these currently co-exist in the SHR strain (Hendley et al., Hypertension 5:211, '83). From an original SHR X WKY cross, selected recombinant inbreeding resulted in the development of 2 new strains, WK-HT with hypertension but not hyperactive behavior, WK-HA with hyperactivity but not hypertension. These 2 strains, along with SHR and WKY rats, were analyzed for the immunohistochemical localization of peptides, monoamines and catecholamine synthetic enzymes.

Normal rats and rats which received an icv. injection of colchicine 36 hours earlier (n=8 for each group) were perfused transcardially. The brains (10 levels) and spinal cords (10 levels) were sectioned (14 µm sections). One brain or spinal cord segment from each of the four strains was sectioned together in one block to allow for direct comparison between the strains. Sections were processed for immunohistochemistry using antisera raised against neuropeptide Y (NPY), peptide YY, galanin (GAL), substance P, proctolin (PROC), neurotensin, methionine-enkephalin, thyrotropin releasing-hormone, phenylethanolamine N-methyltransferase (PNMT), tyrosine hydroxylase, dopamine ß-hydroxylase and 5-hydroxytryptamine The slides were analyzed by four observers, each blind to strain.

PROC-immunoreactive fibers in the medullary reticular formation were denser in the hypertensive strains (SHR and WK-HT) than in the normotensive WKY or WK-HA. The number of NPY- and PNMT-immunoreactive fibers was reduced in the intermediolateral cell column of the thoracic and lumbar spinal cord (T2, T6, T12, L2) in the hypertensive strains. The NPY- and GAL-immunoreactive fibers were less dense in the dorsal horn of the spinal cord at all levels investigated. No strain differences were observed at any level of the spinal cord or brain with the other antisera used.

The specific alterations in NPY, PNMT, PROC and GAL immunoreactivity were all associated with the genetic hypertension among these 4 strains. No differences were observed at the level of the cell bodies where these chemical messengers or enzymes are synthesized, but were observed at the level of the fiber terminals in the medulla and spinal cord. Supported in part by NIH Fellowship NS07287.

206.3 EFFECTS OF ADRENAL DEMEDULLATION ON CARDIOVASCULAR RESPONSES TO ACUTE STRESS IN RATS WITH STRESS-INDUCED HYPERTENSION. S. Knardahl*, B. Sanders, and A. K. Johnson. (SPON: I. Gormezano) Departments of Psychology and Pharmacology, and the Cardiovascular Center, The University of Iowa, Iowa City, IA 52242

Because chronic infusions of adrenaline have been shown to produce hypertension rats, it has been suggested that adrenaline is a mediator in stress-induced hypertension. In order to test the hypothesis that lowering adrenaline will attenuate stress-induced hypertension, the adrenal medulla, which is the main source of adrenaline, was removed (ADM-group). Sham-operated rats served as controls (C). All rats were offspring of a cross between spontaneously hypertensive rats and Wistar-Kyoto rats (borderline hypertensive rats), hence they had a genetic predisposition for hypertension. Prior to the chronic stress, the systolic pressures (measured with a tail-cuff method) were 150.8 mmHg in the ADM and 149.2 mmHg in the C-group. The chronic stress consisted of 60 2-hour sessions of a shock-shock conflict paradigm over an 18-week period. At the end of the chronic stress the rats were implanted with catheters in the femoral arteries and allowed two days to recover. The resting mean arterial pressure (MAP) was 141.2 mmHg in the ADM and 142.3 mmHg in the C (ns). Cardiovascular responses to an acute stressor were then examined. The rats were transferred to a test-box in which they were subjected to 1.0 mAmp pulsed footshocks (0.5 sec duration at 5 sec intervals for 5 min. The increase in MAP after transfer was 22.3 % in the ADM and 4.2% in the C ($p < .001$). Immediately after termination of the shocks, the MAP was increased 22.2% above baseline in the ADM and 8.1% in C ($p < .02$). Five min after footshocks, the increase in MAP was 21.6% in the ADM and 7.2% in C ($p < .02$). The adrenal demedullation increased the pressor response to acute stress. Because adrenal demedullation in all lilelihood reduced adrenaline release in response to stress, the beta-adrenoreceptor mediated vasodilation of skeletal muscle vasculature may have been attenuated, resulting in larger pressor responses to stress. If lowering the level of adrenaline has a beneficial effect it may have been offset by the attenuation of skeletal muscle vessle vasodilation.

Supported by NIH grant F05 TW03623 to S.K.

206.4 CHANGES IN NOREPINEPHRINE (NE) INDUCED ENHANCEMENT OF LATERAL HYPOTHALAMIC NEURONAL RESPONSIVENESS TO PUTATIVE NEUROTRANSMITTERS IN HIGH SALT DIET RATS. F.M. Sessler, J.T. Cheng, S.M. Grady* and B.D. Waterhouse. Dept. of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102-1192.

The lateral hypothalamus (LH) has been implicated in the central integration of fluid and electrolyte balance and ingestive behaviors. The results of a number of studies have suggested the participation of NE in these functions and, in fact, changes in hypothalamic content of NE and numbers of alpha- and beta- adrenoceptors have been observed during disturbances of hydromineral balance. In previous studies, we presented evidence in support of a modulatory role for NE within the local circuitry of the LH, i.e. NE was capable of facilitating responses of LH neurons to synaptic inputs and putative transmitters. In the present experiments we examined interactions of NE with LH neuronal responses to amino acid transmitters in animals maintained on a high salt diet (HSD). Male rats were given 1% NaCl in their drinking water for 1-2 weeks. Control subjects received tap water. Extracellular activity of single. LH neurons was then recorded from anesthetized animals LH neuronal responses to iontophoretic pulses (5-50 nA; 10 sec. duration) of GABA or glutamate (Glu), were examined before, during and after NE microiontophoresis (5-50 nA). In control rats, inhibitory responses of LH cells to pulses of GABA were routinely potentiated above control levels during NE administration (31 of 46 neurons, 67%). On the other hand, Glu-evoked excitation was antagonized by NE iontophoresis in 66% (17 of 26) of LH neurons tested. In HSD rats the ability of NE to potentiate GABA-induced depressant responses was reduced such that GABA augmentation was observed in only 5 of 22 cases (23%). The effect of NE on Glu excitation was also modified in HSD rats: in 2 of 15 cases (13%), Glu-evoked responses were antagonized by NE whereas Glu excitation was enhanced relative to background firing in 60% (n=9) of LH neurons during NE application. In summary, these findings indicate that chronic HSD can alter the modulatory influences of NE on LH neuronal responsiveness to putative amino acid transmitters. Although many, as yet undetermined mechanisms including adrenoceptor changes are likely to be reponsible for these alterations in noradrenergic action, the present results provide evidence of a specific parameter of NE function that is altered in response to a perturbation of hydromineral balance. This plasticity of noradrenergic action may represent the physiologic expression of a mechanism whereby the LH circuitry can change its sensitivity to afferent synaptic signals as a means of compensating for changes in salt intake. (Supported by AFOSR-85-0155 and NS 18081 to B.D.W.)

206.5 GROWTH AND BEATING RATE OF EMBRYONIC SHR AND WKY HEART MATURING IN THE ANTERIOR EYE CHAMBER OF SHR AND WKY HOST RATS. D.C. Tucker and C.H. Gautier*, Dept. of Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

Culture of embryonic heart in the anterior eye chamber is a model system in which control of cardiac growth and beating rate by genetic and neurohumoral influences can be separated. Both increased heart size and tachycardia characterize weaning-aged Spontaneously Hypertensive Rat (SHR) pups. To examine genetic and neurohumoral controls of heart growth and beating rate in SHR rats, we cultured heart tissue from SHR and Wistar-Kyoto (WKY) embryos in the anterior eye chambers of adult SHR or WKY host rats. Differential influences of sympathetic innervation on SHR and WKY hearts developing in oculo were also studied.

A sample of hearts dissected from SHR and WKY fetuses at 13 days gestation did not differ significantly in weight (1.85 + 0.38 vs 1.07 + 0.08 mg). However, the body weight of WKY fetuses was greater than SHR (89.05 + 5.16 vs 59.65 + 3.19 mg), resulting in a significantly higher heart weight to body weight ratio in SHR compared to WKY fetuses (3.13 vs 1.25, $p<.01$). Atria and ventricles were separated at the AV junction and either atria (N=70) or ventricles (N=96) were implanted into anterior eye chambers of SHR or WKY host rats. Neither atria nor ventricles from SHR fetuses cultured in oculo showed greater growth than WKY tissue. Beating rate of transplants was measured from chronically implanted recording electrodes while host rats were awake and freely moving. Functional autonomic innervation was assessed by rate changes in response to changes in ambient light; intrinsic beating rate was estimated after pharmacologic blockade of beta-adrenergic and muscarinic receptors. The intrinsic beating rate of SHR and WKY atria cultured in oculo did not differ as a function of the host strain or the genetic source of the tissue ($p>.10$).

An additional study compared SHR or WKY atria implanted into sympathetically denervated and innervated eye chambers of SHR and WKY hosts (N=80). Sympathetically innervated implants were larger than noninnervated implants ($p<.05$). Neither final size nor intrinsic beating rate of SHR and WKY implants was differentially affected by sympathetic innervation. During weekly measurements from ether-anesthetized hosts, the baseline beating rate of SHR and WKY atria were similar in WKY hosts (226 + 25 vs 235 + 21 bpm). In anesthetized SHR hosts, SHR atria beat significantly faster than WKY tissue (205 + 20 vs 280 + 14; $p<.03$). These results argue against the hypothesis that cardiac hypertrophy and increased intrinsic beating rate are genetically programmed in the SHR rat. (Supported by American Heart Assoc, Ala. Affiliate and R23 HL37045.)

206.6 ENHANCED BINDING OF [3H]HEMICHOLINIUM-3 TO MEMBRANES PREPARED FROM SEVERAL BRAIN REGIONS OF SPONTANEOUSLY HYPERTENSIVE RATS. N.F. Makari*, R.S. Aronstam and J.J. Buccafusco (SPON: D.S. Feldman). Dept. Pharmacology and Toxicology, Medical College of Georgia and Veterans Administration Med. Ctr., Augusta, GA 30912.

Stimulation of cholinergic receptors in unanesthetized rats evokes a hypertensive response. This pressor response has been shown to be exaggerated in spontaneously hypertensive (SH) rats compared to age-matched, normotensive Wistar Kyoto (WK) rats. A biochemical basis for this hypercholinergic state is suggested by our recent demonstration of a marked increase in the capacity of the high affinity choline uptake system in brain synaptosomes derived from SH compared to WK rats (Neurochem. Res. 12:247, 1987). Hemicholinium-3 (HC-3) binds with high affinity to choline uptake sites on synaptosomal membranes, and in vitro [^{3}H]HC-3 binding provides an estimate of the number of these sites as well as an indirect measure of cholinergic activity. The purpose of this study was to compare the binding properties of [^{3}H]HC-3 in synaptosomes prepared from several brain areas of adult (16 week old) SH and WK rats. Systolic pressures (tail cuff) in three SH and WK rats averaged 200 and 125 mmHg, respectively. Brain regions were isolated after decapitation, and rapidly homogenized in cold 50mM Tris buffer. A crude synaptosomal fraction was obtained by centrifugation and the binding of [^{3}H]HC-3 (137 Ci/mmole) was examined using 100ug of membrane protein, an incubation time of 30min at room temperature, and various concentrations of unlabeled HC-3. The reaction was terminated by rapid filtration through glass fiber filters. Competition studies indicated multiple components of [^{3}H]HC-3 binding: 1) a high affinity component having a K_d of < 0.1uM and 2) a low affinity component having a K_d of 5-30 uM. Both components were observed in cortex and medulla. The relative concentrations of high and low affinity sites were determined using 2uM and 1mM unlabeled HC-3, respectively. [^{3}H]HC-3 binding was significantly higher in samples derived from medulla, pons, hypothalamus and midbrain of SH rats compared to WK animals. These differences ranged from about 1.5 to 3 fold and were greater for high affinity binding than for low affinity binding. In contrast, binding of [^{3}H]HC-3 to striatal membranes was not different in the two strains. These results are consistent with our earlier studies (cited above) of high affinity choline uptake, which demonstrated a highly significant correlation between increases in the Vmax for the high affinity uptake system and the development of hypertension in SH rats. This enhancement in choline uptake was observed in hypothalamic and medullary samples, but not striatal samples. These results are also consistent with a role for brain cholinergic neurons in the development and/or maintenance of hypertension. Supported by HL30046 & the Veterans Administration.

206.7 HYPERTENSION FOLLOWING SELECTIVE INHIBITION OF SPINAL ACETYLCHOLINESTERASE IN CONSCIOUS, FREELY-MOVING RATS. V. Magri* and J.J. Buccafusco. Dept. of Pharmacology and Toxicology, and Psychiatry, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

Activation of brain cholinergic receptors in several animal models and in man results in a hypertensive response. Recent studies in this laboratory have indicated that similar responses could be obtained following intrathecal (i.t.) injection of cholinergic agonists in conscious rats. It is possible, however, that the hypertensive response might be due to redistribution of drug from the intrathecal CSF to higher brain structures. The purpose of this study was to determine whether local intraspinal cholinergic neurons mediated the cardiovascular response to i.t. cholinergic agonists. Male, normotensive Wistar rats were surgically implanted with a chronic indwelling i.t. catheter (the length of which was varied so as to terminate at cervical, thoracic or lumbar segments) and a chronic intra-arterial (aortic) catheter. After recovery from surgery rats were freely-moving in their home cages while arterial pressure and heart rate were recorded via the arterial line. I.t. injection of 5 ug of the cholinesterase inhibitor, neostigmine, resulted in a marked increase in mean arterial pressure (MAP) of up to 70 mmHg when the i.t. catheter was at the thoracic level. The pressor response began 2-3 min after injection, became maximal by 30 min and remained significantly higher than pre-injection levels between 1.5-2 hr after injection. Heart rate changes accompanied the pressor response; however, both increases and decreases were observed, often in the same animal. In some cases these changes reached up to 100 beats/min in either direction. The cardiovascular responses observed in animals in which the i.t. catheter was at either the cervical or lumbar regions were qualitatively similar to those for the thoracic level, but significantly reduced in magnitude and duration. Accompanying the cardiovascular response to neostigmine were a number of behavioral and motor changes characterized by tremor, chewing and grooming motions and head bobbing. Several rats which received 5ug of neostigmine at the thoracic level were killed 35 min later and the medulla and spinal cord removed for measurement of cholinesterase activity. No significant enzyme activity was recorded for cervical, thoracic or lumbar spinal segments, however, medullary enzyme activity was near normal. The effects of neostigmine, therefore, are mediated at spinal, not higher brain levels. These results indicate that cholinergic neurons at the thoracic spinal level mediate a hypertensive response. The possibility of an ascending system should be considered in view of the marked behavioral changes observed which accompany the cardiovascular response. Supptd. NIH Grant HL30046 and Veterans Admin.

206.8 ENHANCED PRESSOR RESPONSE TO ACUTE STRESS IN SPONTANEOUSLY HYPERTENSIVE RATS ON A HIGH NaCl DIET. R.M. Thornton*, D.C. Tucker+ and S. Oparil. Hypertension Research Program, Division of Cardiovascular Diseases, Department of Medicine and Department of Psychology+, Univ. of Alabama at Birmingham, AL 35294.

The purpose of this study was to test the hypothesis that spontaneously hypertensive rats (SHR) fed a high NaCl diet manifest enhanced pressor responsiveness to acute environmental stress compared to SHR maintained on a normal NaCl diet. Seven week old male SHR (Taconic Farms) were placed on an 8% NaCl diet (n=20) or a 1% NaCl diet (n=20) for 3 weeks. A catheter was inserted in the femoral artery for measurement of arterial blood pressure and sampling of blood for plasma norepinephrine (NE) and epinephrine (EPI) as indices of sympathoadrenal activity. Two days later, mean arterial pressure (MAP) was recorded for 30 min at room temperature, following which blood was drawn from 6 rats on each diet for measurement of control levels of catecholamines. The remaining animals were placed in 4°C temperature and MAP recorded. Blood was drawn from 7 animals on each diet at 45 and 90 min of cold exposure. In a separate group of animals, multifiber lumbar sympathetic nerve activity (LSNA) was recorded during cold stress in conscious, free moving animals. Prior to cold stress, MAP (163±3mmHg for 1% vs. 159±4mmHg for 8%) was similar in the 1% and 8% groups. MAP increased within 15 min of cold exposure by 27 mmHg in the 8% NaCl group and 14 mmHg in the 1% NaCl group. This elevation in MAP was maintained throughout cold stress in both groups. The maximal increase in MAP and the level at which MAP stabilized in the 8% group were significantly higher than in the 1% group. NE and EPI levels were similar for the two groups at control (NE: 298±43 pg/ml for 1% vs. 255±44 pg/ml for 8%; EPI: 78±31 pg/ml for 1% vs. 61±15 pg/ml for 8%) and increased similarly in both groups at 45 min and at 90 min of cold exposure. LSNA increased within 15 min of cold stress by 296% in the 1% group and 265% in the 8% group and remained elevated at a similar level for both groups throughout cold exposure.

These data support the hypothesis that SHR fed a high NaCl diet manifest increased pressor responsiveness to acute cold stress compared to SHR maintained on a normal NaCl diet. The similar cold induced increment in plasma catecholamines and LSNA in 1% and 8% NaCl groups suggests that the enhanced pressor response in SHR on a high NaCl diet does not result from an exaggerated increase in sympathoadrenal activity. Other mechanisms, such as enhanced responsiveness to a given level of catecholamines at the vascular receptor, or selective enhancement of other pressor systems, e.g. ACTH or AVP, must be invoked to explain the phenomenon.

206.9 NORADRENERGIC INPUT TO ANTERIOR HYPOTHALAMUS OF Dahl-SALT SENSITIVE RATS IS NOT ALTERED FOLLOWING HIGH NaCl INTAKE. Y.F. Chen, Q. Meng*, H. Jin*, J.M. Wyss, S. Oparil. Hypertension Research Program, University of Alabama at Birmingham, Birmingham, AL 35294.

Our previous studies demonstrated that chronic NaCl loading in NaCl-sensitive spontaneously hypertensive rats of the Okamoto strain (SHR-S, Taconic Farms, IBU3 colony) reduced noradrenergic (NA) input to the anterior hypothalamic area (AHA), increased peripheral sympathetic nervous system activity and exacerbated hypertension. The current study tests the hypothesis that reduced NA input to sympathoinhibitory neurons in AHA contributes to NaCl-induced hypertension in a second NaCl sensitive model, the Dahl-S rat. Male Dahl-S rats (4 wks old; Brookhaven National Laboratory) were maintained on a diet containing either 8% (n=20) or 1% (n=20) NaCl for 4 weeks, after which NA stores, synthesis and release was measured in AHA and other brain regions involved in cardiovascular control, including posterior hypothalamic area (PHA), pons and medulla (MED). NA content in each area was measured at time 0 (t_0), and 60 and 120 min after administration of CHMI (50mg/kg, i.p.), a DBH inhibitor. Rats were sacrificed by decapitation without anesthesia, brains were removed rapidly and dissected on ice. Regional NA concentrations were determined by HPLC/EC. NA turnover rate was determined by calculating disappearance rates from tissue following CHMI administration. Results (mean ±SEM) are:

8% NaCl diet			1% NaCl diet		
BP(mmHg)	HR(bpm)	BW(g)	BP(mmHg)	HR(bpm)	BW(g)
171±6Δ	432±11	243±4	138±2	424±9	229±4

	t_0 NA Content (pg/mg)	Turnover Rate (pg/mg/hr)	$t_{1/2}$ (hr)	t_0 NA Content (pg/mg)	Turnover Rate (pg/mg/hr)	$t_{1/2}$ (hr)
AHA	2323±228	715.4	2.25	2356±253	954.3	1.71
PHA	1532±63	407.2	2.61	1605±61	582.4	1.91
PONS	626±17	154.8	2.81	632±14	203.1	2.16
MED	621±22	182.6	2.35	647±56	210.0	2.13

Δp<0.05 compared to 1% NaCl group.

There were no between group differences in NA turnover rates in any brain region studied. These results indicate that NA alterations in NA input to the AHA do not contribute to NaCl induced hypertension in Dahl-S rats, suggesting that the neural mechanisms of dietary NaCl sensitivity are different in Dahl-S and SHR-S models of hypertension.

206.10 PROTEIN CHOICE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) DURING THE DEVELOPMENT OF HYPERTENSION. J.M.B. Pinto and T.J. Maher. Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA., 02115.

Studies in normotensive animals indicate that with age there is a preference for an increased protein/carbohydrate (CHO) ratio. The ingestion of protein increases brain tyrosine levels in the rat, and under certain conditions, i.e., in the SHR, this can lead to enhanced CNS catecholamine synthesis. In the SHR, increments in CNS catecholamines have been shown to lower blood pressure (BP). Our studies were designed to determine whether a preference for dietary protein exists in the SHR during the development of hypertension. Male SHRs and normotensive Wistar-Kyoto rats (WKY) were obtained at 4 weeks of age and maintained on a 20% high quality protein (casein) - 60% CHO diet for 13 weeks, until hypertension, as determined by tail-cuff measurement, was fully established in the SHR. Food choice, at the beginning of the dark cycle, between isocarbohydrate (60%) diets containing 0% or 40% protein was determined at 5-6 day intervals during the 13-week period and each animals choice recorded at various times throughout the day. Results indicated that at 1 hr and 24 hrs a significant difference in preference ($p<0.01$) for SHR compared to WKY during weeks 6-11, when BP increased by 70 mmHg in SHR compared to 40 mmHg in WKY. SHR had a higher protein/CHO ratio choice (range over the 6 weeks of 0.26-0.55, mean ± S.E. 0.37 ± .04 at 1 hr, and 0.30-0.54, 0.42 ± .08 for 24 hr) when compared to WKY (range of 0.16-0.45, mean = 0.27 ± .10 at 1 hr and 0.13-0.40, mean = 0.31 ± .04 at 24 hr). The preference ratio was not significantly different between groups during weeks 1-5, when BP of SHR and WKY did not differ significantly, or after 11 weeks when the rapid increase in BP in SHR was absent. Although there was no significant difference in weight gain between groups during the study. SHR ate more food than WKY (28.2±2.3 g vs 21.7± 1.6 g for SHR and WKY, respectively).

These results suggest the possibility that the increased protein selection by SHR during the phase of rapid development of hypertension may be the result of a requirement for amino acid precursors of neurotransmitters which have been shown to play a significant role in the regulation of BP in this genetically hypertensive model.

206.11 THE BORDERLINE HYPERTENSIVE RAT: RECIPROCAL F1 HYBRIDS COMPARED. C.H. Woodworth, S. Knardahl*, B.J. Sanders, R.F. Kirby, and A.K. Johnson. Departments of Psychology and Pharmacology, and The Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

The borderline hypertensive rat (BHR) is the non-segregating F1 generation produced by crossing the spontaneously hypertensive rat (SHR) with its normotensive progenitor strain, the Wistar-Kyoto (WKY). This mating can result in two genetic variations, depending on which strain is selected to sire the offspring: (SHR♀ X WKY♂)F1 and (WKY♀ X SHR♂)F1. Although no systematic comparison of these reciprocal F1 crosses has been made, there is evidence to suggest that parental effects need to be considered when interpreting results from experiments that use SHR X WKY derivatives. The purpose of our study was to compare reciprocal F1 hybrids with respect to open-field behavior, to resting blood pressure (MAP, mmHg) and heart rate (HR, bpm), and to change in MAP and HR elicited by transfer to a novel chamber. Beginning at 112-120 days of age, 10 (SHR♀ X WKY♂)F1 and 12 (WKY♀ X SHR♂)F1 male offspring from independent litters were tested daily for three days in a dimly-lighted open field. Behaviors recorded in each 15-min session included number of squares entered with all four paws, number of rearings, and number of fecal boli. A week later, animals were anesthetized (ketamine, 0.11 mg/100 g body weight, IP) and catheters implanted in the left femoral artery. Following a 48-hr recovery period, MAP and HR were measured under basal home-cage conditions and immediately after transfer to a novel chamber. Statistical analyses (repeated-measures MANOVA) showed no difference between the reciprocal F1 hybrids in open-field behavior or in cardiovascular measures taken in the home cage or following transfer. Open-field activity showed significant changes over days and over blocks of time within days, although larger blocks were required to detect changes in squares entered than in rearings. These data show that reciprocal F1 hybrids have similar open-field behavior and cardiovascular measurements at 16-17 weeks of age, but do not permit rejection of the hypothesis that parental effects may be manifested at other stages of development or in response to chronic stress. To provide more detailed information on the behavior of BHR in general, intercorrelations were determined between open-field measures for the combined F1 hybrids. Factor analysis of this correlation matrix revealed two factors that load high for activity in the early minutes (Factor 1) or later minutes (Factor 2) of the test sessions, respectively. When these factors were correlated with the cardiovascular measures, a relationship was suggested between Factor 1 (early open-field activity) and change in MAP after transfer to the novel chamber ($r=-.45$, $p<.07$).

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206.12 A DOUBLE DOSE (150 mg SUSTAINED RELEASE) OF PHENYLPROPANOLAMINE (PPA) CAUSES HYPERTENSION. C.R. Lake, R.E. Clymer*, G. Zaloga*, S.M. Hedges* and B. Chernow*. Dept. of Psychiatry, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814.

PPA is a primary ingredient in over 130 medications, including anorectic agents and cough and cold remedies. Over half of these (75) are available without a doctor's prescription or "over the counter" in many drug and grocery stores. Twelve of the 13 diet aids containing PPA on the market are available without prescription. Several billion doses are consumed annually; PPA may be among the top five most frequently used drugs in the United States. Although, to our knowledge, there are no data to suggest the frequency of ingestion of more than the recommended 75 mg, we suspect "double-dosing" occurs often. Whether the recommended dose (75 mg, sustained release) can cause a clinically significant increase in blood pressure (BP), even in some subjects, remains controversial.

In a preliminary study, on 3 separate days, we administered 75 mg PPA, 150 mg PPA and 75 mg PPA plus 400 mg caffeine (all sustained release) to six healthy consenting volunteers, 5 males and 1 female with a mean age of 24 years. We measured supine BP and heart rate (HR) twice before and at 15 minute (min) intervals for 5 hours after single blind,crossover administration of each of the 3 drugs. Once before and at 150 min after drug ingestion, BP and HR also were recorded after a 5 min stand. We observed striking supine BP increases after 150 mg PPA. During the period from 30 to 240 min after drug ingestion, systolic BP (SBP) averaged hypertensive levels of 147 mm Hg after 150 mg PPA versus 125 and 129 for 75 mg PPA and 75 mg PPA plus 400 mg caffeine, respectively ($f=18.95$, $df=2,10$, $p<0.001$). During a broad peak which lasted from 90 to 180 min after drug administration, this difference was even more pronounced: 155 mm Hg versus 125 mm Hg and 132 mm Hg. Increases also were observed in diastolic BP (DBP). Between 30 and 240 min, DBP averaged 81 mm Hg after 150 mg PPA versus 70 after 75 mg PPA and 76 after PPA plus caffeine. This difference was significant with $f=14.2$, $df=2,10$, $p<0.01$. Also interesting were the results of the 5 min stand. Before drug ingestion, the average SBP drop from supine to 5 min of standing was 6 mm Hg (n=18). However, 150 min later, SBP dropped 25 mm Hg after 150 mg PPA, 1 mm Hg with 75 mg PPA and 25 mm Hg with PPA + caffeine. HR was not significantly different across drugs during the 30 to 240 min time frame.